PROJECT COMPLETION REPORT

FOR

MAJOR RESEARCH PROJECT [SCIENCE]

UGC Ref. No.: F. No. 42-230/2013 (SR) dated 25th March 2013

Title of the Project

SYNTHESIS, CHARACTERIZATION OF COUMARIN BASED HETEROCYCLES AND THEIR EFFICACY AS ANTIMICROBIAL AND ANTIOXIDANT AGENTS

Submitted by

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NOVEMBER 2017

Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE
FINAL REPORT OF THE WORK DONE ON THE PROJECT
(TO BE SUBMITTED IN TRIPLICATE)

1. Title of the Project: Synthesis and Characterization of Coumarin Based Heterocycles and Their Efficacy As Antimicrobial and Antioxidant Agents

2. Name and address of the Principal Investigator: Dr. K. Ajay Kumar

3. Name and address of the Institution: Department of Chemistry, Yuvaraja's College, University of Mysore, Mysuru-570005. Karnataka

4. Ugc Approval Letter No. and Date: F. No. 42-230/2013 (SR) dated 25th March 2013

5. Date of Implementation: 28.11.2014

6. Tenure of the Project: 03 Years

7. Total Grant Allocated: 9,44,300.00

8. Total Grant Received: Rs. 6,12,800.00 (Six lakh twelve thousand eight hundred only)

9. Final Expenditure: Rs. 6,31,734=00 (Six lakh thirty one thousand seven hundred and thirty four only)

10. Title of the Project: Synthesis and Characterization of Coumarin Based Heterocycles and Their Efficacy As Antimicrobial and Antioxidant Agents

11. Objectives of the Project:

- Synthesis, purification, characterization the new synthesized molecules as in scheme-1 by spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Screening of the new molecules for their biological activities such as antimicrobial, antioxidant etc.
- Synthesis, purification, characterization the new synthesized molecules as in scheme-2 by spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Screening of the new molecules for their biological activities such as antimicrobial, antioxidant etc.
- Synthesis, purification, characterization the new synthesized molecules as in scheme-3 by spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis.

Screening of the new molecules for their biological activities such as antimicrobial, antioxidant etc.

- Synthesis, purification, characterization the new synthesized molecules as in scheme-4 by spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Screening of the new molecules for their biological activities such as antimicrobial, antioxidant etc.
- Presentation of research findings as in (Scheme-1 to Scheme-4) in an international and in national conferences.
- Publication of the research findings as in (Scheme-1 to Scheme-4) in an appropriate International and National Scientific Journals.

12. Whether Objectives were achieved (Give Details): YES

- Synthesis, purification and characterization of the series of new bis(formylpyrazole) derivatives, screening of the compounds for their antimicrobial, antioxidant activities. The work was published in *Bioorg. Med. Chem. Lett.*
- Synthesis, purification and characterization of the series of new fused pyrans bearing coumarin moiety, screening of the compounds for their antimicrobial activities. The work was published in *Philippines J. Sci.*,
- Stereo selective synthesis, purification and characterization of the series of new synthesis of novel pyrazole and coumarin appended bridged pyrans, screening of the compounds for their antimicrobial activities. *Int. J. Pharm. Pharm. Sci.*,
- Synthesis, purification and characterization of the series of new coumarin appended pyrazolyl-1,3,4-oxadiazoles and pyrazolyl-1,3,4-thiadiazoles, screening of the compounds for their antimicrobial, antioxidant activities. The work was published in *Russ. J. Bioorg. Chem.*
- Synthesis, purification and characterization, crystal structure, molecular structure and Hirshfeld surface analysis of diethyl 2-(4-methylbenzylidine)malonate. The work was published in *Chem. Data Coll.*
- Synthesis, purification and characterization of coumarin appended 1, 3-oxazines, screening of the compounds for their antimicrobial, antioxidant activities. The work was published in *Pharmaceutical Chemistry Journal.*
- Synthesis, purification and characterization of the series of new formylpyrazole derivatives, screening of the compounds for their antimicrobial, antioxidant activities. The work was published in *Bioorg. Med. Chem. Lett*.

- Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate: Synthesis, crystal structure and antimicrobial activities, *Chemical Data Collections*.
- Synthesis, crystal and molecular structure of ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate: Studies on antioxidant, antimicrobial activities and molecular docking. *Chemical Data Collections.*

13. Achievements from the Project: A simple and efficient and accessible approach for the synthesis of coumarin appended hterocycles was developed. The synthesized series of compounds such as coumarin tethered formylpyrazoles, bis(formylpyrazoles), carbazones, thiosemicarbazones, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, fused pyrans have showed remarkable antimicrobial and antioxidant potencies, which paves way for the development of new antimicrobial and antioxidant agents.

14. Summary of the Findings (in 500 words): A series of compounds such as coumarin appended hydroxyl aryl ketones, **3**; hydrazones, **5a-e**; formyl pyrazoles, **6a-e;** Pyrazole fused pyrans, **8a-f**; hydroxy aryl ketone, **10**; phenolic ester of ketone, **11**; hydroxy aryl diketone, **12**; bis-hydrazones, **14a-h**; bis-formyl pyrazoles, **15a-h**; isomeric compounds of fomyl pyrazole tethered pyrazole fused pyrans, **17**(**a-f**) and **18**(**a-f**); semicarbazones, **21a-e** and thiosemicarbazones, **21f-j**; 1,3,4-oxadiazoles **22a-e** and 1,3,4-thiadiazoles, **21f-j**; intermediate compounds such as diethyl 2-(4-methylbenzylidene)malonate, **23** and ethyl 2-(4-substitutedbenzylidene)-3-oxobutanoates **25a-b** have been synthesized by an easy and accessible procedure. The target compounds of the series have been evaluated for their antimicrobial and antioxidant potencies. Quantitative structure-activity relationship (QSAR) analysis and molecular docking studies for some series of compounds were performed to understand the structure activity relationship. The results of the studies showed that some compounds amongst the synthesized series, have excellent antimicrobial and antioxidant properties. This paves way for the researchers to develop new antimicrobial and antioxidant agents of lesser side effects and maximum activities even at lower concentrations.

Amongst the synthesized series, particularly the compounds, **5d**, **6d**, **8d, 8e, 14g, 14h, 17e, 17f, 18e** and **18f** have exhibited promising antifungal and antibacterial activity against the different organisms tested. Compounds **6d**, **15g**, **15h** and **15d** possesses remarkable DPPH radical scavenging abilities and compounds **15g**, **15h** and **15d** showed excellent hydroxyl radical scavenging abilities. The single crystal X-ray diffractions studies of the compounds help to understand the mode of action of these molecules as antimicrobial and antioxidant agents.

15. Contribution to the Society (give details):

The above findings would further encourage the understanding in employing coumarin pyrazole hybrids as potential antibiotic agents for treating infections caused by pathogenic microbes and fungi. Further, detailed QSAR analysis paves the way for design of potent and specific inhibitors for the various microbes assessed in this study. Looking at the results, compounds **5d**, **6d**, **8d, 8e, 14g, 14h, 17e, 17f, 18e** and **18f** as antimicrobial, compounds **6d**, **15g**, **15h** and **15d** as DPPH radical scavengers; and compounds **15g**, **15h, 15d** as hydroxyl radical scavenger drugs for further detailed studies. Further, the present work also paves the way for exploration of these compounds as potential therapeutic agents to treat conditions arising because of excessive oxidative damage.

16. Whether any Ph.D. enrolled/produced out of the Project: YES

- **N. RENUKA,** awarded the Doctor of Philosophy in Chemistry from University of Mysore, [Ex. 9.2/Ph.D/RN/2011-12] with title **"**Synthesis and Characterisation of New Coumarin Based Heterocycles and their Biological Activity"**.**
- **A. DILEEP KUMAR,** Registered for the award of Doctor of Philosophy in Chemistry from University of Mysore, [Enrolment No. WF-0565/ 2016-17].

17. No. of Publications out of the Project (please attach):

- Synthesis of novel coumarin appended bis(formylpyrazole)dervatives: Studies on their antimicrobial and antioxidant activities. *Bioorg. Med. Chem. Lett.,* 26, 2016, 690-694.
- Stereo selective synthesis of novel pyrazole and coumarin appended bridged pyrans as antimicrobial agents. *Int. J. Pharm. Pharm. Sci.*, 2015, 7(10), 69-73.
- Synthesis and biological evaluation of fused pyrans bearing coumarin moiety as potent antimicrobial agents. *Philippines J. Sci.*, 144(1), 2015, 91-96.
- Synthesis of coumarin appended pyrazolyl-1,3,4-oxadiazoles and pyrazolyl-1,3,4-thiadiazoles: Evaluation for their *in vitro* antimicrobial and antioxidant activities and molecular docking studies, *Russian Journal Bioorganic Chemistry*, 2017, 43(2), 197-210.
- Synthesis, crystal structure, molecular structure and Hirshfeld surface analysis of diethyl 2-(4-methylbenzylidine)malonate. *Chem. Data Coll.*, 2016, 2, 17-24.
- Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate: Synthesis, crystal structure and antimicrobial activities, *Chemical Data Collections*, 2016, 5-6, 68-78.

- Synthesis, crystal and molecular structure of ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate: Studies on antioxidant, antimicrobial activities and molecular docking. *Chemical Data Collections*, 2016, 5-6, 36-45.
- Synthesis and biological evaluation of novel formyl-pyrazoles bearing coumarin moiety as potent antimicrobial and antioxidant agents. *Bioorg. Med. Chem. Lett.*, 2013, 23(23), 6406-6409.
- Synthesis of coumarin appended 1, 3-oxazines as potent antimicrobial and antioxidant agents, *Pharmaceutical Chemistry Journal*, **2017**, 51(7), 582-589.

Principal Investigator
(Seal)

Registrar/Principal
(Seal)

COMPLETION REPORT

10. Methodology:

The 4-Methyl-7-hydroxy coumarin on o-acylation followed by Fries rearrangement under suitable reaction conditions afforded the 8-acyl-4-methyl-7-hydroxy coumarin. The compound is then converted to corresponding hydrazone following the usual procedure. The hydrazone on Vilsmeier-Haack formaylation reaction may result in the formation of 3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehyde (formylpyrazole derivative), which on intramolecular cyclisation reaction produce 4-ethyl-8-methyl-2*H*,4*H*-5,11-dioxa-1,2-diaza-cyclopenta[*c*]phenanthren-10-one derivative.

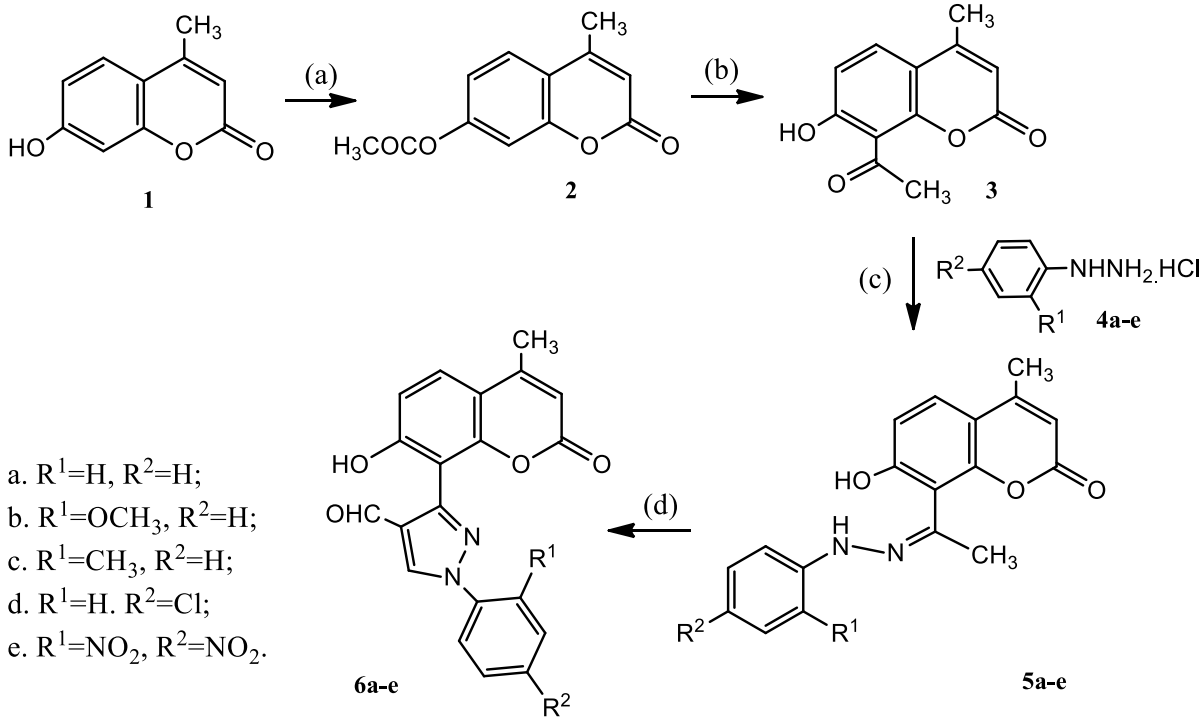
The hydroxyketone on *o*-acylation reaction, followed by Fries rearrangement may produce 6,8-diacyl-7-hydroxy-4-methyl-chromen-2-one derivative. Then the compound is subjected to condensation reaction with, which forms 7-hydroxy-4-methyl-6,8-bis-[1-(hydrazono)-ethyl]-chromen-2-one derivative (dihydrazone derivative). The 7-hydroxy-4-methyl-6,8-bis-[1-(hydrazono)-ethyl]-chromen-2-one is subjected to Vilsmeier-Haack formylation reaction, the reaction is expected to produce bis(formylpyrazole) derivative.

As a part of the project work, we synthesized series of novel bridged pyrans as antimicrobial agents. An isomeric mixture of 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, and 3-(4-ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, were synthesized by the reaction of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-phenyl-1H-pyrazole-4-carbaldehyde) and ethyl alcohol in the presence of conc. H_2SO_4. The synthesized compounds were evaluated for their antimicrobial activity. In a typical procedure, a series of isomeric bridged pyrans tagged to pyrazole and coumarin moiety were synthesized by the condensation reaction of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-aryl-1H-pyrazole-4-carbaldehyde), in ethyl alcohol (10 ml) in the presence of conc. H_2SO_4 (5 ml) under reflux conditions.

As a part and plan of the project work, we synthesized an intermediate diethyl 2-(4-methylbenzylidene)malonate by Knoevenagel condensation reaction of 4-methylbenzaldehyde and diethyl malonate in the presence of catalytic amount of piperidine and trifluoroacetic acid in benzene under reflux conditions. The product obtained was characterized by ^{1}H NMR, Mass spectroscopy and by X-ray diffraction studies.

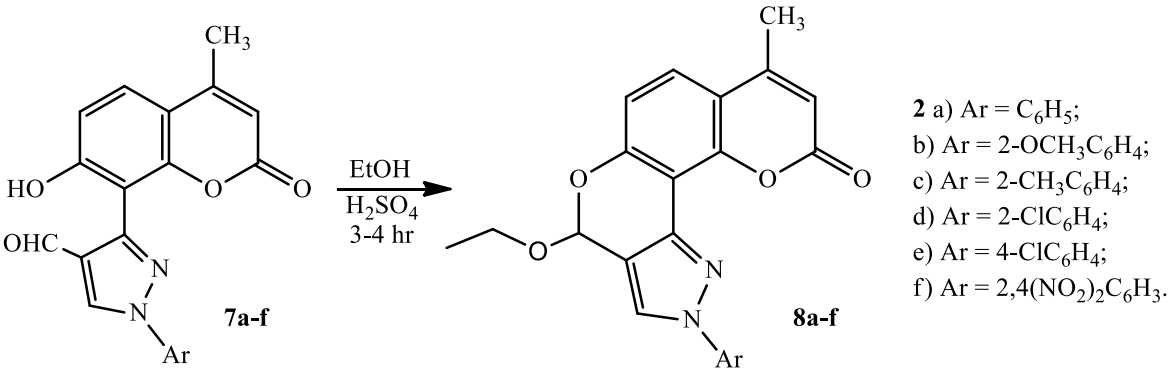
11. Work done so far (please give details):

As per plan of the project, a series of coumarin appended formyl-pyrazoles **6a-e** were synthesized by a simple and accessible approach. 4-Methyl-7-hydroxy coumarin **1** was converted to an ester **2** with acetic anhydride by a standard procedure; then the ester was subjected to Fries rearrangement at 140-160 ºC to get 8-acetyl-4-methyl-7-hydroxy coumarin **3**, the product characteristics are in good agreement with the reported results.[13] The condensation of the compound **3** with substituted phenylhydrazines, **4a-e** in ethyl alcohol and a catalytic amount of acetic acid at water bath reflux conditions, produced the corresponding hydrazones **5a-e**. The hydrazones, **5a-e** (0.0032 mol) were added to the Vilsmeier-Haack reagent prepared by drop-wise addition of $POCl_3$ (1.2 ml) in ice cooled DMF (10 ml). The mixture was stirred at 60-65 ºC for 6 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice cold water, neutralized with $NaHCO_3$, the solid separated was filtered, washed with water and recrystallized from ethanol to obtain target molecules 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehydes, **6a-e** in good yield.[15] The reaction pathway is illustrated in **Scheme-1**. Designed series of molecules **5a-e** and **6a-e** were characterized by spectral and CHN analysis and evaluated for *in vitro* antimicrobial and antioxidant activities.



Scheme-1: Synthesis of compounds **5a-e** and **6a-e**. Conditions: (a) Ac_2O; (b) Anh. $AlCl_3$, 140-160ºC, reflux, 2h; (c) CH_3COOH, C_2H_5OH; (d) DMF, $POCl_3$, 60-65ºC, 5-6 h.

As per plan of the project, we have transformed the synthesized coumarin based formyl pyrazoles in to pyrano pyrazoles. A simple approach for the synthesis of fused pyrans to coumarin moiety is explored. The intramolecular cyclisation of 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehydes under reflux conditions at 80ºC afforded fused pyrans in a relatively excellent yields **Scheme-2**. The synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass and elemental analysis, followed by this; the compounds were screened *in vitro* for their antimicrobial activities against different fungi and bacterium species and antioxidant activity.

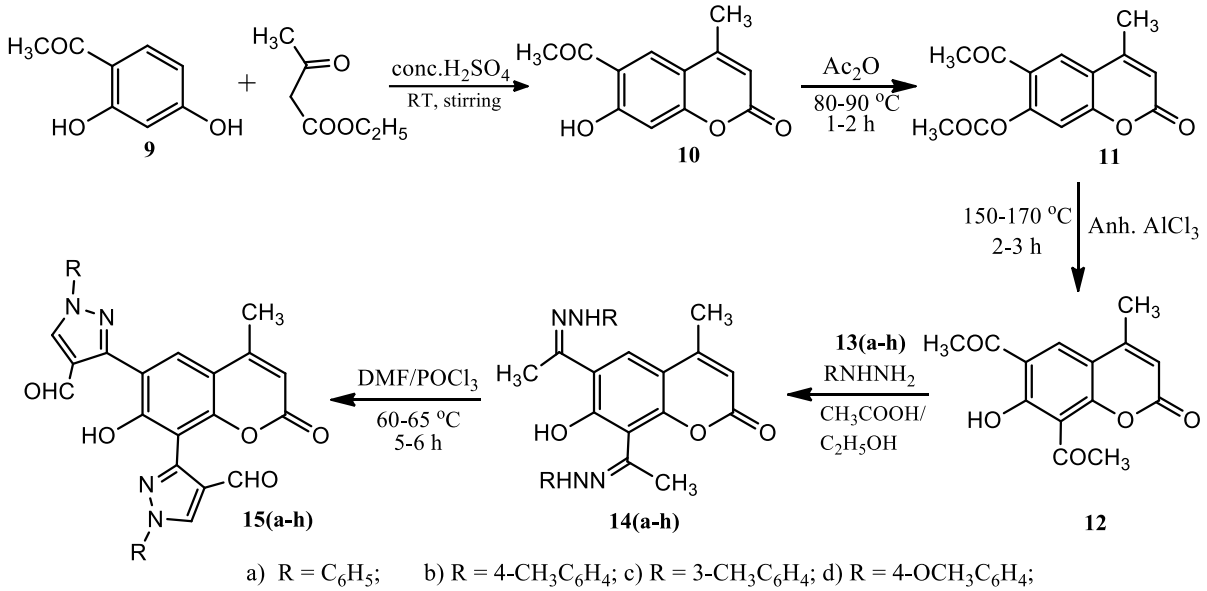


Scheme-2: Synthesis of pyrazole fused pyrans, **8a-f.**

All the new synthesized pyrazole fused pyran compounds **8a-f** exerted a wide range of *in vitro* antibacterial activity against the tested organisms. However, amongst the series, the compound **8e** exhibits inhibition to a greater extent in comparison with the standard against the organisms S. aureus, S. pyogenes and S. Typhimurium. Compound **8d** exhibited promising bacterial activity against the organism tested. Compounds **8d** and **8e** showed potential antifungal activity against all the organisms tested.

As per the plan of project work, in search of new antimicrobial and antioxidant molecules, we have synthesized new bioactive coumarin based bis-hydrazones, bis-carbazones and bis-formyl pyrazole analogues and evaluated them for antimicrobial and antioxidant activities. we have also synthesized coumarin based hydroxy diketones, bis hydrazones and bis(formylpyrazole)derivatives. The starting material 6-acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one **10** was prepared by 1-(2, 4-dihydroxyphenyl)ethanone **9** and ethyl acetoacetate in the presence of sulfuric acid by Pechmann reaction. The treatment of **10** with acetic anhydride to give 6-acetyl-4-methyl-2-oxo-2*H*-chromen-7-yl acetate **11**, this on subjecting to Fries rearrangement using $AlCl_3$ as a catalyst afforded 1,1'-(7-hydroxy-4-methyl-2-oxo-2*H*-chromene-6,8-diyl)diethanone **12**. The condensation of the compound **12** with substituted phenylhydrazines **13(a-f)**, semicarbazine **13g** and thiosemicarbazine **13h** in

ethyl alcohol and a catalytic amount of acetic acid under reflux conditions produced the corresponding bis-hydrazones **14(a-f)**, semicabazones **14g** and thiosemicarbazone **14h**, which were subsequently reacted under Vilsmeier-Haack condition furnished the target molecules **15(a-f),** 3,3'-(7-hydroxy-4-methyl-2-oxo-2*H*-chromene-6,8-diyl)bis(4-formyl-1*H*-pyrazole-1-carboxamide), **15g** and 3,3'-(7-hydroxy-4-methyl-2-oxo-2*H*-chromene-6,8-diyl)bis(4-formyl-1*H*-pyrazole-1-carbothioamide), **15h** in excellent yields **Scheme-3**.



a) R = C_6H_5; b) R = 4-$CH_3C_6H_4$; c) R = 3-$CH_3C_6H_4$; d) R = 4-$OCH_3C_6H_4$; e) R = 4-FC_6H_4; f) R = 4-ClC_6H_4; g) R = $CONH_2$; h) R = $CSNH_2$.

Scheme-3: Synthetic route for coumarin appended bis(formyl pyrazoles)

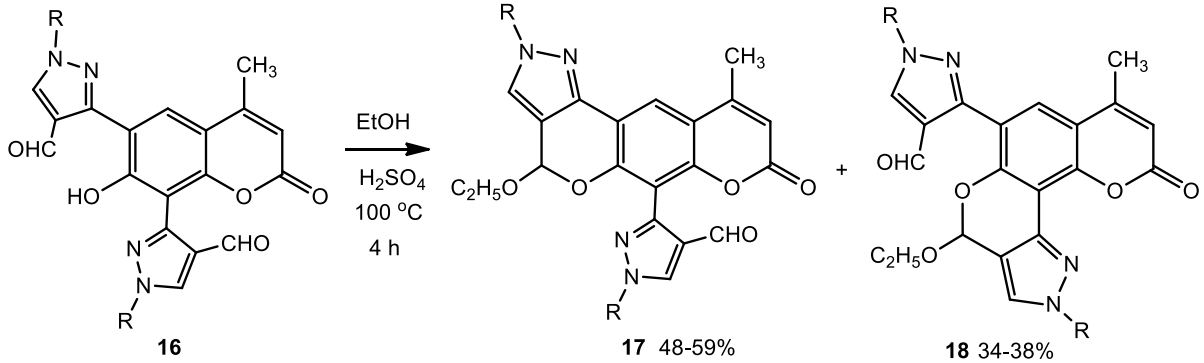
All the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, Mass and CHN analysis, and were screened *in vitro* for their antimicrobial and antioxidant activities. In an attempt to interpret and correlate the molecular parameters of the small molecules with the potency of inhibition against the various microorganisms, detailed quantitative structure-activity relationship (QSAR) analysis was carried out.

Compounds **14g** and **14h** exhibited potent antimicrobial activity among the series synthesised. Compounds **15g**, **15h** and **15d** showed potent antioxidant properties in DPPH and hydroxyl radical scavenging assay. From these biological results, the pyrazole derivatives **15(a-h)** displayed much higher antioxidant activity than those of the carbazones and hydrazone derivatives **14(a-h)**, whereas **14(a-h)** shows higher antimicrobial activity than the pyrazole derivatives **15(a-h).** Further, detailed QSAR analysis paves the way for design of potent and specific inhibitors for the various microbes assessed in this study. Looking at the results, compounds **14g**, **14h** and **15g**, **15h** could be considered as potent antimicrobial and antioxidant drugs for further detailed studies. Efforts have been made for developing new

methodologies to increase the structural complexity while decreasing the number of synthetic steps to facilitate the construction of new coumarin based bis(formylpyrazole) derivatives.

Among the series, compounds **14g** and **14h** showed excellent antimicrobial activity against different bacterial and fungal strains and compounds **15g**, **15h** were found to be potent antioxidant agents in both DPPH and hydroxyl radical scavenging assays. Further, detailed quantitative structure-activity relationship (QSAR) analysis indicated the molecular parameters that contribute to increased potency of inhibition. The above findings would further encourage our understanding in employing coumarin pyrazole hybrids as potential antibiotic agents for treating infections caused by pathogenic microbes and fungi. Further, it also paves the way for exploration of these compounds as potential therapeutic agents to treat conditions arising because of excessive oxidative damage.

As a part of the project work, we have synthesized series of novel bridged pyrans as antimicrobial agents. An isomeric mixture of 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, **17**(**a-f**) and 3-(4-ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, **18**(**a-f**) were synthesized by the reaction of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-phenyl-1H-pyrazole-4-carbaldehyde) **16**(**a-f**) and ethyl alcohol in the presence of conc. H_2SO_4 under reflux conditions **Scheme-4**. The structure proofs of the synthesized compounds were spectral studies and elemental analysis. The synthesized new compounds **17**(**a-f**) and **18**(**a-f**) were evaluated for their antimicrobial activities.



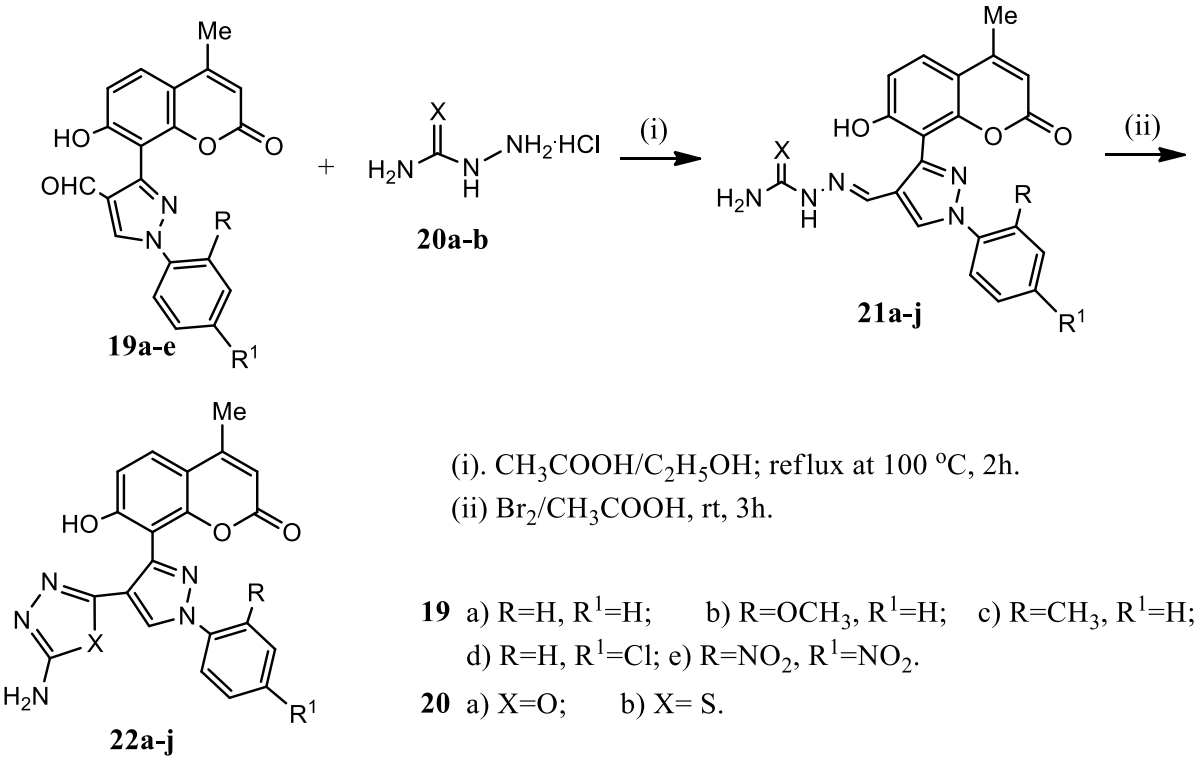
a) R = C_6H_5; b) R = 4-$CH_3C_6H_4$; c) R = 4-$OCH_3C_6H_4$; d) R = 4-FC_6H_4; e) R = $CONH_2$; f) R = $CSNH_2$.

Scheme-4: Synthetic route for coumarin and formylpyrazole appended pyrazole fused pyrans.

The compounds **17e** and **18e** were having -$CONH_2$ substitution and **17f** and **18f** were having -$CSNH_2$ substitutions in the pyrazole rings showed antibacterial at minimum concentrations against all the tested organisms. Results of the antimicrobial activity reveal

that some of the synthesized compounds act as potential antimicrobial agents against different fungal and bacterial organisms.

As a part of the project work, we have synthesized a series of semicarbazones, thiocarbazones, 1,3,4-oxadiazoles and 1,3,4-thiadiazoles bearing coumarin and pyrazole moiety. Initially, precursors 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carboxaldehydes **19a-e** were prepared by the procedure reported earlier [2]. The reaction of 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carboxaldehydes **19a-e** and semicarbazide/thiosemicarbazide hydrochloride **20a-b** in the presence of sodium acetate in ethyl alcohol under reflux conditions at 100ºC to afford corresponding semicarbazones, **21a-e** and thiosemicarbazones, **21f-j**. Then the oxidative cyclisation reaction of the synthesized semicarbazones **21a-e** and thiosemicarbazones, **21f-j** with a solution of brimine-CH_3COOH as oxidant in acetic acid medium at room temperature yielded corresponding 1,3,4-oxadiazoles **22a-e** and 1,3,4-thiadiazoles, **21f-j** in good yields (**Scheme-5**). The synthesized compounds have been evaluated for their antimicrobial and antioxidant activities. In order to understand the structure activity relationship, molecular docking studies has been performed for the designed series of compounds.

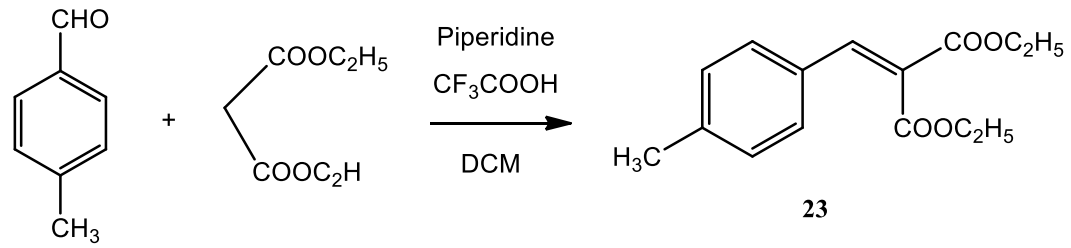


Scheme-5: Synthesis of coumarin appended semicarbazones, **21a-e;** thiosemicarbazones, **21f-j**; 1,3,4-oxadiazoles **22a-e** and 1,3,4-thiadiazoles, **21f-j**.

Structures of all newly synthesized compounds coumarin appended semicarbazones, **21a-e;** thiosemicarbazones, **21f-j**; 1,3,4-oxadiazoles **22a-e** and 1,3,4-thiadiazoles, **21f-j** were provided by spectral and elemental analysis. Preliminary studies on the antimicrobial activities revealed that, all newly synthesized semicarbazones, 1,3,4-oxadiazoles, thiosemicarbazones and 1,3,4-thiadiazoles possess a wide range of antimicrobial activities against the tested organisms. Amongst the series, chloro substituted thiosemicarbazone showed excellent activities against all tested organisms; while, methyl substituted thiosemicarbazone showed greater activity against *E. coli.*

Preliminary studies on the antioxidant activities of the synthesized series of compounds revealed that, chloro substituted 1,3,4-oxadiazole and 1,3,4-thiadiazole demonstrated greater DPPH and hydroxyl radical scavenging abilities. Compounds **22d** and **22i** with chloro substitution in the aromatic ring showed greater DPPH scavenging ability than others. Compounds **22e** and **22j** with electron withdrawing nitro substitution showed lesser activities; while the rest of the series showed moderate activities. Compounds **22d** and **22i** showed a remarkable capacity to scavenge hydroxyl radical, which was significantly higher than that of the standard BHA. Compounds 22**a**, **22f**, **22e** and **22j** were indicative of weak activity against this radical; while the remaining compounds of the series showed moderate activities.

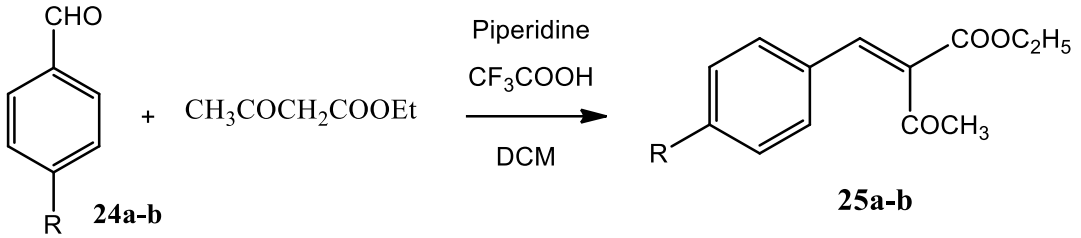
As an additional investigation, in order to develop a new protocol for the synthesis of coumarin tethered heterocycles, we synthesized an intermediate diethyl 2-(4-methylbenzylidene)malonate by Knoevenagel condensation reaction of 4-methylbenzaldehyde and diethyl malonate in the presence of catalytic amount of piperidine and trifluoroacetic acid in DCM under reflux conditions (**Scheme-6**). The synthesized compound **23** was characterized by ^{1}H NMR, Mass spectroscopy and by X-ray diffraction studies.



Scheme-6: Schematic diagram of the synthesis of diethyl 2-(4-methylbenzylidene)malonate

We also synthesized an intermediates ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate and ethyl 2-(4-methylbenzylidene)-3-oxobutanoate were synthesized by Knoevenagel condensation reaction of 4-chlorobenzaldehyde/4-methylbenzaldehyde and ethyl acetoacetate

in the presence of catalytic amount of piperidine and trifluoroacetic acid in benzene under reflux conditions (**Scheme-7**). The synthesised compound was evaluated in vitro for its antimicrobial and antioxidant susceptibilities.



Scheme-7: Schematic diagram of the synthesis of ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate and ethyl 2-(4-methylbenzylidene)-3-oxobutanoate

` The structure proof of the synthesised compounds was obtained by spectral studies and was confirmed by X-ray diffraction studies. The structure adopts a *Z* conformation about the C=C bond.

12. **Work remains to be done (please give details):** Nil

13. **Has the progress been according to original plan of work and towards achieving objectives if not, state reasons:**

Yes, the progress of the project was completed according to the plan of work towards achieving the objectives.

14. **Whether Project work was delayed. If yes, specify reasons:**

Yes, the project work has been delayed, due to late release of FIRST INSTALMENT of the sanctioned amount. Unfortunately, the second installment amount was not released yet. Despite the non release of SECOND INSTALMENT amount of the project, we have completed the project work according to the original plan of work, investing personal money for the expenditure towards chemicals etc. and the project fellow without fellowship.

15. **Please indicate the approximate time by which the project work is likely to be completed:**

COMPLETED

16. **Please indicate the difficulties, if any, experienced in implementing the project:**

We have faced no difficulties as for as implementation and thereby completion of the project. Difficulty we faced is implementation time of the project was delayed, due to non-release of first installment amount by UGC in time and non-release of second installment

amount, led to spend personal money for the purchase of required chemicals and labwares was the major difficulty.

17. Collaboration, if any (with Department, University, Industry etc,):

- **Dr. Bharath Srinivasan,** Center for the Study of Systems Biology, School of Biology, Georgia Institute of Technology, 950, Atlantic Drive, Atlanta, Georgia 30332, USA. : FOR BIOLOGICAL ACTIVITY AND DOCKING STUDIES.
- **Dr. Mylarappa B Ningappa,** Transplant Surgery Section, Rangos Research Center, University Of Pittsburgh, PA 15201, USA. FOR BIOLOGICAL ACTIVITY STUDIES.
- **Dr. N.K. Lokanath,** Department of Studies in Physics, University of Mysore, Mysore, India. FOR X-RAY CRYSTALLOGRAPHIC STUDIES

19. Details of the Publications resulting from the project work (please attach re-prints) letter of Acceptance of paper communicated:

The following is the list of papers published from the project work, Reprints enclosed.

- Synthesis of novel coumarin appended bis(formylpyrazole)dervatives: Studies on their antimicrobial and antioxidant activities. *Bioorg. Med. Chem. Lett.,* 26, 2016, 690-694.
- Stereo selective synthesis of novel pyrazole and coumarin appended bridged pyrans as antimicrobial agents. *Int. J. Pharm. Pharm. Sci.*, 2015, 7(10), 69-73.
- Synthesis and biological evaluation of fused pyrans bearing coumarin moiety as potent antimicrobial agents. *Philippines J. Sci.*, 144(1), 2015, 91-96.
- Synthesis of coumarin appended pyrazolyl-1,3,4-oxadiazoles and pyrazolyl-1,3,4-thiadiazoles: Evaluation for their *in vitro* antimicrobial and antioxidant activities and molecular docking studies, *Russian Journal Bioorganic Chemistry*, 2017, 43(2), 197-210.
- Synthesis, crystal structure, molecular structure and Hirshfeld surface analysis of diethyl 2-(4-methylbenzylidine)malonate. *Chem. Data Coll.*, 2016, 2, 17-24.
- Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate: Synthesis, crystal structure and antimicrobial activities, *Chemical Data Collections*, 2016, 5-6, 68-78.
- Synthesis, crystal and molecular structure of ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate: Studies on antioxidant, antimicrobial activities and molecular docking. *Chemical Data Collections*, 2016, 5-6, 36-45.

- Synthesis and biological evaluation of novel formyl-pyrazoles bearing coumarin moiety as potent antimicrobial and antioxidant agents. *Bioorg. Med. Chem. Lett.*, 2013, 23(23), 6406-6409.
- Synthesis of coumarin appended 1, 3-oxazines as potent antimicrobial and antioxidant agents, *Pharmaceutical Chemistry Journal*, **2017**, 51(7), 582-589.

20. **Any other information which would help in evaluation of work done on the project:**

Please find the details from reprints of publications; which would help in evaluation of work done on the project.

21. **Financial Assistance Provided/Expenditure incurred:**

Sl. No.	Items	Amount Approved	Expenditure incurred so far
1.	Books & Journal	15,000.00	15,000.00
2.	Equipments	2,00,000.00	1,99,916.00
3.	Honorarium/Fellowship to project fellow	2,64,000.00	2,66,030.00 [2,64,000.00 + 2,030.00 interest]
4.	Contingency	22,500.00	22,474.00
5.	Travel/fieldwork	------	------
6.	Chemicals & Glassware	45,000.00	45,000.00
7.	Hiring Services	------	------
8.	Overhead	66,300.00	80,237.00 [66,300.00 + 13,937.00 interest]
9.	Any other items (please specify)	------	------
10.	Honorarium to Principal Investigator	------	------
11.	Staff (date of appointment) (from 28.11.2014 to _____) (please give details of staff appointed in the prescribed format annexure IX as per XI	------	------

	plan guidelines of Major Research Project)		
	Total (approved)	**6,12,800.00**	
	Interest occurred	**18,934.00**	
	Grand Total Rs.	**6,31,734.00**	**6,28,657.00** [6,12,800.00 + 15,887.00 interest]
	Unspent amount Rs. (Returned to UGC account through NEFT		**3,077.00**

It is certified that the grant of Rs. **6,12,800.00** (Rupees Six lakh twelve thousand eight hundred only) [+ Interest amount Rs. **18,934.00**] received from the University Grants Commission under the scheme of support for Major Research Project entitled **Synthesis and Characterization of Coumarin Based Heterocycles and Their Efficacy as Antimicrobial and Antioxidant Agents** vide UGC letter No. **F. No. 42-230/2013 (SR) dated 25th March 2013** has been fully/partly utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

Principal Investigator
(Signatures with Seal)

Registrar/Principal
(Signatures with Seal)

Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator: **Dr. K. Ajay Kumar**

2. Dept. of Principal Investigator: **Department Of Chemistry**
University/College: **Yuvaraja's College, University Of Mysore**

3. UGC approval Letter No. and Date: **F.No.42-230/2013 (SR), dated 25th March 2013**

4. Title of the Research Project: **"Synthesis, characterization of coumarin based heterocycles and their efficiency as antimicrobial and antioxidant agents".**

5. Effective date of starting the project: **28/11/2014.**

6.	**a.**	Period of Expenditure	From: **01/07/2016**	**to 31-08-2017**
	b.	Details of Expenditure: As given below for the unspent amount after utilization certificate submitted to UGC along with Mid-term Evaluation report on November 2016.		
		Sl. No. / **Item**	**Amount Approved (Rs.)**	**Expenditure Incurred (Rs.)**
		i. Books & Journals	---	---
		ii. Equipment	84.00	---
		iii. Contingency	26.00	---
		iv. Field Work/Travel (Give details in the proforma at Annexure- IV)	Nil	Nil
		v. Hiring Services	Nil	Nil
		vi. Chemicals & Glassware	---	---
		vii. Overhead	38,770.00	52,707.00 [38,770.00 + 13,937.00 interest]
		viii. Any other items (Please specify)		
	c.	Staff:		
	Date of Appointment: **28/11/2014**			

Items	**From**	**To**	**Amount Approved (Rs.)**	**Expenditure incurred (Rs.)**
Project fellow:				
i) **NET/GATE qualified-**				

	Rs. 16,000/- p.m. for initial 2 years and Rs. 18,000/- p.m. for the third year.				
	ii) **Non-GATE/Non-NET**- Rs. 14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. for the third year.	01-06-2016	30-06-2016	11,970.00	14,000.00 [11,970.00 + 2,030.00 interest]
		Balance Remaining in fellowship grant			**00.00**

- It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
- If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
- Payment @ revised rates shall be made with arrears on the availability of additional funds.
- It is certified that the grant of Rs. **6,12,800=00** (Rupees: Six Lakhs Twelve thousand and Eight hundred rupees) received from the University Grants Commission under the scheme of support for Major Research Project entitled "**Synthesis, characterization of coumarin based heterocycles and their efficiency as antimicrobial and antioxidant agents**" vide **UGC letter No. F.No.42-230/2013 (SR), dated 25th March 2013** has been completely utilized. The utilization of Rs. **6,28,657.00** [**6,12,800.00** approved + **15,887.00** interest] (Rupees Six Lakhs Twenty Eight Thousand Six Hundred and Fifty Seven Only) for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

Signature of the
Principal Investigator
(Seal)

Registrar/Principal
(Seal)

Statutory Auditor
(Govt. internal Auditor/
Chartered Accountant)
(Seal)

ज्ञान-विज्ञान विमुक्तये

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization Certificate

Certified that Grant-in-aid of Rs **69,784=00** (Rupees Sixty Nine Thousand Seven Hundred and Eighty Four only, which includes the Interest amount of Rs. 4,905=00) was sanctioned by the University Grants Commission vide letter No**. UGC letter No: F.No.42-230/2013 (SR), dated, 20-08-2014** in respect to the research project **" Synthesis, characterization of coumarin based heterocycles and their efficiency as antimicrobial and antioxidant agents"**. Out of released grant, the amount of Rs. **66,707=00** (Rupees Sixty Six Thousand Seven Hundred and Seven Only) was utilized and unspent amount is **Rs. 3,077=00** (Rupees Three Thousand and Seventy Seven only).

Out of the released grant of Rs. **6,12,800=00** (Six Lakh Twelve Thousand and Eight Hudred only), the utilization certificate was submitted to UGC for Rs. **Rs. 5,61,950=00** ((Rupees Five Lakh Sixty One Thousand Nine Hundred and Fifty Only) along with the Mid-term Evaluation report on **September 2016**.

Principal Investigator

Principal
UGC Major Research Project
Department of Chemistry
Yuvaraja's College, Mysore-570 005

Principal

Principal
Yuvaraja's College (Autonomous)
University of Mysore
MYSORE - 570 005

Statutory auditor

For BVM & Co.,
Chartered Accountants
(B.V. MAHESHA)
FRN:012526S M.No.223396
(Proprietor)

Note: The University will submit an audited statement of accounts, duly audited by the statutory auditors of the University as soon as the accounts of the University are audited

23236351, 23232701, 23237721, 23234116
23235733, 23232317, 23236735, 23239437

विश्वविद्यालय अनुदान आयोग
बहादुरशाह जफर मार्ग
नई दिल्ली-110 002
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Annexure -VIII

Annual/Final Report of the work done on the Major Research Project.
(Report to be submitted within 6 weeks after completion of each year)
(TO BE SUBMITTED IN TRIPLICATE)

1. **Project report No. 1st /2nd /3rd/Final:** FINAL (COMPLETION)
2. **UGC Reference:** No. F. No. 42-230/2013 (SR) dated 25th March 2013
3. **Period of report:** from 28.11.2014 to 27.11.2017
4. **Title of research project:** Synthesis and Characterization of Coumarin Based Heterocycles and Their Efficacy As Antimicrobial and Antioxidant Agents
5. **(a) Name of the Principal Investigator:** Dr. K. Ajay Kumar
   (b) Deptt.: Associate Professor, Department of Chemistry,
   (c) University/College where work has progressed: Yuvaraja's College, University of Mysore, Mysuru-570005. Karnataka
6. **Effective date of starting of the project:** 28.11.2014
7. **Grant approved and expenditure incurred during the period of the report:**
   a. Total amount approved Rs. 9,44,300.00
   b. Total expenditure Rs. 5,61,950.00 + 69,784.00 **= Rs. 6,31,734=00**
   c. Report of the work done: (Please attach a separate sheet)

i. Brief objective of the project:

Synthesis of the series of new coumarin based intermedidates such as hydroxyl ketones, hydrazones, and coumarin appended heterocycles such as formylpyrazoles, bis(formyl pyrazoles), fused pyrans, fused oxazines. Purification and characterization synthesized new compounds by spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. Screening of the new molecules for their biological activities such as antimicrobial, antioxidant etc.

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication:

The results of the investigation have been published in journals below

- Synthesis of novel coumarin appended bis(formylpyrazole)dervatives: Studies on their antimicrobial and antioxidant activities. *Bioorg. Med. Chem. Lett.,* 26, 2016, 690-694.

- Stereo selective synthesis of novel pyrazole and coumarin appended bridged pyrans as antimicrobial agents. *Int. J. Pharm. Pharm. Sci.*, 2015, 7(10), 69-73.

- Synthesis and biological evaluation of fused pyrans bearing coumarin moiety as potent antimicrobial agents. *Philippines J. Sci.*, 144(1), 2015, 91-96.

- Synthesis of coumarin appended pyrazolyl-1,3,4-oxadiazoles and pyrazolyl-1,3,4-thiadiazoles: Evaluation for their *in vitro* antimicrobial and antioxidant activities and molecular docking studies, *Russian Journal Bioorganic Chemistry*, 2017, 43(2), 197-210.

- Synthesis, crystal structure, molecular structure and Hirshfeld surface analysis of diethyl 2-(4-methylbenzylidine)malonate. *Chem. Data Coll.*, 2016, 2, 17-24.

- Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate: Synthesis, crystal structure and antimicrobial activities, *Chemical Data Collections*, 2016, 5-6, 68-78.

- Synthesis, crystal and molecular structure of ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate: Studies on antioxidant, antimicrobial activities and molecular docking. *Chemical Data Collections*, 2016, 5-6, 36-45.

- Synthesis and biological evaluation of novel formyl-pyrazoles bearing coumarin moiety as potent antimicrobial and antioxidant agents. *Bioorg. Med. Chem. Lett.*, 2013, 23(23), 6406-6409.

- Synthesis of coumarin appended 1, 3-oxazines as potent antimicrobial and antioxidant agents, *Pharmaceutical Chemistry Journal*, **2017**, 51(7), 582-589.

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons:

Yes, the progress of the project work has been according to original plan of work and towards achieving objectives.

iv. Please indicate the difficulties, if any, experienced in implementing the project:

Yes, the implementation of the project work has delayed for non-release of the first installment amount by UGC. Though, we received applications for appointment of project fellow through an advertisement, no candidate turn-up to join as a project fellow for the reason of non-release of fund by University Grants Commission. Also, suppliers of

chemicals and instruments to denied us to supply the requirements necessary for implementation of the project.

We received the **first installment amount by UGC on August 2014**. After this, again fresh advertisement for the appointment of project fellow has made, selection of the candidate was made according to UGC norms, the project was implemented from **28.11.2014.**

Secondly, non-release of second installment amount by the UGC made difficulties for the completion of the project, but we successfully achieved and completed the project objectives, by spending money from personally earned amount.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.

Not applicable-as the objectives of the project achieved and completed

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission.

Yes, the project has been completed. Summary of the findings of the study has been sent to the University Grants Commission, herewith.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

(a) Manpower trained: 02 (Two)

(b) Ph. D. awarded: 01 candidate awarded Ph.D., and 01 candidate completed his thesis work and submitting his thesis in the month of November 2018, as per his eligibility to him by the University of Mysore.

(c) Publication of results: Research findings have been published in international refuted journals

(d) other impact: Nil.

Signature of the Principal Investigator **Registrar/Principal**
(Seal) **(Seal)**

Annexure-VI

23236351, 23232701, 23237721, 23234116
23235733, 23232317, 23236735, 23239437

विश्वविद्यालय अनुदान आयोग
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NEW DELHI-110 002

PROFORMA FOR SUPPLYING THE INFORMATION IN RESPECT OF THE STAFF APPOINTED UNDER THE SCHEME OF MAJOR RESEARCH PROJECT

UGC FILE NO. **F.No. 42-230/2013 (SR) dated 25th March 2013**
YEAR OF COMMENCEMNT: **28th November 2014**
TITLE OF THE PROJECT**: Synthesis and Characterization of Coumarin Based Heterocycles and Their Efficacy as Antimicrobial and Antioxidant Agents**

1.	Name of the Principal Investigator	Dr. K. Ajay Kumar				
2.	Name of the University/College	Yuvaraja's College, University of Mysore, Mysuru. Karnataka				
3.	Name of the Research Personnel appointed	A. Dileep Kumar				
4.	Academic qualification	S.No.	Qualification	Year	Marks	%age
		1.	M.Sc.	2013		85.26
		2.	M.Phil	-----	-----	-----
		3.	Ph.D	-----	-----	-----
5.	Date of Joining	28/11/2014				
6.	Date of Birth of Research Personnel	18/12/1989				
7.	Amount of HRA, if drawn	Eligible HRA amount of Rs. 67,200.00 (I, II year) + 38,400.00 (III year) =1,05,600.00 is yet to be released by the UGC.				
8.	Number of Candidates applied for the post	12				

Certificate

This is to certify that all the rules and regulations of UGC Major Research Project outlined in the guidelines have been followed. Any lapse on the University will liable to terminate of said UGC project.

Principal Investigator **Registrar / Principal**

Annexure – X

23236351, 23232701, 23237721, 23234116
23235733, 23232317, 23236735, 23239437

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UNIVERSITY GRANTS COMMISSION
BAHADURSHAH ZAFAR MARG
NEW DELHI-110 002

MAJOR RESEARCH PROJECT COPY OF THE SPECIMEN OF HOUSE RENT FOR PROJECT FELLOW

Certified that Sri **Dillep Kumar A** is paying House Rent of Rs. **5000.00 per month** and is eligible to draw House Rent Allowances @**20% (Rs. 2,800.00/month in I and II year) + (Rs. 3,200.00/month in III year)** as per University Rules.

Registrar/ Principal
(Signature with Seal)

Certified that Shri/Dr. _____ is not staying independently and therefore is eligible to draw House Rent @ of Rs. _____ p.m. minimum admissible to a Lecturer as per University Rules.

Registrar/ Principal
(Signature with Seal)

Certified that Shri/Dr. _____ has been provided accommodation in the Hostel. But he/she could not be provided with single seated flat type accommodation as recommended by the Commission, Hostel fee @ Rs. per month w.e.f. is being charged from him/her.

Registrar/ Principal
(Signature with Seal)

Annexure - VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK

Name of the Principal Investigator: Dr. K. Ajay kumar

Name of the Place visited	**Duration of the Visit**		**Mode of Journey**	**Expenditure Incurred (Rs.)**
University Grants Commission New Delhi	**22.02.2017**	**22.02.2017**	**By Air**	**10,033.00**

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

Signature of the Principal Investigator (Seal)

Registrar/Principal (Seal)

Bioorganic & Medicinal Chemistry Letters 26 (2016) 690–694

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of novel coumarin appended bis(formylpyrazole) derivatives: Studies on their antimicrobial and antioxidant activities

Renuka Nagamallu [a], Bharath Srinivasan [b], Mylarappa B. Ningappa [c], Ajay Kumar Kariyappa [a,*]

[a] Department of Chemistry, Yuvaraja College, University of Mysore, Mysore, India
[b] Center for the Study of Systems Biology, School of Biology, Georgia Institute of Technology, 950, Atlantic Drive, Atlanta, GA 30332, USA
[c] Transplant Surgery Section, Rangos Research Center, University of Pittsburgh, PA 15201, USA

ARTICLE INFO

Article history:
Received 11 August 2015
Revised 5 November 2015
Accepted 12 November 2015
Available online 12 November 2015

Keywords:
Antioxidant
Antimicrobial
Coumarin
DPPH
Hydroxyl radical
MIC
Pyrazole

ABSTRACT

A series of novel coumarin pyrazole hybrids of biological interest were synthesized from the hydrazones, carbazones and thiocarbazones via Vilsmeier Haack formylation reaction. These intermediates and formyl pyrazoles were evaluated for antimicrobial and antioxidant activities. Among the series, compounds **6g** and **6h** showed excellent antimicrobial activity against different bacterial and fungal strains and compounds **7g**, **7h** were found to be potent antioxidant agents in both DPPH and hydroxyl radical scavenging assays. Further, detailed quantitative structure–activity relationship (QSAR) analysis indicated the molecular parameters that contribute to increased potency of inhibition. The above findings would further encourage our understanding in employing coumarin pyrazole hybrids as potential antibiotic agents for treating infections caused by pathogenic microbes and fungi. Further, it also paves the way for exploration of these compounds as potential therapeutic agents to treat conditions arising because of excessive oxidative damage.

An increasing interest in antioxidant activity of small molecules, particularly to prevent the deleterious effects caused by free radicals in the human body, is attracting the attention of the wider research community. Free radicals are believed to be associated with multiple diseases conditions such as carcinogenesis, inflammation, mutagenesis, arthritis, cancer and genotoxicity. These conditions arise due to the oxidative stress resulting from an imbalance between free radical generation and their quenching.[1] Despite numerous attempts to search for more effective antioxidant agents, coumarin still remains as the scaffold of choice. It has demonstrated utility in quenching of free radicals and, hence, has tremendous potential for exploration as candidate molecules for drug discovery against conditions that result from oxidative damage. Coumarins are naturally derived and synthetically taken polyphenolic substances giving a wide variety of biological activities and employed as therapeutic agents for multiple diseases.[2] A large number of coumarin derivatives were found to affect the formation and scavenging of reactive oxygen species and reactive nitrogen species displaying tissue protective antioxidant properties.[3] Among them, hydroxyl coumarins are effective metal chelators, free radical scavengers and powerful chain breaking antioxidants. Many coumarin derivatives have unique ability to scavenge the reactive oxygen species, such as hydroxyl free radicals and superoxide, to prevent free radical injury. In addition, coumarin derivatives act as antioxidant agents with application in parasitic diseases.[4]

Coumarin derivatives, such as coumarin hydrazones, have been under investigation in recent years due to their usage as pharmaceuticals,[5] fluorescence sensors,[6] chemoreceptors[7] and precursors for the synthesis of coumarin pyrazoles.[8] Different coumarins are identified as anticoagulants, antioxidants, antidepressants, analgesics and diagnostics.[9] Coumarin derivatives containing a substituted hydroxyl group at the position 7 possess antibiotic and antifungal activities. The incorporation of another heterocyclic moiety, either as a substituent or a fused component into coumarin, alters the properties of the parent material and the resulting compounds generally exhibit promising or unprecedented properties.[10]

In 1883, Knorr synthesized the first pyrazole derived compound that led to the discovery of antipyrine and its derivatives.[11] Since the introduction of antipyrine in 1884, the first pyrazolone derivative, it was used in the treatment of pain, inflammation and fever. Pyrazole derivatives possess wide spectrum of biological activities. They are extensively studied and used as antimicrobial agents.[12,13] In particular, 5-chloropyrazol derivatives are reported to show potential antimicrobial,[14] analgesic and anti-inflammatory activities.[15] Further much attention was given to pyrazoles as antimicrobial agents after the discovery of the natural pyrazole C-glycoside

* Corresponding author.

http://dx.doi.org/10.1016/j.bmcl.2015.11.038
0960-894X/

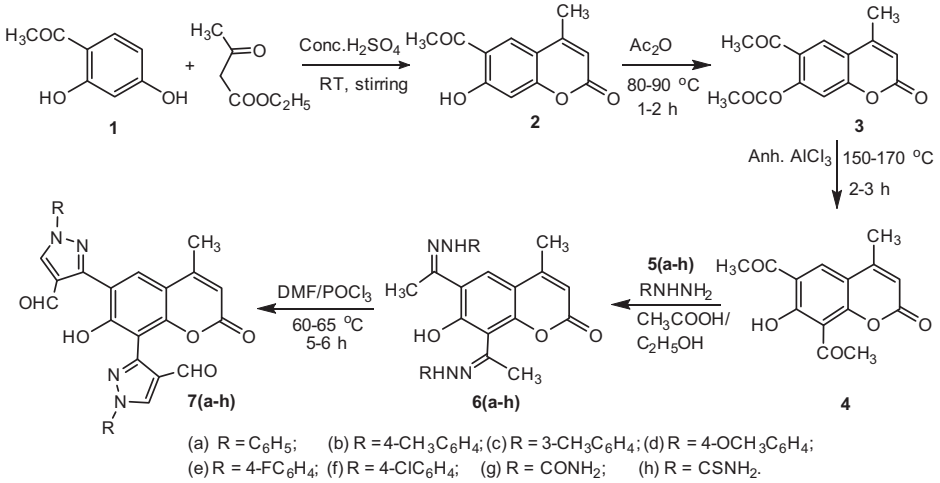
pyrazofurin; 4-hydroxy-3-β-D-ribofuranosyl-1*H*-pyrazole-5-carboxamide which demonstrated a broad spectrum of antimicrobial activities.[16] Hydrazones and semicarbazones of ketones having an α methyl substitution prevents the formylation of pyrazoles upon treatment with Vilsmeier–Haack reagent.[17] Recently Tarik and coworkers[18] synthesized the 4,6-bis(4-formylpyrazol-3-yl)resorcinol from the bis-hydrazones of 4,6-diacetylresorcinol by Vilsmeier–Haack reaction and evaluated them for their antimicrobial activity.

Prompted by the above literature survey results, we planned to study structural variation by attaching biologically active pyrazoles to the coumarin moiety as well as hydrazono, hydrazinecarboxamido and hydrazinecarbothioamido coumarins. These combinations were explored in an attempt to investigate the synergetic influence of these compounds on the antioxidant and antimicrobial activity, thus hoping to get a new lead structure for the further study.

In continuation of our research work aiming at developing a straightforward and flexible synthetic method towards the functionalized coumarins appended pyrazoles with remarkable biological importance, we report the utility of 7-hydroxy-4-methyl-6,8-bis(1-(2-phenylhydrazono)ethyl)-2*H*-chromen-2-one, 2,2′-((7-hydroxy-4-mrthyl-2-oxo-2*H*-chromene-6,8-diyl)bis(ethan-1-yl-1-ylidene))bis(hydrazinecarboxamide) and 2,2′-((7-hydroxy-4-methyl-2-oxo-2*H*-chromene-6,8-diyl)bis(ethan-1-yl-1-ylidene)) bis(hydrazinecarbothioamide) as building blocks for the synthesis of functionalized coumarin based bis(formylpyrazole) derivatives, and study their antimicrobial and antioxidant activity in order to get potent antimicrobial and antioxidant agents.

The synthetic strategies adopted to obtain the target compounds are depicted in Scheme 1. The starting material 6-acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one **2** was prepared by 1-(2,4-dihydroxyphenyl)ethanone **1** and ethyl acetoacetate in the presence of sulfuric acid by Pechmann reaction. The treatment of **2** with acetic anhydride to give 6-acetyl-4-methyl-2-oxo-2*H*-chromen-7-yl acetate **3**, this on subjecting to Fries rearrangement using $AlCl_3$ as a catalyst afforded 1,1′-(7-hydroxy-4-methyl-2-oxo-2*H*-chromene-6,8-diyl)diethanone **4**. The condensation of the compound **4** with substituted phenylhydrazines **5**(**a**–**f**), semicarbazine **5g** and thiosemicarbazine **5h** in ethyl alcohol and a catalytic amount of acetic acid under reflux conditions produced the corresponding bis-hydrazones **6**(**a**–**f**), semicarbazones **6g** and thiosemicarbazone **6h**, which were subsequently reacted under Vilsmeier–Haack condition furnished the target molecules 3,3′-(7-hydroxy-4-methyl-2-oxo-2*H*-chromene-6,8-diyl)bis(1-aryl-1*H*-pyrazole-4-carbaldehyde) **7**(**a**–**f**), 3,3′-(7-hydroxy-4-methyl-2-oxo-2*H*-chromene-6,8-diyl)bis(4-formyl-1*H*-pyrazole-1-carboxamide), **7g** and 3,3′-(7-hydroxy-4-methyl-2-oxo-2*H*-chromene-6,8-diyl)bis(4-formyl-1*H*-pyrazole-1-carbothioamide), **7h** in excellent yield.

All the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, mass and CHN analysis. In ^{1}H NMR spectra of compound **2**, the appearance of one broad singlet for only one OH proton and the presence of one singlet for the C_3-proton at 5.96 ppm confirmed the compound. In ^{13}C NMR, the signal was observed at the region 160.24 ppm due to the presence of C=O group, thus confirming the formation of coumarin. Compound **2** was converted to an ester **3**, which was confirmed by the disappearance of broad singlet for OH proton present in compound **2** spectra and the presence of singlet at the region 2.26 ppm due to the presence of CH_3 group. Further, in the ^{13}C NMR, the appearance of C=O group at the region 170.34 ppm confirms the formation of ester. Fries rearrangement of compounds **3** gave hydroxy ketones **4**, which were confirmed by the appearance of broad singlet for OH proton and decrease in one aromatic proton in ^{1}H NMR. Further, presence of C=O group at C_7 position in compound **3** shifted to C_8 position in compound **4** at the region 196.10 ppm, confirming the formation of ketone. Compound **4** on treatment with substituted phenylhydrazines **5**(**a**–**f**), carbazine **5g** and thiocarbazine **5h** gave respective hydrazones **6**(**a**–**f**), carbazones **6g** and thiocarbazones **6h**. The ^{1}H NMR and ^{13}C NMR observations reveal the appearance of two NH protons at the region 7.01–7.12 ppm in the ^{1}H NMR and the presence of two C=N group at the region 167.44–168.66 ppm in the ^{13}C NMR, confirming the formation of bis hydrazones. On the other hand, bis-carbazones and thiocarbazones were confirmed by the presence of two NH_2 and NH group at the region 6.06–8.92 ppm and 7.10–7.14 ppm in the ^{1}H NMR and in the ^{13}C NMR spectra, two C=N group at the region 167.07 ppm and a C=S carbon at the region 179.22 ppm, respectively. Finally all the compounds **6** (**a**–**h**) on treatment with Vilsmeier–Haack reagent delivered the expected final compounds **7**(**a**–**h**) in good yield, which was clearly evident with the disappearance of NH and NH_2 groups present in the compound **6**(**a**–**h**) and the formation of two CHO groups at the region 9.80–9.93 ppm in the ^{1}H NMR and 183.10–186.14 in ^{13}C NMR. The peak appeared at the region 8.10–8.66 ppm and 130.08–137.72 ppm in ^{1}H NMR and ^{13}C NMR providing the evidence for the formation of bis-pyrazole. All the compounds gave M^+, MH^+ ions as base peak and significantly stable molecular ion peaks with a relative abundance ranging from 10% to 40% and showed satisfactory CHN analysis data with the theatrically calculated values.



(a) R = C_6H_5; (b) R = 4-$CH_3C_6H_4$; (c) R = 3-$CH_3C_6H_4$; (d) R = 4-$OCH_3C_6H_4$; (e) R = 4-FC_6H_4; (f) R = 4-ClC_6H_4; (g) R = $CONH_2$; (h) R = $CSNH_2$.

Scheme 1. Synthetic route for coumarin appended bis(formyl pyrazoles).

The antibacterial and antifungal activities of the compounds were determined by broth dilution technique.[19] The antibacterial tests were conducted against bacterial pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antifungal activity was evaluated against different fungal strains such as *Aspergillus nigar*, *Aspergillus flavus* and *Candida albicans*. The antibiotics ciprofloxacin and fluconazole were used as standard drugs against bacteria and fungal species, respectively. Dimethyl sulfoxide was used as solvent control. The experiments were carried out in triplicate; the results were taken as a mean ± standard deviation (SD). The results of minimum inhibitory concentrations (MIC's), defined as the concentration required for 90% clearance of an organism, of the synthesized compounds against several bacterial and fungal species are summarized in Table 1.

All the compounds exerted a wide range of modest in vitro antibacterial activity having minimum inhibitory concentration (MIC) values ranging from 6.25 to 75 μg/mL. Compounds **6g** and **6h** having $CONH_2$ and $CSNH_2$ substitution in the carbazones showed excellent bacterial activity by inhibiting spore germination of all the tested organisms and compounds **7g** and **7h** having the same substitution in the pyrazole ring showed activity as potent as the standard. Compounds **6d** and **6b** having methoxy and methyl substitution shows good activity but compounds **7d** and **7b** shows less activity than the standard. Fluoro, chloro and methyl substitution in compounds **6e**, **7e**, **6f**, **7f** and **6c**, **7c** exhibit less activity against the organism tested. Compound **6a**, without any substitutions, resulted in moderate antibacterial and antifungal activity against all the organisms tested and compound **7a** having same substitution in pyrazole ring exhibited poor inhibitory effect on the organisms.

All the synthesized bis-carbazones, bis-hydrazones and bis (formylpyrazole) derivatives exerted modest antifungal activity having minimum inhibitory concentration (MIC) value 12.5–100 μg/mL. $CONH_2$ and $CSNH_2$ substitution in compounds **6g** and **6h** demonstrated excellent antifungal activity against the organisms tested. However, **7g** also having $CONH_2$ substitution showed activity similar to that shown by the standard. **7h** having $CSNH_2$ group displayed higher potency against *A. niger*. Compound **6f** having chloro substitution in the hydrazone showed moderate activity while the same substitution in the pyrazole ring (compound **7f**) retard the activity against the fungal strains. However compound **6d** having methoxy group showed activity against *A. niger* and the compound **7d** showed activity similar to that shown by the standard. Methyl substitution present in the compound **6b** exhibits good activity with *A. flavus* and *C. albicans*. Remaining compounds **6e**, **7e** having fluoro; **6c**, **7c** having methyl at *meta* position; **7b** having methyl at *ortho* position and **6a**, **7a** having no substitution exhibit lesser activity against all the organisms tested.

In an attempt to interpret and correlate the molecular parameters of the small molecules with the potency of inhibition against the various microorganisms, detailed quantitative structure–activity relationship (QSAR) analysis was carried out. Physicochemical parameters for the small molecules were computed[20] and both pair-wise and multivariate analysis was carried out as specified in the literature[21,22] and elaborated in Supplementary material. As shown in Figures S1–S7 summarizes the pair-wise correlation analysis. Examination of the figures suggest that parameters like hydrogen-bonding acceptors/donors, polar surface area and presence of double bonds are positively correlated with the ability of compounds **6a–h** and compounds **7a–h** against the various microbes tested. Further, parameters like Log *P*, aromatic bond content, molecular weight, number of atoms and bonds were negatively correlated with inhibition potency.

Further, multivariate analysis using genetic algorithm for compounds **6a–h** yielded good models for *E. coli*, *A. niger* and *C. albicans*. The model for *S. aureus* was poor most probably due to limiting data and lack of pairwise correlation with good *p*-values (Fig. S1A–I). Likewise, when modeling for compounds **7a–h**, the multivariate model for *E. coli* and *C. albicans* were very poor and hence were not considered.

Analysis of the results indicate that the small-molecule features that likely contribute to increased potency of inhibition vary across different microorganisms in spite of overall similarity in the pairwise correlation profiles (Figs. S1–S7). This is an encouraging observation since specific variation of a particular molecular feature would lead to increased specificity towards a particular kind of microorganism. Further, this analysis also points out to the parameters that can be modulated to increase the potency of these compounds in general across the different microorganisms employed. However, care must be exercised in interpreting these results given the small sample size that was employed across

Table 1
MIC's of the test compounds **6**(**a**–**h**) and **7**(**a**–**h**) against bacterial and fungal species

Compound	Minimum inhibitory concentration (MIC's) in μg/mL*					
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Aspergillus flavus*	*Candida albicans*
6a	50 ± 0.11	25 ± 0.40	25 ± 0.45	50 ± 0.50	50 ± 0.4	50 ± 0.56
6b	25 ± 0.17	25 ± 0.3	25 ± 0.15	50 ± 0.66	25 ± 0.70	50 ± 0.70
6c	50 ± 0.35	50 ± 0.28	25 ± 0.25	75 ± 0.61	50 ± 0.75	50 ± 0.66
6d	25 ± 0.45	12.5 ± 0.25	12.5 ± 0.56	12.5 ± 0.62	25 ± 0.94	50 ± 0.61
6e	75 ± 0.65	50 ± 0.45	50 ± 0.40	50 ± 0.51	75 ± 0.25	75 ± 0.23
6f	50 ± 0.40	50 ± 0.45	25 ± 0.35	25 ± 0.55	50 ± 0.40	50 ± 0.15
6g	25 ± 0.20	12.5 ± 0.45	12.5 ± 0.30	12.5 ± 0.46	25 ± 0.60	25 ± 0.97
6h	25 ± 0.51	12.5 ± 0.50	6.25 ± 0.76	12.5 ± 0.30	12.5 ± 0.45	25 ± 0.85
7a	50 ± 0.50	50 ± 0.52	50 ± 0.36	50 ± 0.36	75 ± 0.5	50 ± 0.42
7b	75 ± 0.28	50 ± 0.70	25 ± 0.40	50 ± 0.36	75 ± 0.45	50 ± 0.79
7c	75 ± 0.79	50 ± 0.41	25 ± 0.9	75 ± 0.55	75 ± 0.4	50 ± 0.60
7d	50 ± 0.61	50 ± 0.55	12.5 ± 0.45	25 ± 0.56	50 ± 0.3	50 ± 0.17
7e	75 ± 0.65	75 ± 0.28	50 ± 0.60	75 ± 0.25	75 ± 0.45	100 ± 0.36
7f	50 ± 0.50	25 ± 0.65	25 ± 0.15	50 ± 0.3	50 ± 0.61	100 ± 0.21
7g	50 ± 0.17	12.5 ± 0.81	25 ± 0.70	25 ± 0.41	25 ± 0.21	50 ± 0.46
7h	25 ± 0.41	25 ± 0.41	12.5 ± 0.37	12.5 ± 0.45	25 ± 0.3	50 ± 0.29
Cipro[a]	25 ± 0.75	25 ± 0.70	12.5 ± 0.45	—	—	—
Fluc[b]	—	—	—	25 ± 0.25	25 ± 0.21	50 ± 0.96

* Values are mean ± SD of three replicates.
[a] Ciprofloxacin was used as a positive control against bacteria species.
[b] Fluconazole was used as a positive control against fungi species.

compounds **6a–h** and **7a–h** (8 compounds each) and the fact that MIC, instead of IC-50, was considered as the dependent variable.

Further, various assays were performed to assess the antioxidant activities of the compounds. Radical scavenging potency of all the compounds **6**(**a–h**) and **7**(**a–h**) was assessed in vitro by the DPPH,[23] and hydroxyl radical scavenging assay.[24] The experiments were performed in triplicate; the results were taken as a mean ± standard deviation (SD) and are presented in Tables 2 and 3.

The capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method.[23] A freshly prepared DPPH solution shows a deep purple color with an absorption maximum at 517 nm. When the purple color changes to yellow, it leads to decreased absorbance. This is because of the antioxidant molecule reducing the DPPH free radical through donation of hydrogen atom. Instantaneously or concomitant decrease in absorbance would be indicative of potent antioxidant activity by the compound. Based on the experimental results, we found out those compounds **7g** and **7h** having $CONH_2$ and $CSNH_2$ in the pyrazole ring showed antioxidant activity better than the standard ascorbic acid. Compounds **6g**, **6h** also having the same substituent in the carbazones showed better activity than the standard, but less activity compared to **7g** and **7h**. Compounds **6a**, **7a**, **6d**, and **7d** having no substitution and methoxy group showed moderate activity. Compounds **7b** and **7f** having methyl and chloro substitution showed antioxidant properties closer to the standard but the compounds **6b** and **7f**, having the same substitution, showed less activity compared to standard. Remaining compounds **6c**, **7c** having methyl at *meta* position and fluoro substitution in the compounds **6e**, **7e** showed less activity compared with the standard ascorbic acid.

The hydroxyl radical is a highly reactive free radical formed in biological systems and it is capable of damaging biomolecule found in living cells.[25] The hydroxyl radical has the ability to break DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In this method, compounds **6**(**a–h**) and **7**(**a–h**) were found to be possess stronger to weak hydroxyl radical scavenging activity. Among the compounds studied, $CONH_2$ and $CSNH_2$ substitution in compounds **7g** and **7h** exhibited remarkable capacity for scavenging hydroxyl radical, significantly higher than that of the standard BHA, whereas the same substitution present in compounds **6g** and **6h** also showed good activity against the radical. Compound **7d** having methoxy substitution in the pyrazole ring shows excellent activity while the compound **6d** having the same group showed moderate scavenging activity. However compounds **7a**, **7b** and **7f** having no substitution, methyl and chloro group present in the pyrazole ring exhibit moderate activity but the same group present in compounds **6a**, **6b** and **6f** showed least activity against this radical. Methyl group at *meta* substitution and fluoro substitution present in the compounds **6c**, **7c** and **6e**, **7e** exhibits weak radical scavenging activity.

Table 2
Antioxidant activity of the compounds **6**(**a–h**) and **7**(**a–h**) in DPPH method

Test samples	% radical scavenging activity*			
	25 (μg/mL)	50 (μg/mL)	75 (μg/mL)	100 (μg/mL)
6a	15.03 ± 0.27	16.96 ± 0.42	19.35 ± 0.57	22.22 ± 0.19
6b	14.29 ± 0.44	16.65 ± 0.55	19.43 ± 0.43	22.45 ± 0.66
6c	10.92 ± 0.45	11.80 ± 0.26	13.97 ± 0.23	17.29 ± 0.51
6d	14.57 ± 0.34	17.17 ± 0.45	19.70 ± 0.69	22.40 ± 0.42
6e	8.49 ± 0.42	11.69 ± 0.59	14.01 ± 0.66	17.45 ± 0.12
6f	10.73 ± 0.43	14.75 ± 0.76	17.41 ± 0.17	20.62 ± 1.09
6g	14.65 ± 0.84	17.84 ± 0.46	21.89 ± 0.74	24.09 ± 0.52
6h	15.46 ± 0.50	17.86 ± 0.54	22.24 ± 0.37	26.13 ± 0.37
7a	15.10 ± 0.46	17.34 ± 0.73	20.08 ± 0.59	23.60 ± 0.46
7b	16.00 ± 0.27	17.28 ± 0.14	20.75 ± 0.26	24.56 ± 0.74
7c	13.24 ± 0.70	16.40 ± 0.84	18.78 ± 0.66	24.02 ± 0.52
7d	14.2 ± 0.55	17.87 ± 0.60	21.14 ± 0.34	24.59 ± 0.45
7e	11.62 ± 0.77	14.93 ± 0.39	17.69 ± 0.39	19.44 ± 0.73
7f	15.58 ± 0.52	17.55 ± 0.60	20.63 ± 0.46	23.61 ± 0.32
7g	18.98 ± 0.39	23.36 ± 0.84	25.70 ± 0.58	29.26 ± 0.55
7h	23.93 ± 0.25	26.93 ± 0.45	29.81 ± 0.54	36.15 ± 0.34
aa[a]	15.08 ± 0.83	16.87 ± 0.89	21.98 ± 0.31	24.25 ± 0.22

* Values are mean ± SD of three replicates.
[a] Ascorbic acid was used as a standard antioxidant.

Table 3
Antioxidant activity of the compounds **6**(**a–h**) and **7**(**a–h**) in hydroxyl radical method

Test samples	% radical scavenging activity*			
	25 (μg/mL)	50 (μg/mL)	75 (μg/mL)	100 (μg/mL)
6a	9.31 ± 0.37	12.49 ± 0.81	24.36 ± 0.32	29.65 ± 0.39
6b	9.60 ± 0.28	13.92 ± 0.31	25.54 ± 0.26	30.52 ± 0.38
6c	8.40 ± 0.36	13.56 ± 0.65	24.22 ± 0.38	30.78 ± 0.31
6d	11.82 ± 0.37	18.23 ± 0.22	26.36 ± 0.67	33.01 ± 0.40
6e	6.60 ± 0.46	11.44 ± 0.18	17.33 ± 0.34	26.6 ± 0.27
6f	10.1 ± 0.68	15.56 ± 0.39	23.49 ± 0.79	30.75 ± 0.22
6g	14.12 ± 0.44	19.01 ± 0.65	26.72 ± 0.45	33.92 ± 0.67
6h	14.21 ± 0.31	21.06 ± 0.86	27.78 ± 0.32	34.24 ± 0.18
7a	10.20 ± 0.50	17.77 ± 0.41	25.9 ± 0.33	30.04 ± 0.36
7b	10.85 ± 0.28	19.34 ± 0.47	27.07 ± 0.37	31.48 ± 0.17
7c	10.58 ± 011	18.15 ± 0.24	26.49 ± 0.35	30.1 ± 0.73
7d	14.08 ± 0.46	21.57 ± 0.25	29.40 ± 0.47	35.13 ± 0.76
7e	8.99 ± 0.39	15.30 ± 0.52	21.27 ± 0.26	29.47 ± 0.30
7f	10.46 ± 0.66	16.63 ± 0.50	24.77 ± 0.91	32.07 ± 0.27
7g	17.55 ± 0.66	24.41 ± 0.16	32.33 ± 0.34	36.80 ± 0.33
7h	19.56 ± 0.62	27.83 ± 0.40	35.40 ± 0.25	40.84 ± 0.44
BHA[a]	12.02 ± 0.05	17.95 ± 0.12	25.58 ± 0.20	32.03 ± 0.32

* Values are mean ± SD of three replicates.
[a] Butylated hydroxyanisole was used as a positive control.

In summary, in order to develop antimicrobial and antioxidant molecules, we have synthesized new bioactive coumarin based bis hydrazones, biscarbazones and bis formyl pyrazole analogs and evaluated them for antimicrobial and antioxidant activities. Compounds **6g** and **6h** exhibited potent antimicrobial activity among the series synthesised. Compounds **7g**, **7h** and **7d** showed potent antioxidant properties in DPPH and hydroxyl radical scavenging assay. From these biological results, the pyrazole derivatives **7**(**a–h**) displayed much higher antioxidant activity than those of the carbazones and hydrazone derivatives **6**(**a–h**), whereas **6**(**a–h**) shows higher antimicrobial activity than the pyrazole derivatives **7**(**a–h**). Further, detailed QSAR analysis paves the way for design of potent and specific inhibitors for the various microbes assessed in this study. Looking at the results, compounds **6g**, **6h** and **7g**, **7h** could be considered as potent antimicrobial and antioxidant drugs for further detailed studies. Efforts have been made for developing new methodologies to increase the structural complexity while decreasing the number of synthetic steps to facilitate the construction of new coumarin based bis(formylpyrazole) derivatives.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.11.038.

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Philippine Journal of Science
144 (1): 91-96, June 2015
ISSN 0031 - 7683

Synthesis and Biological Evaluation of Fused Pyrans Bearing Coumarin Moiety as Potent Antimicrobial Agents

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A simple approach for the synthesis of fused pyrans to coumarin moiety is presented. The intramolecular cyclisation of 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1H-pyrazole-4-carbaldehydes under reflux conditions at 80ºC afforded fused pyrans in a relatively good yield. The synthesized compounds were characterized by spectral studies and elemental analysis. The new compounds were evaluated in vitro for their antifungal and antibacterial activity against different fungi and bacterium species.

Key Words: antibacterial, coumarins, intramolecular, MIC, pyrazoles

INTRODUCTION

The growing population of antibiotic resistance of bacteria strains as a result of enzymatic inactivation of the drug, modification of target sites and extrusion by efflux has become one of the major tasks to be addressed in the area of research in drug design and discovery (Spratt 1994). The construction of complex molecular architectures that exhibit greater biological potency in a facile and efficient manner remains an overarching goal for chemists. In the recent years, coumarins have attracted great attention because of their synthetic utility as building blocks for the construction of biologically potent molecules. Coumarin derivatives are known to have a wide range of activities such as antioxidant, antimicrobial, anti-HIV, antibiotic, anticancer, muscle relaxant, anti-inflammatory and anticoagulant properties (Murakami et al. 2000).

Pyrazoles have attracted particular interest over the last few decades due to use of such a ring system as the core nucleus in various drugs. This class of compounds represent a key motif which occupy a prime place in medicinal chemistry due to their competence to exhibit antimicrobial (Gilbert et al. 2006), anticancer (Igor Magedov et al. 2007), anti-inflammatory (Bennamane et al. 2008), anticonvulsant (Ozdemir et al. 2007), antipyretic (Sener et al. 2002), peptide deformylase inhibitor (Cali et al. 2004) activities.

Pyranopyrazoles were first obtained in 1973 by reaction between 3-methyl-1-phenylpyrazolin-5-one and tetracyanoethylene (Junek & Aigner 1973). After this Otto (1974) had proposed the synthesis of the dihydropyrano[2,3-*c*]pyrazoles in 1974 via the base catalyst cycloaddition of 4-aryliden-5-pyrazolone (Otto 1974). Pyran derivatives constitute a useful class of heterocyclic compounds, which are widely distributed in nature (Moriguchi et al. 1997). Pyran and fused pyran derivatives have attracted a great deal of interest due to their association with various kinds of biological properties. Substituted benzo(b)pyran derivatives synthesized were reported to exhibit anticancer activities against three human cell lines even at very low concentrations (Hamman et al. 2005). Pyranochalcones have been reported to exhibit antimutagenic, antimicrobial, antiulcer and antitumor activities (Lee et al. 2007). A regioselective

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palladium-catalyzed allylic alkylation cascade forms furo[3,2-c]pyrans from various cyclic β-dicarbonyl bis-nucleophiles and 3,6-dihydro-2*H*-pyran bis-electrophiles (Bartlett et al. 2013). Pyrano[3,2-*c*]pyran derivatives were synthesized by the reaction of aromatic aldehyde, malononitrile and 4-hydroxy-5-methylpyran-2-one in ethyl alcohol at room temperature catalyzed by KF/Al_2O_3 (Wang et al. 2006).

When a biodynamic heterocyclic system is coupled with other heterocyclic systems, such coupled molecules are expected to show enhanced biological activity. With this in view and considering the importance of pyran and pyrazole derivatives, it was thought worthwhile to synthesize new compounds incorporating both these moieties to the coumarin nucleus with the hope of getting molecules of greater biological potency. We herein report the synthesis of a series of new fused pyrans bearing coumarin moiety and *in vitro* evaluation of their antimicrobial activity.

METHODS

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on a Nujol mull on Shimadzu 8300 spectrometer. The ^1H NMR and ^{13}C NMR spectra were recorded on a Spect 500 MHz and Spect 125 MHz spectrophotometer respectively using DMSO as solvent and TMS as an internal standard. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer (CI). Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Purification of compounds was done by column chromatography on silica gel (70-230 mesh. Merck).

General procedure for the synthesis of 2-aryl-4-ethoxyl-8-methyl-2*H*-pyrano[2',3':5,6] chromeno[4,3-*c*]pyrazol-10(4*H*)-one 2a-f

Precursors 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carboxaldehydes **1a-f** (Scheme-1) were obtained by the procedure reported by us earlier (Renuka & Kumar 2013).

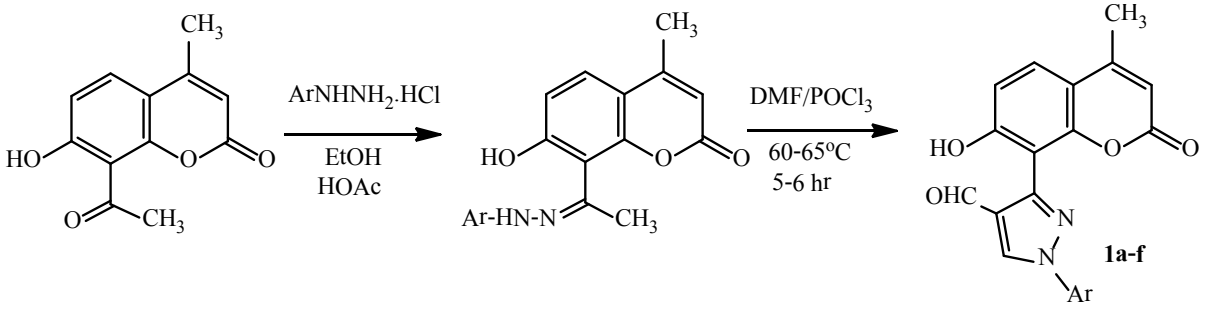
A mixture of 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carboxaldehydes **1a-f** (0.001mol) in ethyl alcohol (10ml) and concentrated sulfuric acid (1mL) was refluxed for 4 hours at 80ºC. The progress of the reaction was monitored by TLC; after completion, the solvent was removed in vacuo. The resulting residue was extracted into ether (30mL), washed successively with NaOH and $NaHCO_3$. The organic phase was dried over anhydrous sodium sulphate. The solvent was evaporated to dryness to get the products **2a-f** (Scheme-2). The products were purified by column chromatography using hexane and ethyl acetate as eluent.

4-Ethoxy-8-methyl-2-phenyl-2*H*-pyrano[2',3':5,6] chromeno[4,3-*c*]pyrazol-10(4H)-one 2a

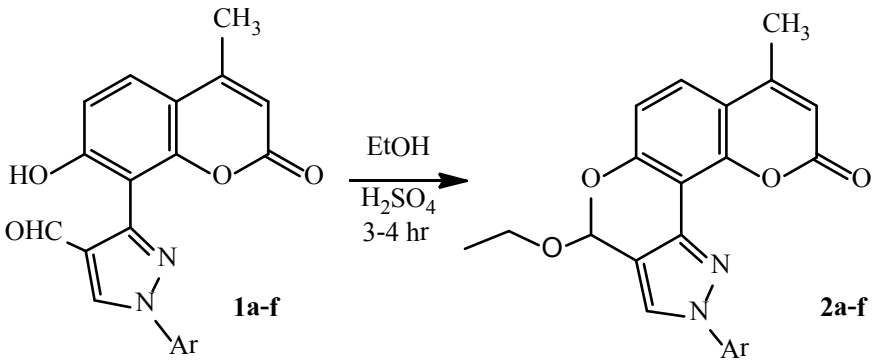
Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-phenyl-1H-pyrazole-4-carboxaldehyde **1a** as a light yellow solid in 85% yield; purified by column chromatography separations using hexane: ethyl acetate (9:2) as eluent. m.p. 196-198°C. ^1H NMR (DMSO-d_6): δ 1.10 (t, 3H, CH_3), 2.30 (q, 2H, CH_2), 3.78 (s, 3H, CH_3), 6.3 (s, 1H, C_9-H), 6.60 (s, 1H, C_4-H), 7.20 (d, 1H, C_6-H), 7.43 (d, 1H, C_7-H), 7.40-8.20 (m, 5H, Ar-H), 8.80 (s, 1H, C_3-H). ^{13}C NMR: (DMSO-d_6): δ 13.62 (1C, CH_3), 18.75 (1C, CH_3), 39.49 (1C, OCH_2), 80.33 (1C, C_6), 115.02 (1C, C_4), 115.6 (1C, C_9), 116.2 (1C, C_{7a}), 117.3 (1C, C_{11b}), 118.6 (1C, $C_{3'}$), 119.61 (2C, Ar), 119.76 (1C, C_3), 127.39 (1C, C_7), 128.11 (1C, Ar), 130.19 (2C, Ar), 134.27 (1C, Ar), 138.23 (1C, C_{11a}), 158.5 (1C, $C_{2'}$), 160.46 (1C, C_8), 166.04 (1C, $C_{5'}$), 174.15 (1C, C_{10}). MS (m/z): 375 [M+1], 359, 345, 329, 283, 270, 238, 177. Anal. Cacld. for $C_{22}H_{18}N_2O_4$: C, 70.58; H, 4.85; N, 7.48%; Found: C, 70.62; H, 5.01; N, 7.58%.

4-Ethoxy-2-(2-methoxyphenyl)-8-methyl-2*H*-pyrano[2',3':5,6]chromeno[4,3-*c*]pyrazol-10(4H)-one 2b

Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-(2-methoxyphenyl)-1H-pyrazole-4-



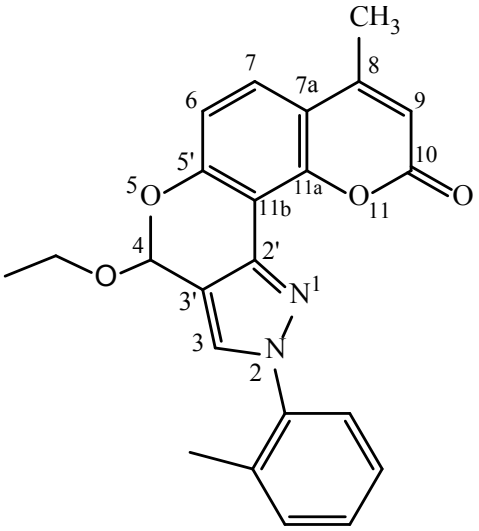
Scheme 1. Synthetic pathway for the preparation of formylpyrazoles **1a-f.**



2 a) Ar = C_6H_5; b) Ar = 2-$OCH_3C_6H_4$; c) Ar = 2-$CH_3C_6H_4$;
d) Ar = 2-ClC_6H_4; e) Ar = 4-ClC_6H_4; f) Ar = 2,4$(NO_2)_2C_6H_3$.

Scheme 2. Synthetic pathway for the preparation of fused pyrans **2a-f.**

Additional figure according to accepted numbering system



carboxaldehyde **1b** as a light yellow solid in 82% yield; purified by column chromatography separations using hexane: ethyl acetate (9:1) as eluent. m.p. 122-124°C. ^{1}H NMR (DMSO-d_6): δ 1.00 (t, 3H, CH_3), 2.22 (q, 2H, CH_2), 3.66 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.32 (s, 1H, C_9-H), 6.60 (s, 1H, C_4-H), 7.22 (d, 1H, C_6-H), 7.40 (d, 1H, C_7-H), 7.42 (d, 1H, Ar-H), 7.52 (t, 1H, Ar-H), 7.88 (t, 1H, Ar-H), 8.08 (d, 1H, Ar-H), 8.76 (s, 1H, C_3-H). ^{13}C NMR: (DMSO-d_6): δ 13.62 (1C, CH_3), 18.75 (1C, CH_3), 39.49 (1C, OCH_2), 60.2 (1C, OCH_3), 80.33 (1C, C_6), 115.02 (1C, C_4), 115.6 (1C, C_9), 116.2 (1C, C_{7a}), 117.3 (1C, C_{11b}), 118.6 (1C, $C_{3'}$), 118.8 (1C, Ar), 119.2 (1C, Ar), 119.76 (1C, C_3), 120.5 (1C, Ar), 121.8 (1C, Ar), 123.4 (1C, Ar), 126.3 (1C, Ar), 127.39 (1C, C_7), 138.23 (1C, C_{11a}), 158.5 (1C, $C_{2'}$), 160.46 (1C, C_8), 166.04 (1C, $C_{5'}$), 174.15 (1C, C_{10}). MS (m/z): 405 [M+1], 388 [M+, base peak], 375, 360, 283, 270, 238, 177. Anal. Cacld. for $C_{23}H_{20}N_2O_5$: C, 68.31; H, 4.98; N, 6.93%; Found: C, 68.11; H, 5.03; N, 7.09%.

4-Ethoxy-8-methyl-2-(2-methylphenyl)-2*H*-pyrano[2',3':5,6]chromeno[4,3-*c*]pyrazol-10(4H)-one 2c

Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-(2-methylphenyl)-1H-pyrazole-4-carboxaldehyde **1c** as a light yellow solid in 92% yield; purified by column chromatography separations using hexane: ethyl acetate (8:2) as eluent.m.p. 156-158°C. ^{1}H NMR (DMSO-d_6): δ 0.99 (t, 3H, CH_3), 2.24 (q, 2H, CH_2), 3.01 (s, 3H, CH_3), 3.62 (s, 3H, CH_3), 6.20 (s, 1H, C_9-H), 6.62 (s, 1H, C_4-H), 7.14 (d, 1H, C_6-H), 7.44 (d, 1H, C_7-H), 7.52 (d, 1H, Ar-H), 7.74 (t, 1H, Ar-H), 7.92 (t, 1H, Ar-H), 8.18 (d, 1H, Ar-H), 8.70 (s, 1H, C_3-H).^{13}C NMR: (DMSO-d_6): δ 13.68 (1C, CH_3), 16.22 (1C, CH_3), 18.90 (1C, OCH_2), 39.42 (1C, CH_3), 80.24 (1C, C_6), 115.12 (1C, C_4), 115.6 (1C, C_9), 116.2 (1C, C_{7a}), 117.3 (1C, C_{11b}), 118.6 (1C, $C_{3'}$), 119.2 (1C, Ar), 119.65 (1C, Ar), 119.72 (1C, C_3), 127.35 (1C, C_7), 128.18 (1C, Ar), 131.5 (1C, Ar), 134.6 (1C, Ar), 136.4 (1C, Ar), 138.32 (1C, C_{11a}), 158.08 (1C, $C_{2'}$), 160.26 (1C, C_8), 166.12 (1C, $C_{5'}$), 174.00 (1C, C_{10}). MS (m/z): 388, 389 [M+1], 373, 359, 344, 283, 270, 238, 177. Anal. Cacld. for $C_{23}H_{20}N_2O_4$: C, 71.12; H, 5.19; N, 7.21%; Found: C, 71.23; H, 5.28; N, 7.27%.

2-(2-Chlorophenyl)-4-ethoxy-8-methyl-2*H*-pyrano[2',3':5,6]chromeno[4,3-*c*]pyrazol-10(4H)-one 2d

Obtained from 1-(2-chlorophenyl)-3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1H-pyrazole-4-carboxaldehyde **1d** as a light yellow solid in 86% yield; purified by column chromatography separations using

hexane: ethyl acetate (8:1) as eluent. m.p. 134-136°C. ^{1}H NMR (DMSO-d_6): δ 1.02 (t, 3H, CH_3), 2.28 (q, 2H, CH_2), 3.04 (s, 3H, CH_3), 6.24 (s, 1H, C_9-H), 6.63 (s, 1H, C_4-H), 7.10 (d, 1H, C_6-H), 7.42 (d, 1H, C_7-H), 7.56 (d, 1H, Ar-H), 7.68 (t, 1H, Ar-H), 7.88 (t, 1H, Ar-H), 8.08 (d, 1H, Ar-H), 8.61 (s, 1H, C_3-H). ^{13}C NMR: (DMSO-d_6): δ 13.80 (1C, CH_3), 18.88 (1C, CH_3), 39.60 (1C, OCH_2), 80.30 (1C, C_6), 115.12 (1C, C_4), 115.6 (1C, C_9), 116.2 (1C, C_{7a}), 117.3 (1C, C_{11b}), 118.68 (1C, $C_{3'}$), 119.40 (1C, Ar), 119.98 (1C, C_3), 123.6 (1C, Ar), 127.32 (1C, C_7), 128.18 (1C, Ar), 130.3 (1C, Ar), 132.4 (1C, Ar), 135.5 (1C, Ar), 138.44 (1C, C_{11a}), 158.40 (1C, $C_{2'}$), 160.40 (1C, C_8), 166.16 (1C, $C_{5'}$), 174.02 (1C, C_{10}). MS (m/z): 408, 409 [M+1], 393, 379, 364, 283, 270, 238, 177. Anal. Cacld. for $C_{22}H_{17}ClN_2O_4$: C, 64.63; H, 4.19; N, 6.85%; Found: C, 64.53; H, 4.12; N, 7.01%.

2-(4-Chlorophenyl)-4-ethoxy-8-methyl-2*H*-pyrano[2',3':5,6]chromeno[4,3-*c*]pyrazol-10(4H)-one 2e

Obtained from 1-(4-chlorophenyl)-3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1H-pyrazole-4-carboxaldehyde **1e** as a pale yellow solid in 74% yield; purified by column chromatography separations using hexane: ethyl acetate (9:2) as eluent. m.p. 113-115°C. ^{1}H NMR: (DMSO-d_6): δ 1.06 (t, 3H, CH_3), 2.24 (q, 2H, CH_2), 3.00 (s, 3H, CH_3), 6.11 (s, 1H, C_9-H), 6.58 (s, 1H, C_4-H), 7.13 (d, 1H, C_6-H), 7.30 (d, 1H, C_7-H), 7.68 (dd, 2H, Ar-H), 8.12 (dd, 2H, Ar-H), 8.58 (s, 1H, C_3-H).^{13}C NMR: (DMSO-d_6): δ 13.86 (1C, CH_3), 18.80 (1C, CH_3), 39.54 (1C, OCH_2), 80.33 (1C, C_6), 115.18 (1C, C_4), 115.6 (1C, C_9), 116.2 (1C, C_{7a}), 117.3 (1C, C_{11b}), 118.62 (1C, $C_{3'}$), 119.73 (2C, Ar), 119.94 (1C, C_3), 127.30 (1C, C_7), 130.22 (2C, Ar), 132.4 (1C, Ar), 136.3 (1C, Ar), 138.48 (1C, C_{11a}), 158.46 (1C, $C_{2'}$), 160.44 (1C, C_8), 166.10 (1C, $C_{5'}$), 174.00 (1C, C_{10}). MS (m/z): 408, 409 [M+1], 393, 379, 364, 283, 270, 238, 177Anal. Cacld. for $C_{22}H_{17}ClN_2O_4$: C, 64.63; H, 4.19; N, 6.85%; Found: C, 64.48; H, 4.30; N, 7.01%.

2-(2,4-Dinitrophenyl)-4-ethoxy-8-methyl-2*H*-pyrano[2',3':5,6]chromeno[4,3-*c*]pyrazol-10(4H)-one 2f

Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-(2,4-dinitrophenyl)-1H-pyrazole-4-carboxaldehyde **1f** as a yellow solid in 81% yield; purified by column chromatography separations using hexane: ethyl acetate (9:1) as eluent. m.p. 196-198°C. ^{1}H NMR: (DMSO-d_6): δ 1.05 (t, 3H, CH_3), 2.25 (q, 2H, CH_2), 3.02 (s, 3H, CH_3), 6.04 (s, 1H, C_9-H), 6.55 (s, 1H, C_4-H), 7.16 (d, 1H, C_6-H), 7.34 (d, 1H, C_5-H), 7.58 (d, 1H, Ar-H), 7.98 (d, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 8.68 (s, 1H, C_3-H). ^{13}C NMR: (DMSO-d_6): δ 13.86 (1C, CH_3), 18.80 (1C-CH_3), 39.54 (1C, OCH_2), 80.33 (1C, C_6), 115.18 (1C, C_4), 115.6 (1C, C_9), 116.2 (1C, C_{7a}), 117.3 (1C, C_{11b}), 118.62 (1C, $C_{3'}$), 119.94 (1C, C_3), 120.6 (1C, Ar), 123.6 (1C, Ar), 127.30 (1C, C_7), 128.4 (1C, Ar), 132.4 (1C, Ar), 138.48 (1C, C_{11a}), 140.5 (1C, Ar), 150.4 (1C, Ar), 158.46 (1C, $C_{2'}$), 160.44 (1C, C_8), 166.10 (1C, $C_{5'}$), 174.00 (1C, C_{10}). MS (m/z): 464, 465 [M+1], 449, 435, 420, 283, 270, 238, 177. Anal. Cacld. for $C_{22}H_{16}N_4O_8$: C, 56.90; H, 3.47; N, 12.06%; Found: C, 56.87; H, 3.52; N, 12.16%.

Antimicrobial activity

Minimum inhibitory concentrations (MICs) of the synthesized compounds **2a-f** against different bacterial and fungal strains were determined by a known method (Kumar et al. 2012). Ciprofloxacin and Nystatin were used as standard drugs against bacteria and fungi species. The experiments were performed in triplicate and the results were taken as a mean ± standard deviation (SD). The results of antibacterial and antifungal activity of the synthesized compounds were summarized in Table 1 and Table 2 respectively.

Table 1. Minimum Inhibitory Concentrations of the synthesized compounds 2a-f against bacteria species.

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*				
	Staphylococcus aureus	*Streptococcus pyogenes*	*Salmonella typhimurium*	*Escherichia coli*	*Pseudomonas aeruginosa*
2a	30^{a}±1.05	75^{a}±1.46	40^{a}±0.50	25^{a}±1.00	25^{a}±0.92
2b	NA	NA	75^{b}±0.53	50^{b}±0.43	NA
2c	50^{b}±0.62	100^{b}±0.46	75^{b}±0.53	75^{c}±0.75	100^{b}±0.62
2d	25^{c}±0.46	60^{c}±0.56	60^{c}±0.80	50^{b}±1.00	50^{c}±0.53
2e	20^{d}±0.36	30^{d}±0.62	50^{d}±0.53	40^{d}±0.46	25^{a}±0.65
2f	50^{e} ±0.30	75^{e}±0.26	NA	NA	NA
Ciprofloxacin	25^{c} ±0.43	50^{f}±1.28	50^{d}±0.55	25^{a}±0.65	12.5^{d}±0.65

The compounds with the different letter in the parenthesis are significantly different at 5% level according to (DMRT) Duncan's Multiple Range Test.

* Values are expressed as mean ± standard deviation (SD).

NA: No activity observed.

Table 2. Minimum Inhibitory Concentrations of the synthesized compounds 2a-f against fungi species.

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*			
	Cryptococcus neoformans	*Aspergillas niger*	*Aspergillas flavus*	*Candida albicans*
2a	50^{a}±0.36	75^{a}±0.82	75^{a}±1.05	50^{a}±1.37
2b	150^{b}±0.62	NA	NA	150^{b}±1.34
2c	100^{c}±1.21	NA	200^{b}±1.81	NA
2d	30^{d}±0.70	50^{b}±2.09	60^{c}±0.79	30^{c}±1.25
2e	25^{e}±0.85	40^{c}±1.65	40^{d}±0.70	25^{d}±0.56
2f	NA	NA	NA	NA
Nystatin	25^{e}±1.55	50^{b}±0.75	50^{e}±0.43	25^{d}±0.85

The compounds with the different letter in the parenthesis are significantly different at 5% level according to (DMRT) Duncan's Multiple Range Test.
* Values are expressed as mean ± standard deviation (SD).
NA: No activity observed.

RESULTS AND DISCUSSION

In the current study, we intended of introduce the pyran moiety to the coumarin skeleton in order to build a novel family of bioactive molecules. Thus, a series of fused pyran derivatives **2a-f** were synthesized by intramolecular cyclisation of 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaxaldehyde **1a-f** in excellent yields.

The structures of the synthesized new compounds were confirmed by spectral and elemental analysis. For instance, in ^{1}H NMR spectrum, the signals observed in the region δ 10.60-10.80 ppm. due to –CHO group; and in the region δ 9.65-9.90 ppm. due to phenolic –OH group of the compounds **1a-f** (Renuka & Kumar 2013) were found absent in all the synthesized compounds **2a-f**. A consistent pattern signals a singlet in the region δ 6.5-6.6 ppm. due to $-C_4$-H function of pyran ring; triplet in the region δ 0.99-1.10 ppm. due to $-CH_3$ protons, and a quartet in the region δ 2.22-2.30 ppm. due to $-CH_2$ protons, which were absent in ^{1}H NMR spectra of **1a-f** confirmed the formation of the products. Further, all showed the signals due to aromatic and substituent protons at the expected region.

The ^{13}C NMR spectra of **2a-f** showed the signals due to aromatic carbons and the substituent carbons at the expected region. The signals observed due to aldehydic carbon of **1a-f** (Renuka & Kumar 2013) in the region δ 165-170 ppm. were absent in **2a-f**. In addition to the signals observed in **1a-f**, compounds **2a-f** showed a consistent pattern of signals due to C_4-carbon of the pyran ring which appears in the region δ 115-116 ppm.; CH_3-carbon in the region δ 13-14 ppm.; OCH_2-carbon in the region δ 39-40 ppm. These additional signals support the formation of products. All the synthesized new molecules showed M+1 ion as a base peak in their mass spectra. Further, satisfactory elemental analysis data confirms the cyclisation of **1a-f** to form the products **2a-f**.

All the new synthesized compounds **2a-f** exerted a wide range of *in vitro* antibacterial activity against the tested organisms. However, compound **2b** failed to inhibit the growth of *Staphylococcus aureus*, *Streptococcus pyogenes,* and *Pseudonomas aeruginosa* even at a higher concentration of 200µg/mL. Similarly, compound **2f** failed to inhibit *Salmonella typhimurium*, *Echerichia coli,* and *Pseudonomas aeruginosa* organisms. Compound **2e** exhibits inhibition to a greater extent in comparison with the standard against the organisms *S. aureus*, *S. pyogenes,* and *S. typhimurium*. Compound **2d** exhibited promising bacterial activity against the organism tested. Compound **2f** displayed lesser or no activity against the organisms tested.

Compounds **2d** and **2e** showed potential antifungal activity against all the organisms tested. However, **2f** showed no activity even at a higher concentration of 200µg/mL. Compound **2a** showed moderate activity against the organisms tested. However, compounds **2b** and **2c** exhibited lesser activity against *Aspergillus niger*, *Aspergillus flavus,* and *Candina albicans,* respectively.

Statistical analysis: All values are expressed as mean ± standard deviation did in triplicates of two independent experiments. Statistical analyses of the MIC values were performed by the One-way ANOVA.

Antibacterial activities of compound **2e** against *S. aureus* and *S. pyogenes*, and compound **2a** against *S. typhimurium* are significantly higher than the standard at $p<0.05$. Likewise, compound **2e** exhibited significantly higher antifungal activity against *A. niger* and *A. flavus* compared with the standard at $p<0.05$ confidence level.

CONCLUSION

The simple easy accessible procedure for the synthesis of fused pyrans and their in vitro antibacterial and antifungal activity results revealed the significance of the study. The synthesized compounds exhibited moderate to good antibacterial and antifungal activity against some of the tested organisms. Compounds, particularly 2d and 2e exhibited greater activity in comparison to the standard drug. The SAR study of the synthesized compounds remains the topic of interest.

ACKNOWLEDGEMENT

The authors are grateful to the University Grants Commission, New Delhi for financial assistance.

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Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 10, 2015

Original Article

STEREO SELECTIVE SYNTHESIS OF NOVEL PYRAZOLE AND COUMARIN APPENDED BRIDGED PYRANS AS ANTIMICROBIAL AGENTS

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Received: 19 Jun 2015 Revised and Accepted: 08 Aug 2015

ABSTRACT

Objectives: The aim of the present study was to synthesize a series of novel bridged pyrans as antimicrobial agents.

Methods: An isomeric mixture of 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, 2(a-f) and 3-(4-ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, 3(a-f) were synthesized by the reaction of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-phenyl-1H-pyrazole-4-carbaldehyde) 1(a-f) and ethyl alcohol in the presence of conc. H_2SO_4. The synthesized compounds were evaluated for their antimicrobial activity.

Results: The structures of the new bridged pyran analogues 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano [3',2':6,7] chromeno [4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, 2(a-f) and 3-(4-ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano [2',3':5,6] chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehydes, 3(a-f) were confirmed by spectral studies and elemental analysis. The compounds 2e and 3e were having-$CONH_2$ substitution and 2f and 3f were having-$CSNH_2$ substitutions in the pyrazole rings showed antibacterial at minimum concentrations against all the tested organisms.

Conclusions: Results of the antimicrobial activity reveal that some of the synthesized compounds act as potential antimicrobial agents against different fungal and bacterial organisms.

Keywords: Antibacterial, Antifungal, Cyclisation, Formyl pyrazoles, Inhibitory.

INTRODUCTION

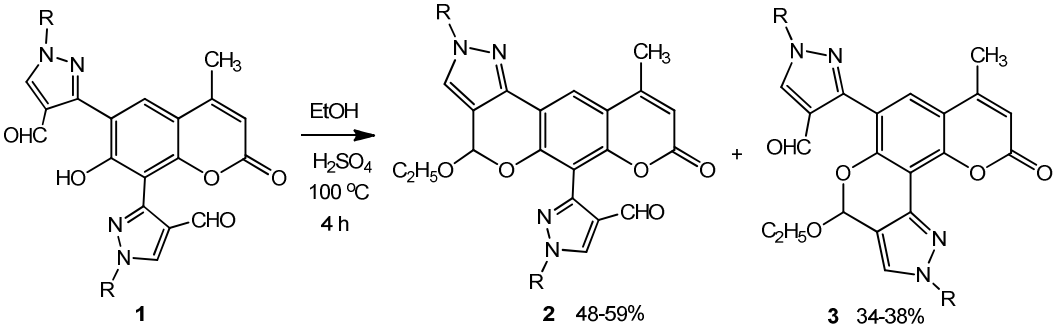
The construction of complex molecular architectures that exhibit greater biological potency in a facile and efficient manner remains an overarching goal for the chemists. In recent years coumarin libraries have attracted great attention because of their synthetic utility as building blocks for the construction of bioactive molecules. Coumarin derivatives are known to a wide range of activities; such as an antioxidant, antimicrobial, anti-HIV, antibiotic, anticancer, muscle relaxant, anti-inflammatory and anticoagulant properties [1]. An efficient synthesis of poly functionalized 4H-pyrans is carried out in one pot synthesis from an aldehyde, malononitrile and an active methylene diketo compound using a heterogeneous Mg/la mixed oxide catalyst [2]. A facile one-pot expeditious synthesis of 2-amino-4H-pyrans and 2-amino-5-oxo-5, 6, 7, 8-tetrahydro-4H-chromenes under solvent-free conditions using magnesium oxide as a catalyst is reported [3].

Pyrazole scaffolds drew a great deal of attention due to its contribution in biological and pharmacological fields [4]. The pyrazole nucleus has pronounced pharmacological applications such as anti-inflammatory [5], antimicrobial [6], antioxidant [7] and analgesic activity [8]. They have a long history of applications in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals.

In search of new antimicrobial agents and in continuation of our work on coumarin analogues we herein report the synthesis of a series of isomeric bridged pyrans tagged to pyrazole and coumarin skeleton and their antimicrobial activities.

Experimental

In a typical procedure, a series of isomeric bridged pyrans tagged to pyrazole and coumarin moiety 2(a-f) and 3(a-f) were synthesized by the condensation reaction of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-aryl-1H-pyrazole-4-carbaldehyde), 1(a-f) in ethyl alcohol (10 ml) in the presence of conc. H_2SO_4 (5 ml) under reflux conditions (Scheme-1).



1 **2** 48-59% **3** 34-38%

a) R = C_6H_5; b) R = 4-$CH_3C_6H_4$; c) R = 4-$OCH_3C_6H_4$;
d) R = 4-FC_6H_4; e) R = $CONH_2$; f) R = $CSNH_2$.

Scheme-1: Synthetic route for the bridged pyrans

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique [9]. The nutrient broth, which contains logarithmic serially two-fold diluted amount of test compound and controls was inoculated with approximately 5 x 10^5 c. f. u of actively dividing bacteria cells. The bacterial cultures were incubated for 24 h at 37 °C and fungi cultures were incubated for 72 h at 37 °C; the growth was monitored visually and spectrophotometrically. The lowest concentration required to arrest the growth of bacteria and fungi was regarded as minimum inhibitory concentration (MIC). The synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Bacillus substilis*, *Staphylococus aureus* and antifungal activity against *Aspergillus niger, Aspergillus flavus, C. albicans*. The antibiotics streptomycin and nystatin were used as standard drugs against bacteria and fungi species respectively. The experiments were carried out in triplicate; the results were taken as a mean of three determinations.

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on a Nujol mull on Shimadzu 8300 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 MHz and 125 MHz spectrophotometer respectively in $CDCl_3$ with TMS as an internal standard. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrophotometer TOF mode. Elemental analysis was performed on a Thermo Finnigan Flash EA 1112 CHN analyzer. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane: ethyl acetate (9:2) as eluent.

Typical procedure for synthesis of 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde, 2a

To a solution of 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-phenyl-1H-pyrazole-4-carbaldehyde), 1a (2.58g, 0.005 mol) in ethyl alcohol (10 ml), concentrated sulfuric acid (5 ml) was drop wise and the mixture was refluxed for 4 h at 80 °C. The progress of the reaction was monitored by TLC. After completion, the solvent was removed in vacuo. The resulting mass was extracted in to ether (30 ml), washed successively with NaOH and $NaHCO_3$. The organic phase was dried over anhydrous sodium sulphate. The solvent was evaporated to dryness to get isomeric mixture of 3-(4-ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano [3',2':6,7] chromeno [4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde, 2a and 3-(4-ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano [2',3': 5,6]chromeno[4,3-c]pyrazol -6-yl)-1-phenyl-1H-pyrazole-4-carbal-dehyde, 3a (Scheme-1). The products were purified by column chromatography using hexane and ethyl acetate (9:2 v/v) as eluent. The same procedure was used in all cases.

RESULTS AND DISCUSSION

3-(4-Ethoxy-10-methyl-8-oxo-2-phenyl-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde, 2a

Solid in 59% (3.20g) yield; m. p. 192-194 °C. IR (Nujol, γ cm^{-1}): 1772 (s) (lactone C=O str), 1733 (s) (aldehyde C=O str), 1219 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.051 (t, 3H, CH_3), 2.423 (s, 3H, CH_3), 3.920 (q, 2H, OCH_2), 6.026 (s, 1H, C_9-H), 6.304 (s, 1H, C_4-H), 7.423-7.728 (m, 12H, Ar-H), 8.320 (s, 1H, 5m ring-H), 9.782 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.06 (1C, CH_3), 20.13 (1C, CH_3), 62.33 (1C, OCH_2), 112.59 (1C), 113.64 (1C), 114.87 (1C), 115.30 (1C), 116.02 (1C), 116.86 (1C), 117.40 (1C), 119.72 (4C), 123.22 (1C), 126.16 (2C), 128.13 (1C), 128.90 (4C), 130.17 (1C), 138.46 (2C), 147.80 (1C), 148.63 (1C), 151.12 (1C), 152.94 (1C), 156.10 (1C), 161.18 (1C, C_8), 181.32 (1C, CHO). MS (m/z): 545 (MH^+), 544 (M^+), 516, 488, 280 (100%, base peak), 236. Anal. Calcd. for $C_{32}H_{24}N_4O_5$: C, 70.58; H, 4.44; N, 10.29%; Found: C, 70.49; H, 4.26; N, 10.21%.

3-(4-Ethoxy-2-(4-methylphenyl)-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde, 2b

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis (1-(4-methyl phenyl)-1H-pyrazole-4-carbaldehyde), **1b** (2.72g, 0.005 mol) as solid in 48% (2.75g) yield; m. p. 144-146 °C. IR (Nujol, γ cm^{-1}): 1765 (s) (lactone C=O str), 1726 (s) (aldehyde C=O str), 1213 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.089 (t, 3H, CH_3), 2.314 (s, 6H, CH_3), 2.453 (s, 3H, CH_3), 3.984 (q, 2H, OCH_2), 6.066 (s, 1H, C_9-H), 6.202 (s, 1H, C_4-H), 7.405-7.748 (m, 10H, Ar-H), 8.263 (s, 1H, 5m ring-H), 9.707 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.62 (1C, CH_3), 20.30 (1C, CH_3), 21.40 (2C, CH_3), 62.66 (1C, OCH_2), 112.16 (1C), 113.04 (1C), 114.44 (1C), 115.33 (1C), 116.21 (1C), 119.42 (4C), 123.12 (1C), 128.76 (1C), 129.32 (4C), 130.37 (1C), 134.78 (2C), 135.14 (2C), 147.88 (1C), 148.66 (1C), 150.32 (1C), 151.64 (1C), 152.49 (2C), 156.11 (1C), 161.36 (1C, C_8), 180.66 (1C, CHO). MS (m/z): 573 (MH^+), 572 (M^+), 544, 516, 280 (100%, base peak), 236. Anal. Calcd. for $C_{34}H_{28}N_4O_5$: C, 71.32; H, 4.93; N, 9.78%; Found: C, 71.20; H, 4.78; N, 9.73%.

3-(4-Ethoxy-2-(4-methoxyphenyl)-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde, 2c

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde), **1c** (2.88g, 0.005 mol) as solid in 49% (2.95g) yield; m. p. 178-180 °C. IR (Nujol, γ cm^{-1}): 1769 (s) (lactone C=O str), 1732 (s) (aldehyde C=O str), 1221 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.126 (t, 3H, CH_3), 2.394 (s, 3H, CH_3), 3.813 (s, 6H, OCH_3), 3.955 (q, 2H, OCH_2), 6.148 (s, 1H, C_9-H), 6.322 (s, 1H, C_4-H), 7.361-7.707 (m, 10H, Ar-H), 8.123 (s, 1H, 5m ring-H), 9.869 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.62 (1C, CH_3), 20.30 (1C, CH_3), 55.40 (2C, OCH_3), 62.10 (1C, OCH_2), 111.32 (1C, C_9), 112.04 (4C), 113.20 (1C), 114.12 (4C), 114.93 (1C), 115.32 (1C), 116.00 (1C), 117.56 (1C), 123.22 (1C), 130.30 (1C), 131.18 (1C), 132.78 (2C), 145.06 (1C), 147.33 (2C), 151.44 (1C), 153.56 (1C), 154.86 (1C), 157.08 (2C), 162.10 (1C, C_8), 179.86 (1C, CHO). MS (m/z): 605 (MH^+), 604 (M^+), 576, 548, 280 (100%, base peak), 236. Anal. Calcd. for $C_{34}H_{28}N_4O_7$: C, 67.54; H, 4.67; N, 9.27%; Found: C, 67.40; H, 4.57; N, 9.14%.

3-(4-Ethoxy-2-(4-fluorophenyl)-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazol-6-yl)-1-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde, 2d

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl) bis (1-(4-fluoro phenyl)-1H-pyrazole-4-carbaldehyde), **1d** (2.76g, 0.005 mol) as solid in 54% (3.15g) yield; m. p. 182-185 °C. IR (Nujol, γ cm^{-1}): 1763 (s) (lactone C=O str), 1724 (s) (aldehyde C=O str), 1213 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.105 (t, 3H, CH_3), 2.343 (s, 3H, CH_3), 3.908 (q, 2H, OCH_2), 6.244 (s, 1H, C_9-H), 6.440 (s, 1H, C_4-H), 7.224-7.682 (m, 10H, Ar-H), 8.189 (s, 1H, 5m ring-H), 9.804 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.02 (1C, CH_3), 20.14 (1C, CH_3), 63.00 (1C, OCH_2), 112.02 (1C, C_9), 113.16 (1C), 113.53 (1C), 114.32 (1C), 115.14 (4C), 115.52 (1C), 116.32 (4C), 116.68 (1C), 117.26 (1C), 123.12 (1C), 128.18 (1C), 130.14 (1C), 134.18 (2C), 146.23 (1C), 148.13 (1C), 150.65 (1C), 152.36 (1C), 155.46 (1C), 159.28 (2C), 162.34 (1C, C_8), 180.22 (1C, CHO). MS (m/z): 581 (MH^+), 580 (M^+), 552, 524, 280 (100%, base peak), 236. Anal. Calcd. for $C_{32}H_{22}F_2N_4O_5$: C, 66.20; H, 3.82; N, 9.65%; Found: C, 66.07; H, 3.66; N, 9.49%.

6-(1-Carbamoyl-4-formyl-1H-pyrazol-3-yl)-4-ethoxy-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c] pyrazole-2-carboxamide, 2e

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(4-formyl-1H-pyrazole-1-carboxamide), **1e** (4) (2.25g, 0.005 mol) as solid in 50% (2.39g) yield; m. p. 200-203 °C. IR (Nujol, γ cm^{-1}): 1771 (s) (lactone C=O str), 1730 (s) (aldehyde C=O str), 1217 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.166 (t, 3H, CH_3), 2.404 (s, 3H, CH_3), 3.902 (q, 2H, OCH_2), 6.215 (s, 1H, C_9-H), 6.363 (s, 1H, C_4-H), 7.636 (s, 1H, C_{11}-H), 7.923 (s, 4H, NH_2), 8.26 (s, 1H, 5m ring-H), 8.677 (s, 1H, 5m ring-H), 9.803 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.56 (1C, CH_3), 21.10 (1C, CH_3), 62.24 (1C, OCH_2), 110.72 (1C), 112.46 (1C), 112.82 (1C, C_9), 114.02 (1C), 114.51 (1C), 115.66 (1C), 126.48 (1C), 128.88 (1C), 135.12 (1C), 136.28 (1C), 142.33 (1C), 144.63 (1C), 147.54 (1C), 152.10 (1C), 156.31 (1C), 157.30 (2C, $CONH_2$), 161.14 (1C, C_8), 167.22 (1C, CHO). MS (m/z): 479 (MH^+), 478 (M^+), 450, 422, 280 (100%, base peak), 236. Anal. Calcd. for $C_{22}H_{18}N_6O_7$: C, 55.23; H, 3.79; N, 17.57%; Found: C, 55.20; H, 3.64; N, 17.44%.

6-(1-Carbamoyl-4-formyl-1H-pyrazol-3-yl)-4-ethoxy-10-methyl-8-oxo-4,8-dihydro-2H-pyrano[3',2':6,7]chromeno[4,3-c]pyrazole-2-carbothioamide, 2f

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(4-formyl-1H-pyrazole-1-carbothioamide), **1f** (4) (2.41g, 0.005 mol) as solid in 56% (2.85g) yield; m. p. 174-178 °C. IR (Nujol, γ cm^{-1}): 1766 (s) (lactone C=O str), 1735 (s) (aldehyde C=O str), 1215 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.101 (t, 3H, CH_3), 2.414 (s, 3H, CH_3), 3.882 (q, 2H, OCH_2), 6.128 (s, 1H, C_9-H), 6.343 (s, 1H, C_4-H), 7.638 (s, 1H, C_{11}-H), 7.864 (s, 1H, 5m ring-H), 8.076 (s, 1H, 5m ring-H), 8.622 (s, 4H, NH_2), 9.844 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.78 (1C, CH_3), 20.86 (1C, CH_3), 62.68 (1C, OCH_2), 111.72 (1C), 112.54 (1C, C_9), 112.96 (1C), 114.88 (1C), 115.24 (1C), 116.12 (1C), 126.72 (1C), 128.74 (1C), 135.27 (1C), 136.7 (1C), 142.21 (1C), 144.51 (1C), 147.33 (1C), 152.32 (1C), 155.44 (1C), 162.36 (1C, C_8), 174.10 (2C, $CSNH_2$), 180.26 (1C, CHO). MS (m/z): 511 (MH^+), 510 (M^+), 482, 454, 280 (100%, base peak), 236. Anal. Calcd. for $C_{22}H_{18}N_6O_5S_2$: C, 51.76; H, 3.55; N, 16.46%; Found: C, 51.82; H, 3.45; N, 16.30%.

3-(4-Ethoxy-8-methyl-10-oxo-2-phenyl-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde, 3a

Solid in 36% (1.95g) yield; m. p. 189-190 °C. IR (Nujol, γ cm^{-1}): 1765 (s) (lactone C=O str), 1728 (s) (aldehyde C=O str), 1214 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.052 (t, 3H, CH_3), 2.422 (s, 3H, CH_3), 3.940 (q, 2H, OCH_2), 6.126 (s, 1H, C_9-H), 6.316 (s, 1H, C_4-H), 7.416-7.745 (m, 12H, Ar-H), 8.319 (s, 1H, 5m ring-H), 9.770 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.22 (1C, CH_3), 20.18 (1C, CH_3), 62.21 (1C, OCH_2), 112.50 (1C), 113.50 (1C), 114.66 (1C), 115.43 (1C), 116.32 (1C), 116.88 (1C), 117.68 (1C), 119.74 (4C), 123.98 (1C), 126.02 (2C), 128.10 (1C), 128.94 (4C), 130.16 (1C), 138.41 (2C), 147.36 (1C), 148.60 (1C), 151.06 (1C), 152.54 (1C), 152.10 (1C, C_8), 156.15 (1C), 185.30 (1C, CHO). MS (m/z): 545 (MH^+), 544 (M^+), 516, 488, 442, 353, 249 (100%, base peak). Anal. Calcd. for $C_{32}H_{24}N_4O_5$: C, 70.58; H, 4.44; N, 10.29%; Found: C, 70.36; H, 4.28; N, 10.11%.

3-(4-Ethoxy-8-methyl-10-oxo-2-(4-methylphenyl))-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde, 3b

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(1-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde), **1b** (2.72g, 0.005 mol) as solid in 38% (2.17g) yield; m. p. 147-149 °C. IR (Nujol, γ cm^{-1}): 1772 (s) (lactone C=O str), 1731 (s) (aldehyde C=O str), 1216 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.123 (t, 3H, CH_3), 2.281 (s, 6H, CH_3), 2.487 (s, 3H, CH_3), 3.760 (q, 2H, OCH_2), 6.112 (s, 1H, C_9-H), 6.306 (s, 1H, C_4-H), 7.452-7.790 (m, 10H, Ar-H), 8.436 (s, 1H, 5m ring-H), 9.780 (s, 1H, CHO). MS (m/z): 573 (MH^+), 572 (M^+), 516, 470, 249 (100%, base peak). Anal. Calcd. for $C_{34}H_{28}N_4O_5$: C, 71.32; H, 4.93; N, 9.78%; Found: C, 71.24; H, 4.77; N, 9.56%.

3-(4-Ethoxy-2-(4-methoxyphenyl)-8-methyl-10-oxo-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde, 3c

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis (1-(4-methoxy phenyl)-1H-pyrazole-4-carbaldehyde), **1c** (2.88g, 0.005 mol) as solid in 37% (2.23g) yield; m. p. 172-174 °C. IR (Nujol, γ cm^{-1}): 1770 (s) (lactone C=O str), 1730 (s) (aldehyde C=O str), 1222 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.099 (t, 3H, CH_3), 2.346 (s, 3H, CH_3), 3.855 (s, 6H, OCH_3), 3.980 (q, 2H, OCH_2), 6.133 (s, 1H, C_9-H), 6.338 (s, 1H, C_4-H), 7.380-7.745 (m, 10H, Ar-H), 8.469 (s, 1H, 5m ring-H), 9.789 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.66 (1C, CH_3), 20.38 (1C, CH_3), 55.56 (2C, OCH_3), 62.19 (1C, OCH_2), 112.08 (1C, C_9), 112.22 (4C), 113.26 (1C), 114.56 (4C), 114.98 (1C), 115.74 (1C), 116.08 (1C), 117.88 (1C), 123.20 (1C), 130.34 (1C), 131.28 (1C), 132.26 (2C), 145.38 (1C), 147.40 (2C), 151.40 (1C), 152.10 (1C, C_8), 153.55 (1C), 154.92 (1C), 157.18 (2C), 183.32 (1C, CHO). Anal. Calcd. for $C_{34}H_{28}N_4O_7$: C, 67.54; H, 4.67; N, 9.27%; Found: C, 67.42; H, 4.48; N, 9.36%.

3-(4-Ethoxy-2-(4-fluorophenyl)-8-methyl-10-oxo-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazol-6-yl)-1-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde, 3d

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis (1-(4-fluoro phenyl)-1H-pyrazole-4-carbaldehyde), **1d** (2.76g, 0.005 mol) as solid in 36% (2.08g) yield; m. p. 177-178 °C. IR (Nujol, γ cm^{-1}): 1775 (s) (lactone C=O str), 1734 (s) (aldehyde C=O str), 1224 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.219 (t, 3H, CH_3), 2.338 (s, 3H, CH_3), 3.914 (q, 2H, OCH_2), 6.241 (s, 1H, C_9-H), 6.466 (s, 1H, C_4-H), 7.220-7.686 (m, 10H, Ar-H), 8.185 (s, 1H, 5m ring-H), 9.820 (s, 1H, CHO). Anal. Calcd. for $C_{32}H_{22}F_2N_4O_5$: C, 66.20; H, 3.82; N, 9.65%; Found: C, 66.10; H, 3.64; N, 9.44%.

6-(1-Carbamoyl-4-formyl-1H-pyrazol-3-yl)-4-ethoxy-8-methyl-10-oxo-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazole-2-carboxamide, 3e

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(4-formyl-1H-pyrazole-1-carboxamide), **1e** (2.25g, 0.005 mol) and ethyl alcohol (0.002 mol) as solid in 36% (1.72g) yield; m. p. 205-206 °C. IR (Nujol, γ cm^{-1}): 1762 (s) (lactone C=O str), 1722 (s) (aldehyde C=O str), 1112 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.162 (t, 3H, CH_3), 2.404 (s, 3H, CH_3), 3.923 (q, 2H, OCH_2), 6.210 (s, 1H, C_9-H), 6.362 (s, 1H, C_4-H), 7.646 (s, 1H, C_7-H), 7.923 (s, 4H, NH_2), 8.262 (s, 1H, 5m ring-H), 8.672 (s, 1H, 5m ring-H), 9.834 (s, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ 15.52 (1C, CH_3), 21.48 (1C, CH_3), 62.36 (1C, OCH_2), 110.70 (1C), 112.38 (1C), 112.80 (1C, C_9), 114.00 (1C), 114.50 (1C), 115.42 (1C), 126.43 (1C), 128.81 (1C), 135.10 (1C), 136.24 (1C), 142.30 (1C), 144.23 (1C), 147.30 (1C), 152.56 (1C), 156.26 (1C), 151.30 (2C, $CONH_2$), 152.14 (1C, C_8), 187.20 (1C, CHO). MS (m/z): 479 (MH^+), 478 (M^+), 450, 422, 249 (100%, base peak). Anal. Calcd. for $C_{22}H_{18}N_6O_7$: C, 55.23; H, 3.79; N, 17.57%; Found: C, 55.08; H, 3.60; N, 17.39%.

6-(1-Carbamothioyl-4-formyl-1H-pyrazol-3-yl)-4-ethoxy-8-methyl-10-oxo-4,10-dihydro-2H-pyrano[2',3':5,6]chromeno[4,3-c]pyrazole-2-carbothioamide, 3f

Obtained from 3,3'-(7-hydroxy-4-methyl-2-oxo-2H-chromene-6,8-diyl)bis(4-formyl-1H-pyrazole-1-carbothioamide), **1f** (4) (2.41g, 0.005 mol) as solid in 38% (1.93g) yield; m. p. 170-171 °C. IR (Nujol, γ cm^{-1}): 1778 (s) (lactone C=O str), 1738 (s) (aldehyde C=O str), 1226 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.118 (t, 3H, CH_3), 2.414 (s, 3H, CH_3), 3.895 (q, 2H, OCH_2), 6.129 (s, 1H, C_9-H), 6.348 (s, 1H, C_4-H), 7.684 (s, 1H, C_{11}-H), 7.846(s, 1H, 5m ring-H), 8.022 (s, 1H, 5m ring-H), 8.560 (s, 4H, NH_2), 9.789 (s, 1H, CHO). Anal. Calcd. for $C_{22}H_{18}N_6O_5S_2$: C, 51.76; H, 3.55; N, 16.46%; Found: C, 51.88; H, 3.33; N, 16.26%.

The structures of the synthesized compounds were provided by IR, ^{1}H NMR, ^{13}C NMR, MS studies and elemental analysis. For instance, in IR spectra, the stretching frequencies of the compounds 2(a-f) and 3(b-e) showed a strong absorption bands in the region 1778-1762 $cm^{-1}$and 1738-1722 cm^{-1} for lactone and aldehydic C=O bonds respectively. A strong and intense absorption band is absorbed in the region 1226-1112 $cm^{-1}$was assigned to C-O bonds.

In ^{1}H NMR spectra, the compounds 2(a-f) and 3(a-f) showed the absorption signals due to aromatic and substituent protons in the expected region. The compounds 2(a-f) showed consistent pattern signals due to C_9-H and C_4-H appeared as singlet in the region δ 6.000-6.100 ppm, and δ 6.200-6.300 ppm. The–CHO proton appeared as singlet in the region δ 9.700-9.900 ppm. While the compounds 3(a-f) showed consistent pattern signals due to C_9-H and C_4-H appeared as singlet in the region δ 6.120-6.150 ppm, and δ 6.300-6.400 ppm. The–CHO proton appeared as singlet in the region δ 9.770-9.980 ppm.

In ^{13}C NMR, all compounds showed the signals due to aromatic and substituent carbons at the expected region. The carbonyl carbons absorbed in the region δ 179.00-185.00 ppm. The synthesized compounds 2(a-f) showed significantly stable molecular ion peaks with a relative abundance ranging up to 18-66% and the base peak at m/z 280; While 3(a-f) showed a base peak at m/z 249. Further, all compounds showed satisfactorily CHN analysis with a deviation of±0.20% from the theoretically calculated values, which strongly favor the formation of the products.

The results of MICs) of the synthesized compounds 2(a-f) and 3(a-f) tested against different bacterium were tabulated in table-1.

Table 1: MIC's of the synthesized compounds 2(a-f) and 3(a-f) tested against bacteria species

Compound	Minimum inhibitory concentrations in µg/ml		
	S. aureus	*B. substilis*	*E. coli*
2a	50	**	200
2b	100	**	100
2c	50	**	150
2d	50	**	200
2e	25	150	50
2f	25	100	50
3a	150	200	150
3b	200	200	150
3c	150	150	200
3d	150	200	150
3e	25	50	25
3f	25	25	50
Streptomycin	25	50	25

Values are expressed as mean of the three determinations (n=3), **No inhibition observed even at a higher concentration of 200 µg/ml

The synthesized compounds exerted moderate to good antibacterial activity against the tested organisms. Compounds 2(a-d) failed to show antibacterial activity against *B. substilis* even at a concentration higher than 200 µg/ml, but showed moderate activity against the species *S. aureus* and *E. coli*. Compounds 3(a-d) showed moderate activities against all the bacterium tested. Compounds 2e and 3e were having-$CONH_2$ substitution and 2f and 3f were having-$CSNH_2$ substitutions in the pyrazole rings showed antibacterial at minimum concentrations against all the tested organisms.

The results of MICs) of the synthesized compounds 2(a-f) and 3(a-f) tested against different fungi species were tabulated in table-2.

Table 2: MIC's of the synthesized compounds 2(a-f) and 3(a-f) tested against fungi species

Compound	Minimum inhibitory concentrations in µg/ml		
	A. niger	*A. flavus*	*C. albicans*
2a	100	200	100
2b	100	150	150
2c	200	150	150
2d	150	200	100
2e	50	50	25
2f	25	50	25
3a	100	150	150
3b	150	150	100
3c	200	150	200
3d	100	20	150
3e	25	50	25
3f	25	50	50
Nystatin	25	50	25

Values are expressed as mean of the three determinations (n=3).

The synthesized compounds showed promising antifungal activity against the tested organisms. Compounds 2(a-d) and 3(a-d) showed moderate activities against all the bacterium tested in comparison with that of the reference standard. Compounds 2e and 3e were having-$CONH_2$ substitution and 2f and 3f were having-$CSNH_2$ substitutions in the pyrazole rings showed antifungal activities at minimum concentrations against all the tested organisms.

CONCLUSION

The synthesized compounds showed promising *in vitro* antifungal activity against the tested organisms. The compounds 2e and 3e were having-$CONH_2$ substitution and 2f and 3f were having-$CSNH_2$ substitutions in the pyrazole rings showed antibacterial and antifungal activity at minimum concentrations against all the tested organisms. Their MIC values indicate that these compounds act as potential antimicrobial agents. The compounds 2(a-d) and 3(a-d) showed antimicrobial activity at a higher concentration comparison with those of standard antibiotics used as reference drugs.

ACKNOWLEDGEMENT

The authors are grateful to the University Grants Commission, New Delhi for financial assistance. IOE, University of Mysore and SAIF, Madras for spectral analysis of the compounds reported.

CONFLICT OF INTERESTS

Declared None

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ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2017, Vol. 43, No. 2, pp. 197–210. © Pleiades Publishing, Ltd., 2017.

Synthesis of Coumarin Appended Pyrazolyl-1,3,4-Oxadiazoles and Pyrazolyl-1,3,4-Thiadiazoles: Evaluation of Their In Vitro Antimicrobial and Antioxidant Activities and Molecular Docking Studies[1]

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Received April 25, 2016; in final form, October 25, 2016

Abstract—A series of semicarbazones, thiocarbazones, 1,3,4-oxadiazoles, and 1,3,4-thiadiazoles bearing coumarin and pyrazole moiety have been synthesized. The new synthesized compounds were screened in vitro for their antimicrobial and antioxidant activities. Preliminary studies showed that among the synthesized new compounds, chloro-substituted thiosemicarbazone showed excellent activities against all tested organisms; at the same time, methyl substituted thiosemicarbazone showed greater activity against *E. coli*. Chloro-substituted 1,3,4-oxadiazole and 1,3,4-thiadiazole demonstrated greater DPPH and hydroxyl radical scavenging abilities. Molecular docking studies indicate that 1,3,4-oxadiazoles and 1,3,4-thiadiazoles manifest better interaction with CAT (catalase) and GPx (glutathione peroxidase) than that with SOD (superoxide dismutase). Studies on the antimicrobial and antioxidant activities of the synthesized compounds compared with those of their starting compounds are discussed.

Keywords: antimicrobial, antioxidant, coumarin, MIC, molecular docking, pyrazole

DOI: 10.1134/S106816201702011X

INTRODUCTION

Coumarins and their derivatives have attracted considerable attention from organic and medicinal chemists as large number of biologically potent natural products contain coumarin nucleus. A number of natural and synthetic coumarin derivatives display a wide range of pharmacological properties [1]. Synthetic analogues of coumarins have shown good antioxidant and antimicrobial activities [2]. Semicarbazones are known to exhibit anticonvulsant [3] and antitubercular properties [4]. 1,3,4-Oxadiazole system has been identified as the main core for many bioactive molecules. The latter compounds have attracted attention in recent times due to their broad spectrum of pharmacological activities. They are known to exhibit antimicrobial [5, 6] and anticonvulsant [7] activities. Recent review on 1,3,4-oxadiazoles clearly accounted for the chemical and pharmaceutical significances of this class of compounds [8].

Thiosemicarbazones obtained by the reaction of aldehydes or ketones are well recognized for their various physiological activities. These classes of compounds have been studied for their antitumor, antituberculosis, antimalarial, antiprotozoal, and antiviral activities [9–11]. 1,3,4-Thiadiazoles occupied a prime position in bioorganic chemistry for their synthetic utility as useful synthons for the construction of bioactive molecules with wide range of biological activities. They have been extensively used in pharmaceutical and agrochemical fields. They have been reported as antibacterial, antifungal, antioxidant, and anti-inflammatory agents and are used as pesticides in the field of agriculture. Apart from the pharmacological applications, thiadiazoles and their derivatives have been known to exhibit varied physical properties like anticorrosion, liquid crystal, optical brightening, and fluorescent properties [12]. A series of synthesized 1,2,4-triazolo-thiadiazoles were examined for their antimicrobial activities. The results of the study have shown that these compounds possess moderate to good antibacterial and antifungal activities against pathogenic strains [13].

In view of the enormous synthetic and biological applications of semicarbazones, thiocarbazones, 1,3,4-oxadiazoles, and 1,3,4-thiadiazoles bearing coumarin and pyrazole moiety and in search of new potent antimicrobial and antioxidant agents, we herein

[1] The article is published in the original.

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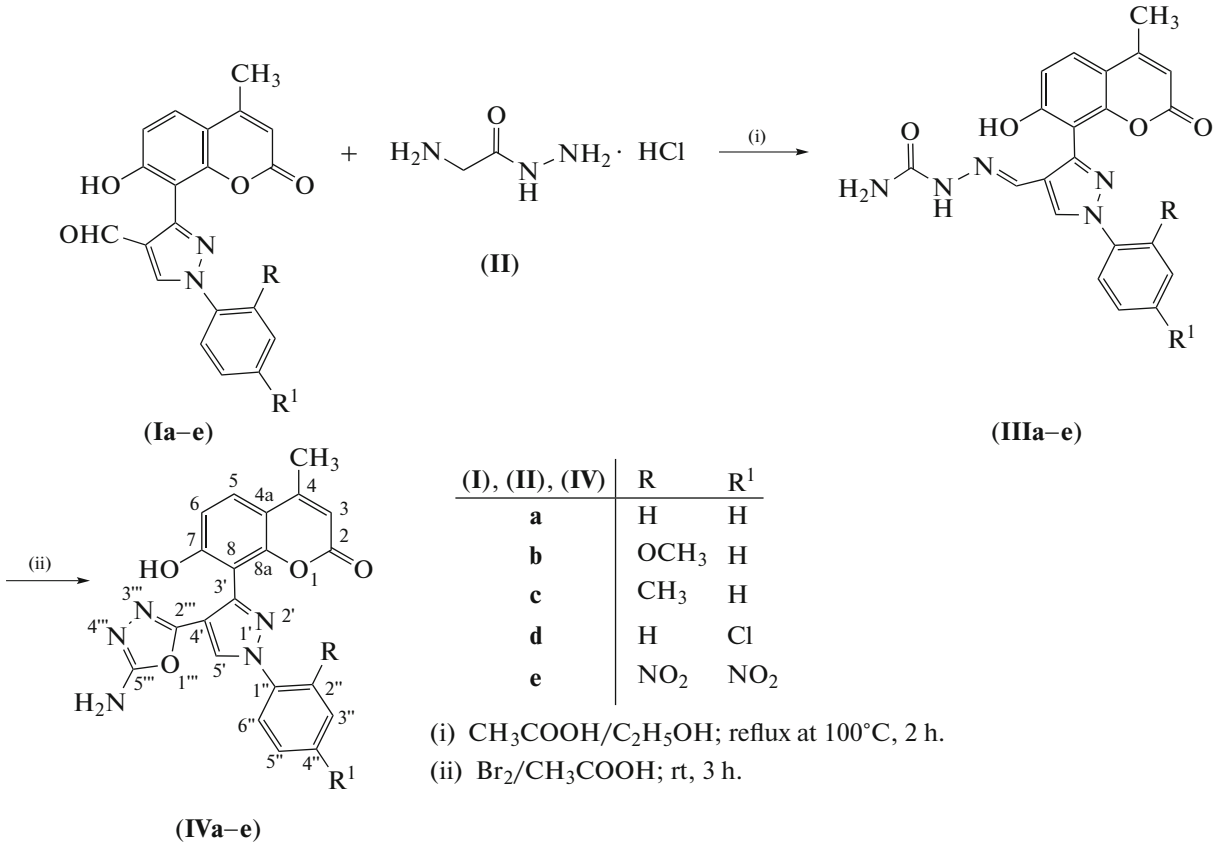
Abbreviations: CAT, catalase; GPx, glutathione peroxidase; DPPH, α,α-diphenyl-β-picrylhydrazyl; SOD, superoxide dismutase.

report the synthesis of a series of semicarbazones, thiocarbazones, 1,3,4-oxadiazoles, and 1,3,4-thiadiazoles bearing coumarin and pyrazole moieties and their antibacterial, antifungal, and antioxidant activities.

RESULTS AND DISCUSSION

Chemistry

The precursors, 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carboxaldehydes (**Ia**–**e**), were prepared by a procedure reported earlier [2]. Their reaction with semicarbazide hydrochloride (**II**) in the presence of sodium acetate in ethyl alcohol under reflux conditions afforded corresponding semicarbazones (**IIIa**)–(**IIIe**). The oxidative cyclisation of semicarbazones (**IIIa**–**e**) using bromine as oxidant in acetic acid at room temperature yielded corresponding 1,3,4-oxadiazoles (**IVa**)–(**IVe**) (Scheme 1).



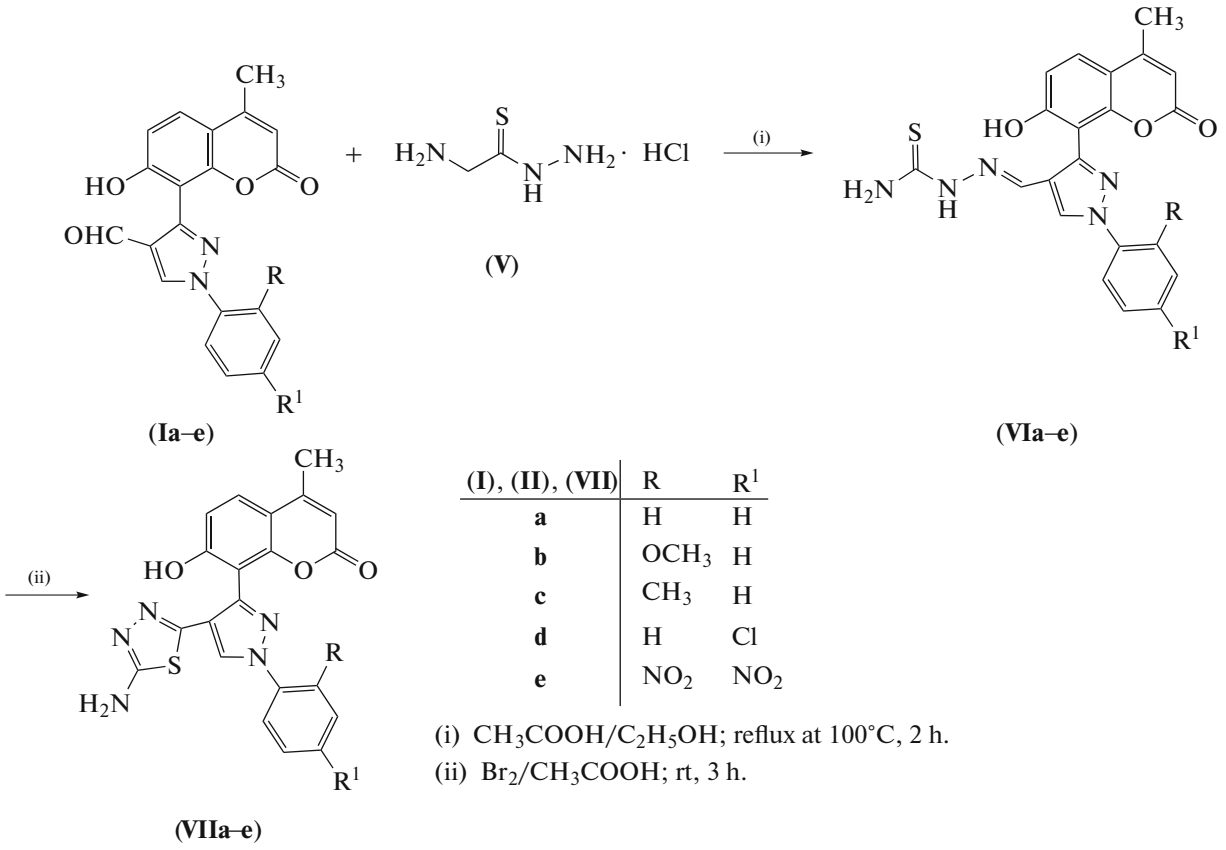
(**Ia**–**e**) (**II**) (**IIIa**–**e**) (**IVa**–**e**)

Scheme 1. Synthesis of 2-((3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-aryl-1*H*-pyrazol-4-yl)methylene)hydrazinecarboxamides (**IIIa**–**e**) and 8-(4-(5-amino-1,3,4-oxadiazolyl)-1-aryl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-ones (**IVa**–**e**).

The structures of newly synthesized compounds (**IIIa**–**e**) and (**IVa**–**e**) were confirmed by spectral and elemental analysis data. The IR spectra of (**IIIa**–**e**) and (**IVa**–**e**) showed the absorption bands of C=O, NH, and NH_2 groups in the regions 1671–1695, 3282–3380, and 3426–3575 cm^{-1}, respectively. The ^1H NMR spectra of compounds (**IIIa**–**e**) showed signals of NH_2, CONH, and CH=N protons in the regions of δ 4.49–4.81, 6.09–6.42, and 8.22–8.66 ppm. In the ^{13}C NMR spectra, the signals of $CONH_2$ and C=N carbons were observed at δ 156.44–158.52 and 142.22–143.06 ppm, respectively. In IR spectra, compounds (**IVa**–**e**) showed an absorption band at 1081–1099 cm^{-1} due to C–O str., whereas the absorption bands due to –NH and –C=N str. of the starting compounds (**IIIa**–**e**) were absent. In ^1H NMR spectra, compounds (**IVa**–**e**) showed a signal of two protons as singlet at δ 6.50–6.82 ppm due to $-NH_2$ protons. Signals of CONH proton of their precursors (**IIIa**–**e**) were absent in spectra of products (**IVa**–**e**). In ^{13}C NMR spectra, C=N carbon gave signal at δ 142.14–143.32 ppm. In their mass spectra, compounds (**IIIa**–**e**) and (**IVa**–**e**) showed signals corresponding to their molecular masses M^+ and a base

peaks for MH^+ ions with significantly stable molecular ion peaks with relative abundance ranging from 10 to 40%. All synthesized compounds showed satisfactory CHN analysis data when compared with the theatrically calculated values.

The reaction of 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carboxaldehydes (**Ia**–**e**) with thiosemicarbazide hydrochloride (**V**) in the presence of sodium acetate in ethyl alcohol under reflux conditions afforded corresponding thiosemicarbazones (**VIa**–**e**). Oxidative cyclisation of the thiosemicarbazones (**VIa**–**e**) using bromine as an oxidant in acetic acid at room temperature afforded corresponding 1,3,4-thiaoxadiazoles (**VIIa**–**e**) (Scheme 2).



(I), (II), (VII)	R	R^1
a	H	H
b	OCH_3	H
c	CH_3	H
d	H	Cl
e	NO_2	NO_2

Scheme 2. Synthesis of 2-((3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-aryl-1*H*-pyrazol-4-yl)methylene)hydrazinecarbothiomides (**VIa**–**e**) and 8-(4-(5-amino-1,3,4-thiadiazolyl)-1-aryl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-ones (**VIIa**–**e**).

The structures of compounds (**VIa**–**e**) and (**VIIa**–**e**) were confirmed by spectral and elemental analyses data. In IR spectra, these compounds showed the absorption bands of C=S, NH, and NH_2 groups in the regions 1261–1313, 3282–3380, and 3426–3575 cm^{-1}, respectively. In ^1H NMR spectra, compounds (**VIa**–**e**) showed signals of NH_2, CSNH, and CH=N protons in the regions of δ 4.49–4.78, 6.21–6.59, and 8.22–8.62 ppm. In ^{13}C NMR spectra, the signals of $CSNH_2$ and C=N carbons were observed at δ 171.20–176.52 and 142.14–143.32 ppm, respectively. In IR spectrum, compounds (**VIa**–**e**) and (**VIIa**–**e**) showed an absorption band at 1047–1060 cm^{-1} due to C–S str. The absorption bands due to –NH and –C=N str. of their precursor (**VIa**–**e**) were absent. In ^1H NMR, compounds (**VIIa**–**e**) showed a signal of two protons as singlet at δ 6.50–6.82 ppm due to $-NH_2$ protons. Signals of CSNH proton of their precursors (**VIa**–**e**) were absent in the spectra of products (**VIIa**–**e**). In ^{13}C NMR spectra, C=N carbons were deshielded due to adjacent sulphur atom and appeared at δ 165.54–175.11 ppm. In mass spectra, compounds (**VIa**–**e**) and (**VIIa**–**e**) showed signals corresponding to their molecular masses M^+ and a base peak for MH^+ ions with significantly stable molecular ion peaks with relative abundance ranging from 10 to 40%. All synthesized compounds showed satisfactory CHN analysis data when compared with theatrically calculated values.

Antimicrobial Activity

Preliminary studies on the antimicrobial activities revealed that all newly synthesized semicarbazones (**IIIa**–**e**), 1,3,4-oxadiazoles (**IVa**–**e**), thiosemicarbazones (**VIa**–**e**), and 1,3,4-thiadiazoles (**VIIa**–**e**) possess a wide range of antimicrobial activities against the tested organisms (Tables 1 and 2).

Compounds (**IIIa**) and (**VIa**) having no substituents, (**IIIb**) and (**VIb**) having methoxy substituent, and (**IIIc**) having CH_3 substituent in the aromatic ring showed moderate activity against all tested species. Compound (**VIc**) with CH_3 substituent showed greater activity against *E. coli*. Chloro-substitution in (**VId**) demonstrated excellent activity against all the organisms tested; on contrary to this, same substituent in compound (**IIId**) showed the highest activity against *E. coli* only. Compounds (**IIIa**) and (**VIa**) showed moderate activity against all the tested fungi organisms. Compounds (**IIIb**) and (**VIb**) having methoxy substituent, compounds (**IIIc**) and (**VIc**) having methyl substituent, and compounds (**IIId**) and (**VId**) having chloro-substitution showed greater extent of inhibition against *C. albicans*. An electron-withdrawing nitrosubstitution in (**IIIe**) and (**VIe**) retarded the inhibitory effect against the entire bacterial and fungal organism tested.

Among 1,3,4-oxadiazoles (**IVa**–**e**), and 1,3,4-thiadiazoles (**VIIa**–**e**) compounds (**IVa**) and (**VIIa**) with no substituents in the aromatic ring showed greater activity against *S. aureus*. Compounds (**IVb**) and (**VIIb**) with methoxy substituent and (**IVc**) with methyl substituent showed moderate activities against all tested organisms. Compounds (**VIIc**) with methyl substituent displayed sensitivity against *E. coli*; (**VIId**) with chlorosubstitution showed excellent antibacterial activity against the tested species; while (**IVd**) showed the highest activity against *E. coli*. Compounds (**IVa**), (**VIIa**), (**IVb**), and (**VIIb**) showed moderate activity against all the fugal species tested. Compounds (**IVc**), (**VIIc**) (**IVd**), and (**VIId**) displayed activity against *C. albicans*. Compounds (**IVe**) and (**VIIe**) with nitrosubstituent exhibited lower activity against all the tested bacterial and fungal organisms.

Antimicrobial activities of the synthesized compounds (**IIIa**–**e**), (**IVa**–**e**), (**VIa**–**e**), and (**VIIa**–**e**) were compared with the activities of their starting compounds 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carboxaldehydes (**Ia**–**g**) [2] reported earlier. Comparative study shows that compound (**Ia**) failed to show activity, but its derivatives (**IIIa**), (**VIa**), (**IVa**), and (**VIIa**) showed promising activity against *S. aureus*. Compared to (**Ia**), its derivatives (**IIIa**), (**IVa**), and (**VIIa**) showed improved activity against *E. coli* and *P. aeruginosa*; (**IVa**) showed lower activity against *E. coli*. Compared to (**Ib**), improved activities were observed for its derivatives (**IIIb**), (**VIb**), and (**VIIb**) against *P. aeruginosa*; and for derivatives (**VIb**) and (**VIIb**), against *S. aureus* and *E. coli*. Derivatives of (**Ic**) showed enhanced activities against all tested organisms. Among the derivatives of (**Id**) and (**Ie**), compounds (**VId**), (**VIId**), and (**IVe**) showed lower activities against all organisms and not much change in an inhibition was observed in the rest of the series. Compounds (**Ia**) and (**Ic**) have not showed activities against *A. flavus* or *C. albicans,* but their derivatives (**IIIa**), (**IIIc**), (**VIa**), (**VIc**), (**IVa**) (**IVc**), (**VIIa**), and (**VIIc**) showed promising activities against these organisms. Compound (**Ie**) was found not active against *A. niger*, *A. flavus*, or *C. albicans*; nevertheless, compounds (**IIIe**), (**VIe**), (**IVe**), and (**VIIe**) showed promising activities against these organisms. There was not much change in inhibition for compounds derived from (**Ia**) and (**Id**) against *A. flavus* and *C. albicans.*

DPPH Radical Scavenging Activity

A freshly prepared DPPH solution shows a deep purple color with an absorption maximum at 517 nm. The change of the purple color to yellow indicates the decreased in absorbance, this is due to reduction of the DPPH free radical through donation of hydrogen atom by an antioxidant molecule. Preliminary studies on the antioxidant properties of newly synthesized 1,3,4-oxadiazoles (**IVa**–**e**) and 1,3,4-thiadiazoles (**VIIa**–**e**) showed promising DPPH radical scavenging abilities (Table 3). Among the designated series, compounds (**IVd**) and (**VIId**) with chloro-substitution in the aromatic ring showed greater DPPH scavenging ability than others. Compounds (**IVe**) and (**VIIe**) with electron withdrawing nitrosubstitution showed lower activities; the rest of the series showed moderate activities.

Comparison of the antioxidant activities of the synthesized compounds (**IVa**–**e**) and (**VIIa**–**e**) with their precursors (**Ia**–**e**) were made by taking the results of 50 μg/mL concentrations. It was observed that compounds (**IVc**) and (**VIIc**) possess greater DPPH radical scavenging ability compared to (**Ic**). However, the remaining compounds of the series showed lower activities compared to the corresponding precursors.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical is a highly reactive free radical formed in biological systems and it is able to damage the biomolecules found in living cells [14]. It has the ability to break DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity. Preliminary studies indicate that synthesized 1,3,4-oxadiazoles (**IVa**–**e**) and 1,3,4-thiadiazoles (**VIIa**–**e**) possess varied hydroxyl radical scavenging activities (Table 4). Among the series, compounds (**IVd**) and (**VIId**) showed a remarkable capacity to scavenge hydroxyl radical, significantly higher than that of the standard BHA. Compounds (**IVa**), (**VIIa**), (**IVe**), and (**VIIe**) were indicative of

weak activity against this radical; the remaining compounds of the series showed moderate activities.

Molecular Docking and ADME Predictions

Molecular docking has been successfully carried out to design novel potential leads. A total of twenty validated potential leads are suggested from the in vitro studies, among which compounds (**IVd**) and (**VIId**) satisfy both the docking and ADME drug criteria. Docking results induced comparative investigation based on docking score of the hits with that of the reported inhibitors. Compounds (**IVd**) and (**VIId**) of parental nucleus imparted a specific geometrical space around the Cu–Zn binding loop. Oxidative stress induced by ROS brings about various enzymatic activities to scavenge the free radicals. Among the latter ones, activity of superoxide dismutase (SOD) gets exuberated during the process and is followed by catalase (CAT) and glutathione peroxidase (GPx) [15]. Active site of Cu,Zn-SOD has His48, His46, His61, His120, and His63 that are linked with copper and zinc ions forming imidazolate bridge; these are important residues for activity of SOD. Activity of Cu,Zn-SOD has been studied based on protein engineering to increase activity by modifying the electrostatic environment of the active site [16]. Strong binding of compound at Cu–Zn domain of SOD causes increase in antioxidant activity of SOD and decrease in oxidative stress [17].

Compound (**VIId**) showed better binding mode with Lys134, His78, and Lys67 residing at Cu-Zn loop of the SOD. Compound (**IVd**) showed binding with His61, His78, and Lys134 (Fig. 1). Compounds (**IVd**) and (**VIId**) did not show strong binding with the catalase and glutathione peroxidase (Table 5), which indicate that the activities of these compounds were not affected during the autoactivation of CAT and GPx. Compounds (**IVa**) to (**VIIc**) showed comparatively better interaction with CAT and GPx than SOD. These data suggest that compounds (**IVd**) and (**VIId**) possess better antioxidant properties than the rest of the synthesized compounds (Fig. 2).

The prediction program QikProp was used to calculate ADME properties consisting of principal descriptors and physiochemical properties. QikProp modules provide ranges of molecular predicting properties for comparing the properties of a particular molecule with those of 95% of known drugs [17]. All the ligands obey the Lipinski's rules: a molecular weight below 500 Da, hydrogen bond donor (less than five), and acceptor (less than ten). QPlogPo/w (octanol/water partition coefficient) for all the ligands is less than five [18]. All the synthesized compounds, except (**VId**), (**IIIe**), (**VIe**), (**IVe**), and (**IVe**), satisfy the values of partitioning coefficients in octanol/gas (QPlogPoct), water/gas (QPlogPw), and brain/blood (QPlogBB) systems, as well as skin permeability (QPlogKp) and aqueous solubility (QPlogS) predicted for permissible range of compounds. Qualitative Model for human oral absorption was predicted; compound (**VIId**) showed high oral absorption (Table 6).

EXPERIMENTAL

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on a Nujol mull on Shimadzu 8300 spectrophotometer (ν, cm^{-1}). The ^1H NMR and ^{13}C NMR spectra were recorded on a Spect 500 MHz and Spect 100 MHz spectrometers, respectively, using DMSO as solvent and Me_3Si as an internal standard. Chemical shifts are expressed in δ, ppm. Mass spectra were obtained on a Shimadzu LCMS-2010A spectrometer (CI). Elemental analysis of the compounds was performed on a Thermo Finnigan Flash EA 1112 CHN analyzer.

*General Procedure for the Synthesis of 2-((3-(7-Hydroxy-4-Methyl-2-Oxo-2H-Chromen-8-yl)-1-Phenyl-1H-Pyrazol-4-yl)methylene)-hydrazinecarboxamides (**IIIa–e**)*

To the solution of 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-aryl-1*H*-pyrazole-4-carboxaldehydes (**Ia–e**) (1.0 mmol) in ethyl alcohol (20 mL), a solution of semicarbazide hydrochloride (**II**) (1.0 mmol) and sodium acetate (1.0 mmol) in water was added. The mixture was refluxed on a water bath for 3–4 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into crushed ice; solid separated was filtered and recrystallized from methanol to obtain the compounds (**IIIa–e**) in good yields.

2-((3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazol-4-yl) methylene)hydrazinecarboxamide (IIIa). Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**Ia**) (1.0 mmol) and semicarbazide hydrochloride (**II**) (1.0 mmol). Yellow solid; yield: 85%, mp 160–162°C; IR: 3473 (NH, amide), 3312 (NH), 1682 (C=O, amide), 1675 (C=O, coumarin), 1571 (C=N); ^1H NMR: 2.42 (s, 3H, CH_3), 4.49 (s, 2H, NH_2), 5.35 (s, 1H, OH), 6.09 (s, 1H, CONH), 6.67 (s, 1H, H-3), 6.99 (d, 1H, H-6), 7.16-7.65 (m, 5H, Ar-H), 7.72 (d, 1H, H-5), 8.45 (s, 1H, H-5'), 8.66 (s, 1H, CH=N); ^{13}C NMR: 21.2 (1C, CH_3), 112.65 (1C, C-3), 113.64 (1C, C-4'), 114.21 (1C, C-4a), 114.53 (1C, C-6), 117.23 (1C, C-8), 119.78 (2C, C-3", C-1"), 124.25 (1C, C-5), 126.22 (1C, C-5"), 128.88 (2C, C-4", C-6"), 130.02 (1C, C-5'), 138.54 (1C, C-2"), 142.22 (1C, C=N), 146.25 (1C, C-8a), 150.02 (1C, C-3'), 152.65 (1C, C-4), 153.26 (1C, C-7), 156.44 (1C, $CONH_2$), 160.04 (1C, C=O). MS (*m/z*, %): 404 (M + 1, 100%). Anal. calcd. for $C_{21}H_{17}N_5O_4$: C, 62.73; H, 4.18; N, 17.22%. Found: C, 62.53; H, 4.25; N, 17.36%.

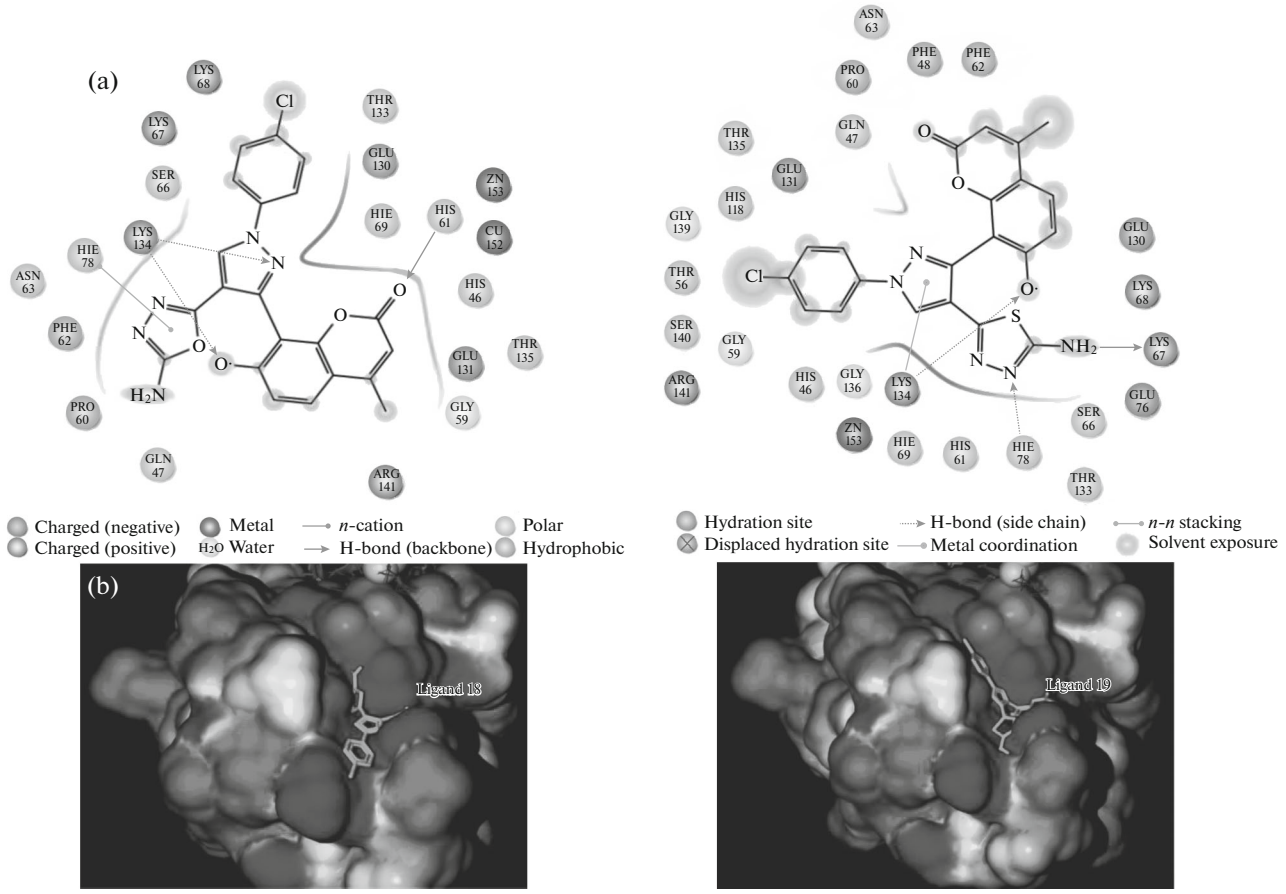


Fig. 1. (a) Molecular interactions of SOD enzyme (PDB ID: 1CB4) with compounds (**IVd**) and (**VIId**). (b) Electrostatic binding mode of compounds (**IVd**) and (**VIId**) to the active site of SOD.

2-((3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(2-methoxyphenyl)-1*H*-pyrazol-4-yl) methylene)hydrazinecarboxamide (IIIb). Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(2-methoxy-phenyl)-1*H*-pyrazole-4-carbaldehyde (**Ib**) (1.0 mmol) and semicarbazide hydrochloride (**II**) (1.0 mmol). Yellow solid; yield: 88%, mp 106–108°C; IR: 3575 (NH, amide), 3298 (NH), 1691 (C=O, amide), 1673 (C=O, coumarin), 1582 (C=N); ^{1}H NMR: 2.42 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 4.52 (s, 2H, NH_2), 5.32 (s, 1H, OH), 6.24 (s, 1H, CONH), 6.35 (s, 1H, H-3), 6.92 (d, 1H, H-6), 7.17–7.68 (m, 4H, Ar-H), 7.71 (d, 1H, H-5), 8.47 (s, 1H, H-5'), 8.63 (s, 1H, CH=N); ^{13}C NMR: 21.2 (1C, CH_3), 55.81 (1C, OCH_3), 112.50 (1C, C-3), 113.02 (1C, C-4'), 114.10 (1C, C-4a), 114.53 (1C, C-6), 114.90 (1C, C-6''), 117.43 (1C, C-8), 119.12 (1C, C-3''), 121.72 (1C, C-4''), 124.92 (1C, C-2''), 126.21 (1C, C-5), 128.64 (1C, C-5''), 130.00 (1C, C-5'), 143.76 (1C, C=N), 148.54 (1C, C-1''), 146.25 (1C, C-8a), 150.07 (1C, C-3'), 152.51 (1C, C-4), 154.31 (1C, C-7), 157.12 (1C, $CONH_2$), 160.31 (1C, C=O). MS (*m/z*, %): 434 (M + 1, 100%).

2-((3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(o-tolyl)-1*H*-pyrazol-4-yl) methylene)hydrazinecarboxamide (IIIc). Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(2-methylphenyl)-1*H*-pyrazole-4-carbaldehyde (**Ic**) (1.0 mmol) and semicarbazide hydrochloride (**II**) (1.0 mmol). Brown solid; yield: 74%, mp 176–177°C; IR: 3426 (NH, amide), 3320 (NH), 1671 (C=O, amide), 1665 (C=O, coumarin), 1621 (C=N); ^{1}H NMR: 2.02 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 4.54 (s, 2H, NH_2), 5.41 (s, 1H, OH), 6.28 (s, 1H, CONH), 6.31 (s, 1H, H-3), 6.82 (d, 1H, H-6), 7.28–7.68 (m, 4H, Ar-H), 7.72 (d, 1H, H-5), 8.40 (s, 1H, H-5'), 8.52 (s, 1H, CH=N); ^{13}C NMR: 17.54 (1C, CH_3), 21.2 (1C, CH_3), 112.05 (1C, C-3), 113.10 (1C, C-4'), 114.10 (1C, C-4a), 114.42 (1C, C-6), 116.52 (1C, C-8), 125.62 (1C, C-2''), 126.22 (1C, C-5), 127.04 (1C, C-3''), 128.52 (1C, C-4''), 130.07 (1C, C-5'), 131.05 (1C, C-5''), 132.74 (1C, C-6''), 137.34 (1C, C-1''), 143.05 (1C, C=N), 146.92 (1C, C-8a), 150.42 (1C, C-3'), 152.47 (1C, C-4), 154.48 (1C, C-7), 158.21 (1C, $CONH_2$), 160.42 (1C, C=O). MS (*m/z*, %): 418 (M + 1, 100%).

2-((1-(4-Chlorophenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazol-4-yl) methy-

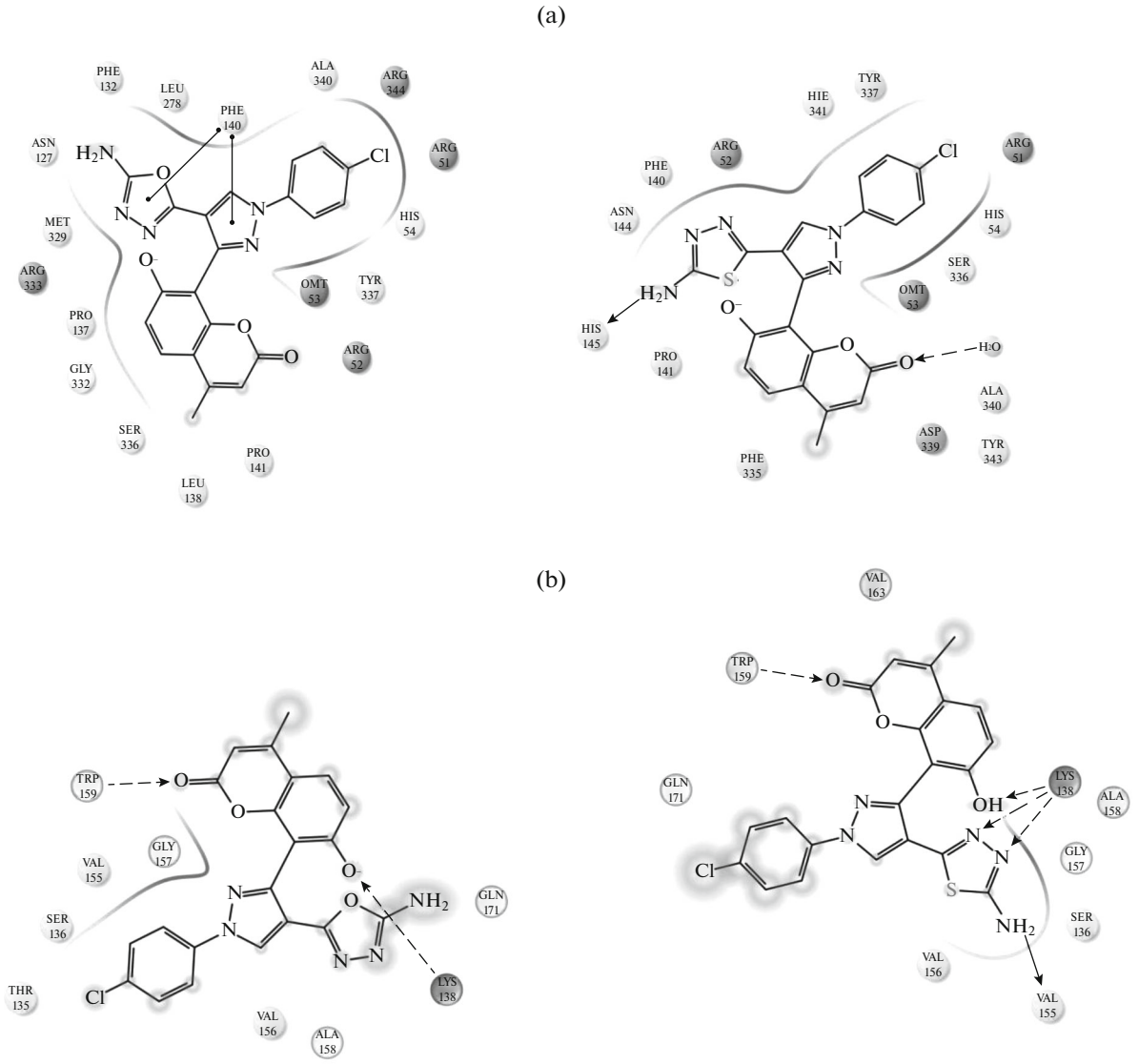


Fig. 2. Molecular interactions of CAT (PDB ID: 2CAG) (A) and GPx (PDB ID: 2P31) enzymes (B) with compounds (**IVd**) (on the left) and (**VIId**) (on the right).

lene)hydrazinecarboxamide (IIId). Obtained from 1-(4-chloro-phenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehyde (**Id**) (1.0 mmol) and semicarbazide hydrochloride (**II**) (1.0 mmol). Yellow solid; yield: 86%, mp 158–160°C; IR: 3501 (NH, amide), 3323 (NH), 1685 (C=O, amide), 1669 (C=O, coumarin), 1499 (C=N); ^{1}H NMR: 2.38 (s, 3H, CH_3), 4.67 (s, 2H, NH_2), 5.38 (s, 1H, OH), 6.42 (s, 1H, CONH), 6.51 (s, 1H, H-3), 6.84 (d, 1H, H-6), 7.18 (dd, 2H, Ar-H), 7.69 (dd, 2H, Ar-H), 7.72 (d, 1H, H-5), 8.24 (s, 1H, CH=N), 8.60 (s, 1H, H-5'); ^{13}C NMR: 21.3 (1C, CH_3), 112.02 (1C, C-3), 113.09 (1C, C-4'), 114.12 (1C, C-4a), 114.52 (1C, C-6), 116.51 (1C, C-8), 119.82 (2C, C-2", C-6"), 126.21 (1C, C-5), 129.41 (2C, C-3", C-5"), 130.02 (1C, C-5'), 131.62 (1C, C-4"), 139.02 (1C, C-1"), 143.06 (1C, C=N), 146.83 (1C, C-8a), 150.21 (1C, C-3'), 152.72 (1C, C-4), 154.61 (1C, C-7), 158.52 (1C, $CONH_2$), 160.39 (1C, C=O). MS (*m/z*, %): 439 (M + 2, ^{37}Cl, 33%), 437 (M^+, ^{35}Cl, 100%). Anal. calcd. for $C_{21}H_{16}ClN_5O_4$: C, 57.61; H, 3.68; N, 16.00%. Found: C, 58.11; H, 3.51; N, 16.12%.

2-((1-(2,4-Dinitrophenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazol-4-yl) methylene)hydrazinecarboxamide (IIIe). Obtained from 1-(2,4-dinitro-phenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehyde (**Ie**) (1.0 mmol) and semicarbazide hydrochloride (**II**) (1.0 mmol). Brown solid; yield: 72%, mp 192–193°C; IR: 3548 (NH, amide), 3291 (NH), 1686 (C=O, amide), 1672 (C=O, coumarin), 1481 (C=N); ^{1}H NMR: 2.42 (s, 3H, CH_3), 4.81 (s, 2H, NH_2), 5.32

(s, 1H, OH), 6.41 (s, 1H, CONH), 6.52 (s, 1H, H-3), 6.81 (d, 1H, H-6), 7.72 (d, 1H, H-5), 8.12 (d, 1H, Ar-H), 8.22 (s, 1H, CH=N), 8.40 (s, 1H, H-5'), 8.76 (d, 1H, Ar-H), 8.88 (s, 1H, Ar-H); MS (*m/z*, %): 493 (M^+, 100%). Anal. calcd. for $C_{21}H_{15}N_7O_8$: C, 51.12; H, 3.06; N, 19.87%. Found: C, 51.28; H, 3.21; N, 19.94%.

*General Procedure for the Preparation of 8-(4-(5-Amino-1,3,4-Oxadiazol-2-cyl)-1-Aryl-1H-Pyrazol-3-yl)-7-Hydroxy-4-Methyl-2H-Chromen-2-one (**IVa**–**e**)*

A solution of semicarbazones (**IIIa**–**e**) (1.0 mmol) in glacial acetic acid (10 mL) was introduced into a round bottom flask equipped with a separating funnel. To this, a solution of bromine (0.7 mL) in glacial acetic acid (5 mL) was added drop-wise through a separating funnel with stirring. The mixture was stirred at room temperature for 3–4 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solution was poured on crushed ice. The solid separated was filtered and washed thoroughly with water to obtain the crude products. The compounds were purified by passing through a column of silica gel (60–120 mesh) using ethyl acetate and hexane (1 : 4 v/v) as eluent to obtain the products (**IVa**–**e**) in good yields.

8-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IVa). Obtained from (**IIIa**) (1.0 mmol). Brown solid; yield: 72%, mp 121–123°C; IR: 3275 (NH_2), 1090 (C–O); H^1 NMR: 2.32 (s, 3H, CH_3), 5.32 (s, 1H, OH), 6.39 (s, 1H, H-3), 6.82 (s, 2H, NH_2), 7.98 (d, 1H, H-6), 7.35 (d, 1H, H-5), 7.7–8.0 (m, 5H, Ar-H), 8.34 (s, 1H, H-5'); ^{13}C NMR: 20.10 (1C, CH_3), 107.45 (1C, C-4'), 112.42 (1C, C-3), 114.01 (1C, C-4a), 114.56 (1C, C-6), 116.04 (1C, C-8), 119.31 (1C, C-5'), 120.22 (2C, C-2", C-6"), 125.7 (1C, C-5), 126.52 (1C, C-4"), 129.52 (2C, C-3", C-5"), 143.10 (1C, C-1"), 144.32 (1C, C-3'), 147.42 (1C, C-8a), 152.13 (1C, C-4), 154.54 (1C, C-7), 160.12 (1C, C=O), 165.21 (1C, C-2'"), 170.52 (1C, C-5'"). MS (*m/z*, %): 402 (M + 1, 58%), 355 (47%), 233 (100%), 149 (22%), 111 (12%). Anal. calcd. for $C_{21}H_{15}N_5O_4$: C, 62.84; H, 3.77; N, 17.45%. Found: C, 62.71; H, 3.23; N, 17.35%.

8-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-(2-methoxyphenyl)-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IVb). Obtained from (**IIIb**) (1.0 mmol) as brown solid; yield: 67%, mp 163–165°C; IR: 3241 (NH_2), 1088 (C–O); H^1 NMR: 2.54 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 5.46 (s, 1H, OH), 6.59 (s, 2H, NH_2), 7.29 (s, 1H, H-3), 7.46 (d, 1H, H-6), 7.46 (d, 1H, H-5), 7.72–8.0 (m, 4H, Ar-H), 8.42 (s, 1H, H-5'); ^{13}C NMR: 19.23 (1C, CH_3), 55.65 (1C, OCH_3), 107.33 (1C, C-4'), 112.50 (1C, C-3), 114.12 (1C, C-4a), 114.42 (1C, C-6), 114.91 (1C, C-5"), 116.25 (1C, C-8), 118.54 (1C, C-2"), 119.43 (1C, C-5'), 121.27 (1C, C-3"), 125.21 (1C, C-1"), 126.23 (1C, C-5), 127.31 (1C, C-4"), 144.08 (1C, C-6"), 145.54 (1C, C-3'), 147.42 (1C, C-8a), 152.34 (1C, C-4), 154.63 (1C, C-7), 160.65 (1C, C=O), 163.54 (1C, C-2'"), 168.93 (1C, C-5'"). MS (*m/z*, %): 432 (M + 1, 67%), 356 (43%), 233 (100%), 156 (31%), 142 (13%).

8-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-*o*-tolyl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IVc). Obtained from (**IIIc**) (1.0 mmol) as a light yellow solid; yield: 66%, mp 168–170°C; IR: 3308 (NH_2), 1095 (C–O); H^1 NMR: 2.32 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 5.02 (s, 1H, OH), 6.78 (s, 2H, NH_2), 7.25 (s, 1H, H-3), 7.46 (d, 1H, H-6), 7.50 (d, 1H, H-6), 7.65–8.42 (m, 4H, Ar-H), 8.55 (s, 1H, H-5'); ^{13}C NMR: 19.65 (1C, CH_3), 108.04 (1C, C-4'), 112.50 (1C, C-3), 114.12 (1C, C-4a), 114.42 (1C, C-6), 116.42 (1C, C-8), 119.43 (1C, C-5'), 125.43 (1C, C-2"), 126.23 (1C, C-5), 126.34 (1C, C-3"), 128.75 (1C, C-4"), 132.23 (1C, C-5"), 134.23 (1C, C-6"), 138.43 (1C, C-1"), 145.65 (1C, C-3'), 147.65 (1C, C-8a), 152.34 (1C, C-4), 154.63 (1C, C-7), 160.72 (1C, C=O), 163.54 (1C, C-2'"), 165.54 (1C, C-5'"). MS (*m/z*, %): 416 (M + 1, 68%), 343 (51%), 233 (100%), 221 (42%), 145 (21%), 110 (11%). Anal. calcd. for $C_{22}H_{17}N_5O_4$: C, 63.61; H, 4.12; N, 16.86%. Found: C, 63.21; H, 4.54; N, 16.75%.

8-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-(4-chlorophenyl)-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IVd). Obtained from (**IIId**) (1.0 mmol) as light yellow solid; yield: 61%, mp 182–184°C; IR: 3279 (NH_2), 1099 (C–O); H^1 NMR: 2.34 (s, 3H, CH_3), 5.34 (s, 1H, OH), 6.66 (s, 2H, NH_2), 6.74 (s, 1H, H-3), 7.23 (d, 1H, H-6), 7.42 (d, 1H, H-5), 7.62 (dd, 2H, Ar-H), 7.91 (dd, 2H, Ar-H), 8.44 (s, 1H, H-5'); ^{13}C NMR: 19.43 (1C, CH_3), 107.44 (1C, C-4'), 112.50 (1C, C-3), 114.16 (1C, C-4a), 114.48 (1C, C-6), 116.50 (1C, C-8), 119.32 (1C, C-5'), 119.82 (2C, C-2", C-6"), 126.23 (1C, C-5), 129.40 (2C, C-3", C-5"), 131.81 (1C, C-4"), 137.43 (1C, C-1"), 145.25 (1C, C-3'), 147.45 (1C, C-8a), 152.70 (1C, C-4), 154.76 (1C, C-7), 160.80 (1C, C=O), 163.43 (1C, C-2'"), 169.23 (1C, C-5'"). MS (*m/z*, %): 437 (M + 2, ^{37}Cl, 33%), 435 (M^+, ^{35}Cl, 100%), 341 (61%), 233 (94%), 101 (17%). Anal. calcd. for $C_{21}H_{14}ClN_5O_4$: C, 57.87; H, 3.24; N, 16.07%. Found: C, 57.78; H, 3.32; N, 16.31%.

8-(4-(5-Amino-1,3,4-oxadiazol-2-yl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IVe). Obtained from (**IIIe**) (1.0 mmol) as brown solid; yield: 72%, mp 176–178°C; IR: 3288 (NH_2), 1081 (C–O); H^1 NMR: 2.34 (s, 3H, CH_3), 5.42 (s, 1H, OH), 6.62 (s, 2H, NH_2), 6.78 (s, 1H, H-3), 7.24 (d, 1H, H-6), 7.53 (d, 1H, H-5), 7.64 (d, 1H, Ar-H), 7.89 (d, 1H, Ar-H), 8.26 (s, 1H, Ar-H), 8.44 (s, 1H, H-5'). MS (*m/z*, %): 492 (M + 1,

65%), 301 (51%), 233 (100%), 165 (31%), 131 (20%), 105 (5%). Anal. calcd. for $C_{21}H_{13}N_7O_8$: C, 51.33; H, 2.67; N, 19.95%. Found: C, 51.45; H, 2.76; N, 19.89%.

*General Procedure for the Synthesis of 2-((3-(7-Hydroxy-4-Methyl-2-Oxo-2H-Chromen-8-yl)-1-Phenyl-1H-Pyrazol-4-yl)methylene)hydrazinecarbothioamides (**VIa–e**)*

To the solution of 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-aryl-1*H*-pyrazole-4-carboxaldehydes (**Ia–e**) (1.0 mmol) in ethyl alcohol (20 mL), a solution of thiosemicarbazide hydrochloride (**V**) (1.0 mmol) and sodium acetate (1.0 mmol) in water was added. The mixture was refluxed on a water bath for 3–4 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into crushed ice; solid separated was filtered and recrystallized from methanol to obtain the compounds (**VIa–e**) in good yields.

2-((3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazol-4-yl) methylene)hydrazinecarbothioamide (VIa). Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**Ia**) (1.0 mmol) and thiosemicarbazide hydrochloride (**V**) (1.0 mmol). Yellow solid; yield: 84%, mp 168–170°C; IR: 3490 (NH, amide), 3305 (NH), 1678 (C=O, coumarin), 1575 (C=N), 1276 (C=S str, amide); ^{1}H NMR: 2.46 (s, 3H, CH_3), 4.51 (s, 2H, NH_2), 5.68 (s, 1H, OH), 6.21 (s, 1H, CSNH), 6.64 (s, 1H, H-3), 7.12 (d, 1H, H-6), 7.16–7.67 (m, 5H, Ar-H), 7.69 (d, 1H, H-5), 8.44 (s, 1H, H-5'), 8.62 (s, 1H, CH=N); ^{13}C NMR: 19.23 (1C, CH_3), 112.16 (1C, C-3), 113.17 (1C, C-4'), 114.12 (1C, C-4a), 114.42 (1C, C-6), 116.52 (1C, C-8), 119.34 (2C, C-3", C-1"), 125.21 (1C, C-5"), 126.34 (1C, C-5), 128.54 (2C, C-4", C-6"), 130.02 (1C, C-5'), 138.42 (1C, C-2"), 143.43 (1C, C=N), 147.42 (1C, C-8a), 150.42 (1C, C-3'), 152.54 (1C, C-4), 154.65 (1C, C-7), 160.60 (1C, C=O), 176.55 (1C, $CSNH_2$). MS (*m/z*, %): 420 (M + 1, 100%). Anal. calcd. for $C_{21}H_{17}N_5O_3S$: C, 60.13; H, 4.09; N, 16.70% %. Found: C, 60.04; H, 4.12; N, 16.87%.

2-((3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(2-methoxyphenyl)-1*H*-pyrazol-4-yl) methylene)hydrazinecarbothioamide (VIb). Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(2-methoxy-phenyl)-1*H*-pyrazole-4-carbaldehyde (**Ib**) (1.0 mmol) and thiosemicarbazide hydrochloride (**V**) (1.0 mmol). Yellow solid; yield: 83%, mp 148–150°C; IR: 3461 (NH, amide), 3282 (NH), 1669 (C=O, coumarin), 1618 (C=N), 1281 (C=S, amide); ^{1}H NMR: 2.44 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 4.49 (s, 2H, NH_2), 5.38 (s, 1H, OH), 6.25 (s, 1H, CSNH), 6.37 (s, 1H, H-3), 6.84 (d, 1H, H-6), 7.14–7.62 (m, 4H, Ar-H), 7.68 (d, 1H, H-5), 8.42 (s, 1H, H-5'), 8.60 (s, 1H, CH=N); ^{13}C NMR: 21.3 (1C, CH_3), 55.23 (1C, OCH_3), 112.12 (1C, C-3), 113.17 (1C, C-4'), 114.17 (1C, C-4a), 114.45 (1C, C-6), 114.78 (1C, C-6"), 116.67 (1C, C-8), 118.80 (1C, C-3"), 122.32 (1C, C-4"), 125.02 (1C, C-2"), 126.32 (1C, C-5), 127.22 (1C, C-5"), 130.05 (1C, C-5'), 142.14 (1C, C=N), 144.72 (1C, C-1"), 147.43 (1C, C-8a), 150.42 (1C, C-3'), 152.72 (1C, C-4), 154.42 (1C, C-7), 160.25 (1C, C=O), 174.13(1C, $CSNH_2$). MS (*m/z*, %): 450 (M + 1, 100%). Anal. calcd. for $C_{22}H_{19}N_5O_4S$: C, 58.79; H, 4.26; N, 15.58%. Found: C, 58.91; H, 4.46; N, 16.04%.

(2-((3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(o-tolyl)-1*H*-pyrazol-4-yl) methylene)hydrazinecarbothioamide (VIc). Obtained from 3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(2-methylphenyl)-1*H*-pyrazole-4-carbaldehyde (**Ic**) (1.0 mmol) and thiosemicarbazide hydrochloride (**V**) (1.0 mmol). Brown solid; yield: 78%, mp 152–154°C; IR: 3472 (NH, amide), 3280 (NH), 1664 (C=O, coumarin), 1569 (C=N), 1313 (C=S, amide); ^{1}H NMR: δ 2.12 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 4.73 (s, 2H, NH_2), 5.41 (s, 1H, OH), 6.28 (s, 1H, CSNH), 6.33 (s, 1H, H-3), 6.82 (d, 1H, H-6), 7.18–7.72 (m, 4H, Ar-H), 7.79 (d, 1H, H-5), 8.42 (s, 1H, H-5'), 8.55 (s, 1H, CH=N); ^{13}C NMR: δ 18.91 (1C, CH_3), 21.6 (1C, CH_3), 112.05 (1C, C-3), 113.16 (1C, C-4'), 114.13 (1C, C-4a), 114.48 (1C, C-6), 116.57 (1C, C-8), 125.73 (1C, C-3"), 126.35 (1C, C-5), 127.4 (1C, C-4"), 128.62 (1C, C-5"), 130.08 (1C, C-5'), 131.42 (1C, C-6"), 133.14 (1C, C-1"), 138.12 (1C, C-2"), 143.24 (1C, C=N), 147.51 (1C, C-8a), 150.55 (1C, C-3'), 152.62 (1C, C-4), 154.67 (1C, C-7), 160.73 (1C, C=O), 171.20 (1C, $CSNH_2$). MS (*m/z*, %): 434 (M+1, 100%).

2-((1-(4-Chlorophenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazol-4-yl) methylene)hydrazinecarbothioamide (VId). Obtained from 1-(4-chloro-phenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehyde (**Id**) (1.0 mmol) and thiosemicarbazide hydrochloride (**V**) (1.0 mmol). Yellow solid; yield: 82%, mp 155–156°C; IR: 3541 (NH, amide), 3338 (NH), 1691 (C=O, coumarin), 1564 (C=N), 1293 (C=S, amide); ^{1}H NMR: 2.41 (s, 3H, CH_3), 4.78 (s, 2H, NH_2), 5.23 (s, 1H, OH), 6.32 (s, 1H, CSNH), 6.42 (s, 1H, H-3), 6.76 (d, 1H, H-6), 7.12 (dd, 2H, Ar-H), 7.74 (dd, 2H, Ar-H), 7.79 (d, 1H, H-5), 8.27 (s, 1H, CH=N), 8.44 (s, 1H, H-5'); ^{13}C NMR: 21.21 (1C, CH_3), 112.51 (1C, C-3), 113.02 (1C, C-4'), 114.10 (1C, C-4a), 114.41 (1C, C-6), 116.87 (1C, C-8), 119.61 (2C, C-3", C-1"), 126.32 (1C, C-5), 129.92 (2C, C-4", C-6"), 130.24 (1C, C-5'), 131.80 (1C, C-5"), 138.21 (1C, C-2"), 143.30 (1C, C=N), 147.52 (1C, C-8a), 150.32 (1C, C-3'), 152.70 (1C, C-4), 154.77 (1C, C-7), 160.69 (1C, C=O), 179.02 (1C, $CSNH_2$). MS (*m/z*, %): 455 (M + 2, ^{37}Cl, 35%), 453 (M^+, ^{35}Cl, 100%).

2-((1-(2,4-Dinitrophenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazol-4-yl) methylene)hydrazinecarbothioamide (VIe). Obtained from 1-(2,4-dinitro-phenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehyde (**Ie**) (1.0 mmol) and semicarbazide hydrochloride (**II**) (1.0 mmol). Dark brown solid; yield: 70%, mp 212–214°C; IR: 3488 (NH, amide), 3344 (NH), 1695 (C=O, coumarin), 1579 (C=N), 1261 (C=S, amide); ^{1}H NMR: 2.46 (s, 3H, CH_3), 4.52 (s, 2H, NH_2), 5.34 (s, 1H, OH), 6.59 (s, 1H, CSNH), 6.63 (s, 1H, H-3), 6.92 (d, 1H, H-6), 7.66 (d, 1H, H-5), 8.16 (d, 1H, Ar-H), 8.22 (s, 1H, CH=N), 8.44 (s, 1H, H-5'), 8.74 (d, 1H, Ar-H), 8.86 (s, 1H, Ar-H); ^{13}C NMR: 20.4 (1C, CH_3), 112.21 (1C, C-3), 113.09 (1C, C-4'), 114.04 (1C, C-4a), 114.51 (1C, C-6), 116.52 (1C, C-8), 120.52 (1C, C-6''), 124.62 (1C, C-3''), 126.23 (1C, C-5), 127.64 (1C, C-4''), 130.31 (1C, C-5'), 135.93 (1C, C-2''), 142.12 (1C, C-1''), 143.32 (1C, C=N), 145.46 (1C, C-5''), 147.42 (1C, C-8a), 150.44 (1C, C-3'), 152.62 (1C, C-4), 154.71 (1C, C-7), 160.81 (1C, C=O), 176.52 (1C, $CSNH_2$). MS (*m/z*, %): 509 (M^+, 100%).

*General Procedure for the Preparation of 8-(4-(5-Amino-1,3,4-Thiadiazol-2-yl)-1-Aryl-1H-Pyrazol-3-yl)-7-Hydroxy-4-Methyl-2H-Chromen-2-ones (**VIIa–e**)*

A solution of semicarbazones (**VIa–e**) (1.0 mmol) in glacial acetic acid (10 mL) was introduced into a round bottom flask equipped with a separating funnel. To this, a solution of bromine (0.7 mL) in glacial acetic acid (5 mL) was added drop-wise through a separating funnel with stirring. The mixture was stirred at room temperature for 3–4 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solution was poured on crushed ice. The solid separated was filtered and washed thoroughly with water to obtain the crude products. The compounds were purified by passing through a column of silica gel (60–120 mesh) using ethyl acetate and hexane (1 : 4 v/v) as eluent to obtain the products (**VIIa–e**) in good yields.

8-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (VIIa). Obtained from (**VIa**) (1.0 mmol) as light yellow solid; yield: 70%, mp 132–134°C; IR: 3258 (NH_2), 1055 (C–S); H^1 NMR: 2.35 (s, 3H, CH_3), 5.86 (s, 1H, OH), 6.50 (s, 2H, NH_2), 7.27 (s, 1H, H-3), 7.34 (d, 1H, H-6), 7.35 (d, 1H, H-5), 7.6–8.2 (m, 5H, Ar-H), 8.54 (s, 1H, H-5'); ^{13}C NMR: 20.14 (1C, CH_3), 104.22 (1C, C-4'), 112.42 (1C, C-3), 114.12 (1C, C-4a), 114.48 (1C, C-6), 116.25 (1C, C-8), 119.47 (2C, C-2'', C-6''), 126.23 (1C, C-5), 126.82 (1C, C-4''), 129.12 (2C, C-3'', C-5''), 131.23 (1C, C-5'), 143.45 (1C, C-1''), 145.04 (1C, C-3'), 147.42 (1C, C-8a), 152.13 (1C, C-4), 154.54 (1C, C-7), 160.42 (1C, C=O), 162.21 (1C, C-5'''), 172.54 (1C, C-2'''). MS (*m/z*, %): 418 (M + 1, 78%), 343 (84%), 233 (100%), 132 (53%), 121 (34%), 111 (22%). Anal. calcd. for $C_{21}H_{15}N_5O_3S$: C, 60.42; H, 3.62; N, 16.78%. Found: C, 60.11; H, 3.14; N, 16.87%.

8-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-(2-methoxyphenyl)-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (VIIb). Obtained from (**VIb**) (1.0 mmol) as a light yellow solid; yield 66%, mp 202–204°C; IR: 3312 (NH_2), 1058 (C–S); H^1 NMR: 2.32 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 5.32 (s, 1H, OH), 6.84 (s, 2H, NH_2), 7.18 (s, 1H, H-3), 7.24 (d, 1H, H-6), 7.32 (d, 1H, H-5), 7.46–7.98 (m, 4H, Ar-H), 8.51 (s, 1H, H-5'); ^{13}C NMR: 19.23 (1C, CH_3), 55.72 (1C, OCH_3), 104.11 (1C, C-4'), 112.50 (1C, C-3), 114.12 (1C, C-4a), 114.42 (1C, C-6), 114.91 (1C, C-5''), 116.32 (1C, C-8), 118.65 (1C, C-2''), 120.54 (1C, C-3''), 124.86 (1C, C-1''), 126.21 (1C, C-5), 127.31 (1C, C-4''), 144.08 (1C, C-6''), 145.54 (1C, C-3'), 147.42 (1C, C-8a), 152.34 (1C, C-4), 154.32 (1C, C-7), 160.65 (1C, C=O), 162.12 (1C, C-5'''), 173.76 (1C, C-2'''). MS (*m/z*, %): 448 (M + 1, 65%), 341 (45%), 326 (14%), 243 (12%), 177 (100%). Anal. calcd. for $C_{22}H_{17}N_5O_3S$: C, 61.24; H, 3.97; N, 16.23%. Found: C, 61.12; H, 3.54; N, 16.45%.

8-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-o-tolyl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H* chromen-2-one (VIIc). Obtained from (**VIc**) (1.0 mmol) as a light yellow solid; yield: 63%, mp 180–182°C; IR: 3268 (NH_2), 1047 (C–S); H^1 NMR: 2.21 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 5.52 (s, 1H, OH), 6.68 (s, 2H, NH_2), 7.16 (s, 1H, H-3), 7.34 (d, 1H, H-6); 7.52 (d, 1H, H-5), 7.66–8.24 (m, 4H, Ar-H), 8.30 (s, 1H, H-5'); ^{13}C NMR: 19.62 (1C, CH_3), 104.12 (1C, C-4'), 112.42 (1C, C-3), 114.16 (1C, C-4a), 114.48 (1C, C-6), 116.50 (1C, C-8), 125.55 (1C, C-2''), 126.23 (1C, C-5), 126.34 (1C, C-3''), 128.82 (1C, C-4''), 131.62 (1C, C-5''), 132.23 (1C, C-5'), 133.22 (1C, C-6''), 138.43 (1C, C-1''), 145.65 (1C, C-3'), 147.65 (1C, C-8a), 152.65 (1C, C-4), 154.76 (1C, C-7), 160.82 (1C, C=O), 162.33 (1C, C-5'''), 175.11 (1C, C-2'''). MS (*m/z*, %): 432 (M + 1, 100%), 342 (22%), 326 (45%), 243 (31%), 177 (34%).

8-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-(4-chlorophenyl)-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (VIId). Obtained from (**VId**) (1.0 mmol) as yellow solid; yield: 67%, mp 196–198°C; IR: 3281 (NH_2), 1060 (C–S); H^1 NMR: 2.32 (s, 3H, CH_3), 5.64 (s, 1H, OH), 6.61 (s, 2H, NH_2), 6.70 (s, 1H, H-3), 7.36 (d, 1H, H-6), 7.48 (d, 1H, H-5), 7.64 (dd, 2H, Ar-H), 8.04 (dd, 2H, Ar-H), 8.32 (s, 1H, Ar-H); ^{13}C NMR: 19.93 (1C, CH_3), 104.12 (1C, C-4'), 112.50 (1C, C-3), 114.12 (1C, C-4a), 114.43 (1C, C-6), 116.50 (1C, C-8), 119.80 (2C, C-2'', C-6''), 126.20 (1C, C-5), 129.40 (2C, C-3'', C-5''), 130.24 (1C, C-5'), 131.81 (1C, C-4''), 137.63 (1C, C-1''), 145.53 (1C, C-3'), 147.40 (1C, C-8a), 152.70 (1C, C-4), 154.82

Table 1. MIC of the synthesized compounds (**IIIa**–**e**) and (**VIa**–**e**) against bacterial and fungal species

Compound	Minimum inhibitory concentration (MIC), μg/mL*					
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Aspergillus flavus*	*Candida albicans*
(IIIa)	25 ± 0.50	25 ± 0.40	25 ± 0.45	25 ± 0.26	50 ± 0.30	50 ± 0.47
(IIIb)	25 ± 0.65	25 ± 0.53	25 ± 0.40	25 ± 0.55	50 ± 0.45	25 ± 0.40
(IIIc)	25 ± 0.40	25 ± 0.60	25 ± 0.23	25 ± 0.29	25 ± 0.45	25 ± 0.40
(IIId)	25 ± 0.21	12.5 ± 0.78	12.5 ± 0.70	50 ± 0.35	25 ± 0.78	12.5 ± 0.53
(IIIe)	50 ± 0.56	75 ± 0.26	100 ± 0.55	25 ± 0.25	75 ± 0.51	75 ± 0.55
(VIa)	50 ± 0.35	50 ± 0.56	12.5 ± 0.53	25 ± 0.32	50 ± 0.23	50 ± 0.47
(VIb)	50 ± 0.45	25 ± 0.30	12.5 ± 0.62	50 ± 0.61	50 ± 0.40	25 ± 0.21
(VIc)	50 ± 0.55	12.5 ± 0.56	12.5 ± 0.75	25 ± 0.32	50 ± 0.46	12.5 ± 0.40
(VId)	12.5 ± 0.55	12.5 ± 0.65	12.5 ± 0.30	50 ± 0.55	25 ± 0.11	12.5 ± 0.2
(VIe)	50 ± 0.29	100 ± 0.70	100 ± 0.40	25 ± 0.45	75 ± 0.70	100 ± 0.25
Ciprofloxacin	25 ± 0.95	25 ± 0.66	12.5 ± 0.78	–	–	–
Fluconazole	–	–	–	25 ± 0.61	25 ± 0.56	50 ± 0.96

* Values are mean of three determinations ($n = 3$) ± SD.

Table 2. MIC of the test compounds (**IVa**–**e**) and (**VIIa**–**e**) against bacterial and fungal species

Compound	Minimum inhibitory concentration (MIC), μg/mL*					
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Aspergillus flavus*	*Candida albicans*
(IVa)	12.5 ± 0.34	50 ± 0.65	25 ± 0.76	25 ± 0.45	25 ± 0.85	50 ± 0.23
(IVb)	25 ± 0.76	25 ± 0.34	12.5 ± 0.40	25 ± 0.74	75 ± 0.65	50 ± 0.34
(IVc)	25 ± 0.87	25 ± 0.54	25 ± 0.61	50 ± 0.65	25 ± 0.87	25 ± 0.45
(IVd)	25 ± 0.78	12.5 ± 0.55	12.5 ± 0.67	25 ± 0.48	50 ± 0.76	12.5 ± 0.43
(IVe)	25 ± 0.67	75 ± 0.34	100 ± 0.85	75 ± 0.31	100 ± 0.65	50 ± 0.42
(VIIa)	12.5 ± 0.12	50 ± 0.54	50 ± 0.45	25 ± 0.31	50 ± 0.53	50 ± 0.15
(VIIb)	50 ± 0.91	25 ± 0.97	12.5 ± 0.54	25 ± 0.51	75 ± 0.42	50 ± 0.18
(VIIc)	25 ± 0.43	12.5 ± 0.37	12.5 ± 0.22	50 ± 0.43	25 ± 0.66	25 ± 0.46
(VIId)	12.5 ± 0.77	12.5 ± 0.54	12.5 ± 0.18	25 ± 0.65	50 ± 0.32	12.5 ± 0.44
(VIIe)	25 ± 0.97	100 ± 0.51	75 ± 0.42	100 ± 0.22	50 ± 0.85	50 ± 0.63
Ciprofloxacin	25 ± 0.65	25 ± 0.52	12.5 ± 0.76	–	–	–
Fluconazole	–	–	–	25 ± 0.77	25 ± 0.14	50 ± 0.29

* Values are mean of three determinations ($n = 3$) ± SD.

(1C, C-7), 160.80 (1C, C=O), 162.04 (1C, C-5'''), 174.06 (1C, C-2'''). MS (*m/z*, %): 453 (M^+, ^{37}Cl, 37%), 451 (M^+, ^{35}Cl, 100%), 298 (54%), 233 (92%), 133 (24%). Anal. calcd. for $C_{21}H_{14}ClN_5O_3S$: C, 55.82; H, 3.12; N, 15.50%. Found: C, 55.72; H, 3.32; N, 15.61%.

8-(4-(5-Amino-1,3,4-thiadiazol-2-yl)-1-(2,4-dinitrophenyl)-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (VIIe). Obtained from (**VIe**) (0.5 mmol) as brown solid; yield: 76%, mp 196–198°C; IR: 3273 (NH_2), 1052 (C–S); H^1 NMR: 2.32 (s, 3H, CH_3), 5.66 (s, 1H, OH), 6.60 (s, 2H, NH_2), 6.78 (s, 1H, H-3), 7.24 (d, 1H, H-6), 7.53 (d, 1H, H-5), 7.64 (d, 1H, Ar-H), 7.89 (d, 1H, Ar-H), 8.26 (s, 1H, Ar-H), 8.44 (s, 1H, H-5'); ^{13}C NMR: 20.64 (1C, CH_3), 103.62 (1C, C-4'), 112.50 (1C, C-3), 114.10 (1C, C-4a), 114.41 (1C, C-6), 116.21 (1C, C-8), 120.59 (1C, C-5''), 124.24 (1C, C-2''), 126.20 (1C, C-5), 127.43 (1C, C-2''), 132.12 (1C, C-5'), 135.12 (1C, C-1''), 144.05 (1C, C-6''), 145.61 (1C, C-3'), 146.21 (1C, C-4''), 147.43 (1C, C-8a), 152.70 (1C, C-4), 154.86 (1C, C-7), 160.83 (1C, C=O), 163.21 (1C, C-5'''), 173.94 (1C, C-2'''). MS (*m/z*, %): 508 (M + 1, 100%), 342 (65%), 243 (34%), 177 (12%).

Table 3. Antioxidant activity of compounds (**IVa**–**e**) and (**VIIa**–**e**) in DPPH method

Compound	Radical scavenging activity, %			
	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL
(IVa)	25.11 ± 0.70	34.60 ± 0.51	37.49 ± 1.72	49.39 ± 0.17
(IVb)	28.71 ± 0.22	37.42 ± 0.32	42.25 ± 0.54	49.89 ± 0.45
(IVc)	24.86 ± 0.26	34.44 ± 0.46	39.14 ± 0.25	47.68 ± 0.54
(IVd)	33.19 ± 0.17	43.02 ± 0.35	48.96 ± 0.41	53.45 ± 0.97
(IVe)	20.70 ± 0.42	30.49 ± 0.45	35.13 ± 0.29	46.95 ± 0.85
(VIIa)	26.35 ± 1.48	36.02 ± 0.37	39.41 ± 0.37	50.52 ± 0.46
(VIIb)	29.52 ± 0.31	37.47 ± 0.20	43.53 ± 0.14	51.02 ± 0.13
(VIIc)	25.80 ± 0.38	35.73 ± 0.29	40.49 ± 0.18	48.34 ± 0.12
(VIId)	35.24 ± 0.78	46.10 ± 0.86	51.05 ± 0.40	54.99 ± 0.80
(VIIe)	23.21 ± 0.65	31.73 ± 0.67	38.06 ± 0.24	49.67 ± 0.14
Ascorbic acid	24.91 ± 0.30	33.98 ± 0.50	39.01 ± 0.38	48.19 ± 0.18

* Values are mean of three determinations ($n = 3$) ± SD

Table 4. Antioxidant activity of the compounds (**IVa**–**e**) and (**VIIa**–**e**) in hydroxyl radical method

Compound	Radical scavenging activity, %			
	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL
(IVa)	8.77 ± 0.62	14.31 ± 0.82	28.60 ± 0.81	32.63 ± 0.58
(IVb)	12.86 ± 0.22	18.30 ± 0.84	31.53 ± 0.75	35.71 ± 0.38
(IVc)	12.15 ± 0.50	16.38 ± 0.30	29.22 ± 0.83	32.06 ± 0.47
(IVd)	20.21 ± 0.40	24.79 ± 0.49	38.00 ± 0.88	40.88 ± 0.77
(IVe)	2.65 ± 0.60	11.51 ± 0.86	15.70 ± 0.46	28.15 ± 3.37
(VIIa)	8.84 ± 0.56	12.94 ± 0.22	29.44 ± 0.18	31.99 ± 0.39
(VIIb)	13.44 ± 0.61	19.36 ± 0.29	32.13 ± 0.67	34.86 ± 0.43
(VIIc)	12.29 ± 0.68	18.89 ± 0.57	31.20 ± 0.17	33.07 ± 0.89
(VIId)	23.21 ± 0.57	28.10 ± 0.66	36.32 ± 1.05	42.15 ± 0.17
(VIIe)	6.41 ± 0.42	12.61 ± 0.32	24.60 ± 0.83	30.50 ± 0.89
(BHA)	9.32 ± 0.39	14.11 ± 0.61	27.35 ± 0.59	32.62 ± 0.56

* Values are mean of three determinations ($n = 3$) ± SD.

Antimicrobial Activity

All the newly synthesized semicarbazones, thiocarbazones, 1,3,4-oxadiazole, and 1,3,4-thiadiazole derivatives were screened for their antibacterial and antifungal activity. Antimicrobial activities of the synthesized compounds were assessed by minimum inhibitory concentration (MIC) by serial dilution method using DMSO–water (1 : 10 v/v) as solvent control [2]. The experiments were carried out in triplicate; the results were taken as a mean ± standard deviation (SD). For antibacterial studies, microorganisms employed were gram-negative bacteria species *Escherichia coli* and *Pseudomonas aeruginosa* and gram-positive bacteria *Staphylococcus aureus*; for antifungal studies *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* were used as fungal strains. The antibiotics ciprofloxacin and fluconazole were used as standard drugs against bacteria and fungi species, respectively. The results of MIC's of the synthesized compounds—carbazones, thiocarbazones, 1,3,4-oxadiazole, and 1,3,4-thiadiazole derivatives—against bacterial and fungal species are summarized in Tables 1 and 2, respectively.

DPPH Radical Scavenging Activity

Antioxidants are characterized by their ability to scavenge the free radicals. DPPH radical scavenging assay was performed by a method reported by Padmaja et al. [19]. Briefly, 1 mL of DPPH solution (0.1 mM in 95% methanol) was mixed with different aliquots of test compounds (25, 50, 75, and 100 μg/mL) in methanol. Then, the solution was allowed to stand for 20 min at room temperature. The absorbance was read

Table 5. Docking scores of all synthesized compounds (**IVa**–**e**) and (**VIIa**–**e**) against Cu-ZnSOD, catalase and glutathione peroxidase. Glide scores and average van der Waal (E_{evdw}) and Glide energies (kcal/mol) as obtained through Glide docking

Compound	SOD				Catalase		Glutathione peroxidase	
	Glide score	Glide hbond	Glide evdw	Glide energy	Glide score	Glide energy	Glide score	Glide energy
(IVa)	−4.89	−0.37	−36.26	−40.53	−6.11	−50.99	−1.76	−30.13
(IVb)	−4.75	−0.35	−37.63	−42.31	−5.78	−37.20	−2.46	−28.61
(IVc)	−5.04	−0.33	−28.30	−37.72	−6.19	−42.88	−2.34	−30.35
(IVd)	−6.02	−0.69	−35.08	−41.13	−4.68	−41.93	−1.67	−28.95
(IVe)	−4.15	0.00	−30.62	−39.86	−5.80	−46.33	−1.34	−27.05
(VIIa)	−4.97	−0.30	−35.97	−41.02	−5.13	−42.79	−1.52	−26.70
(VIIb)	−5.20	−0.32	−38.86	−46.43	−6.08	−42.09	−3.26	−32.22
(VIIc)	−4.44	0.00	−35.73	−40.17	−5.64	−31.13	−1.69	−30.88
(VIId)	−6.05	−0.72	−37.98	−43.61	−3.81	−40.89	−2.19	−32.35
(VIIe)	−4.45	−0.47	−32.38	−40.56	−4.06	−46.93	−1.39	−24.18

Table 6. Computer aided ADME screening of the synthesized compounds (**IVa**–**e**) and (**VIIa**–**e**)

Compound	Mol MW	QPlogHERG	QPPCaco	QPlogBB	QPlogKp	(a)*	(b)*	(c)*	(d)*
(IVa)	401.4	−6.3	83.3	−1.8	−4.1	0.3	74.5	33.7	0
(IVb)	431.4	−5.7	78.8	−1.7	−4.2	0.3	74.1	31.8	0
(IVc)	415.4	−5.9	72.0	−1.8	−4.4	0.4	74.6	28.8	0
(IVd)	435.8	−5.9	74.1	−1.6	−4.4	0.4	76.0	73.3	0
(IVe)	459.4	−5.9	4.1	−3.3	−6.8	−0.1	29.0	1.3	1
(VIIa)	417.4	−6.2	86.5	−1.7	−4.1	0.4	77.4	48.7	0
(VIIb)	447.5	−5.7	89.4	−1.6	−4.1	0.4	77.7	48.0	0
(VIIc)	431.5	−6.0	81.5	−1.7	−4.3	0.5	78.3	44.4	0
(VIId)	451.9	−6.1	86.4	−1.6	−4.3	0.5	80.2	120.1	0
(VIIe)	475.4	−6.5	5.6	−3.4	−6.4	0.1	35.5	2.5	1
Range 95% of drugs	130.0 to 725.0	<−5	<25 poor, >500 great	−3.0 to 1.2	−8.0 to −1.0	−1.5 to 1.5	>80% is high	<25 poor, >500 great	0–4

*(a) QPlogKhsa; (b) Human oral absorption, %; (c) QPPMDCK; and (d) Volition of Lipinski's rule.

against blank at 517 nm in an ELICO SL 159 UV visible spectrophotometer. The free radical scavenging potential was calculated as a percentage (I, %) of DPPH decoloration using the equation

$$I, \% \text{ of scavenging} = (A_0 - A_1/A_0) \times 100,$$

where A_0 is the absorbance of the control reaction mixture excluding the test compounds and A_1 is the absorbance of the test compounds. Experiments were carried out in triplicate and the results are expressed as I, % ± standard deviation and are summarized in Table 3.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging assay was performed by a known procedure [20]. Mixture of 0.1 mL of phosphate buffer, 0.2 mL of 2-deoxyribose, test compounds (25, 50, 75, and 100 μg/mL in methanol), 0.1 mL of H_2O_2 (10 mM), 0.1 mL of ascorbic acid (1 mM), 0.1 mL of EDTA, and 0.01 mL of $FeCl_3$ (100 mM) was incubated at 37°C for 60 min. Thereafter, the reaction was terminated by adding 1 mL of cold 2.8% trichloroacetic acid and the reaction product was measured by adding 1 mL of 1% thiobarbituric acid (1g in 100 mL of 0.05 N NaOH) in boiling water for 15 min. The absorbance was measured at 535 nm. BHA was used as a positive control. Decrease in absorbance of the reaction mixture indicates hydroxyl radical scavenging activity. The experiment was carried out in triplicate, results were expressed as I, % ± standard deviations and was summarized in Table 4.

Molecular Docking and ADME Predictions

The co-ordinates of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were obtained from the Brookhaven Protein Data Bank; whose PDB ID's are 1CB4, 2CAG, and 2P31, respectively. Ligands were drawn using the Maestro 2D sketcher and energy minimization was computed by OPLS 2005. Proteins were prepared by retrieving into the Maestro 9.3 platform (Schrödinger, Inc.). Prime software module of Schrödinger was used to correct the missing loops in the proteins. Water molecules from SOD, CAT, and GPx were removed which were beyond 5 Å from the hetero atoms. Water molecules that are thought to be important in aiding the interaction between the receptor and the ligand were optimized during protein pepwizard. Automated, necessary bonds, bond orders, hybridization, explicit hydrogens, and charges were assigned. OPLS 2005 force field was applied to the proteins to restrain minimization and RMSD of 0.30 Å was set to converge heavy atoms during the pre-processing of protein before starting docking. Using extra-precision (XP) docking each compound was docked into the receptor grid of radii 20Å and the docking calculations were judge based on the Glide score, and Glide energy. QikProp, the prediction program, was used to calculate ADME properties of all the ligands, and molecular visualization was done with Maestro 9.3.

CONCLUSIONS

In summary, the synthetic strategy of incorporating the 1,3,4-oxadiazole and 1,3,4-thiadiazole ring onto a coumarin and pyrazole scaffold was proven to be quite an efficient way to synthesize 8-(4-(5-amino-1,3,4-oxadiazol-2-yl)-1-(aryl)-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-ones and 8-(4-(5-amino-1,3,4-thiadiazol-2-yl)-1-(aryl)-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-ones as designed. The newly synthesized series of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and their intermediates semicarbazones and thiosemicarbazones were evaluated for their biological activities. Results revealed that all compounds showed moderate to excellent antimicrobial activities. Compounds (**IVd**) and (**VIId**) exhibited significant antioxidant activities. Docking studies show that compounds (**IVd**) and (**VIId**) might act as good antioxidant compounds with satisfactory ADME properties and are the suitable candidates to be carried forward as potential leads targeting various diseases affected by free radicals.

ACKNOWLEDGMENTS

The authors thank the University Grants Commission, New Delhi, for financial assistance through major research project grant (F: 42-230/2013 (SR) dated March 23, 2013). N. Renuka is grateful to the UGC for awarding the NON-NET Fellowship (Order no. DV9/192/NON-NETFS/2013-14, dated 11.11.2013).

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DOI 10.1007/s11094-017-1657-5
Pharmaceutical Chemistry Journal, Vol. 51, No. 7, October, 2017 (Russian Original Vol. 51, No. 7, July, 2017)

SYNTHESIS OF COUMARIN APPENDED 1,3-OXAZINES AS POTENT ANTIMICROBIAL AND ANTIOXIDANT AGENTS

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Original article submitted March 18, 2016.

A convenient protocol for the synthesis of coumarin appended 1,3-benzoxazine derivatives (**4a – 4g**) is described. Cyclisation of hydrazones (**3a – 3g**) using triphosgene in dichloromethane gave the corresponding 1,3-oxazines in a relatively good yield. The proposed structures of newly synthesized compounds were confirmed by spectral methods and elemental analysis. Oxazine derivatives **4a – 4g** were evaluated for their *in vitro* antimicrobial activity against various bacteria and fungi. Compounds **4b** and **4d** inhibited growth of test microbes thus proving significant antimicrobial activity. In addition, *in vitro* antioxidant activity of the synthesized compounds was evaluated and compared to that of standards, showing DPPH, NO, and OH radical scavenging properties. Compounds **4b** and **4f** exhibited higher antioxidant activity than a standard drug.

Keywords: antioxidant; antimicrobial; coumarin; hydrazones; oxazine; triphosgene.

1. INTRODUCTION

Heterocyclic compounds play a prominent role in synthetic and bioorganic chemistry [1]. The synthesis of new heterocycles that possess biological potency is a challenging task for organic chemists. Researchers all over the world are now focusing on the development of new innovative and accessible procedures for the synthesis of heterocycles. Condensed heterocycles are considered as the most important class among the heterocyclic compounds for their diverse pharmaceutical applications [2]. Moreover, coumarin nucleus is prevalent in nature and the synthesis of coumarins is an important reaction in organic chemistry because of their wide application in medicinal chemistry [3]. Various coumarin compounds are used as intermediates for the synthesis of other heterocyclic ring systems. Furthermore, these compounds have been found to possess a broad range of biological and controlled therapeutic activities in view of their occurrence and also due to low toxicity.

In addition, oxazine derivatives create more interest in scientists because of their challenging synthetic structure. Oxazines were first synthesized in 1944 by Holly and Cope through Mannich reaction. 1,3-Benzoxazines attract more attention as they constitute an important class of both natural and synthetic products and show useful biological activities [4]. Oxazines are more important fundamentally due to their varied biological and chemical properties. Benzo-1,3-oxazines are known to be biologically active, demonstrating antihypertensive effects [5], cytotoxicity [6], anti-osteoclastic bone resorption activity [7], and anticonvulsant [8] and antimicrobial activities [9]. Recently triphosgene has been used in the cyclisation of hydrazones for the synthesis of 1.3-oxazines [10]. Antioxidants are good oxidizing agents, they oxidize free radicals and prevent cell death caused by the release of free radicals. Antioxidants are considered as important factor for the prevention and treatment of cancer and it is assumed that compounds with potential antioxidant properties can be used for chemotherapy [11].

Therefore, efforts have been made in order to develop efficient approaches to the synthesis of benzoxazine derivatives fused to coumarin nucleus. Prompted by the biological potency of oxazines and in continuation of our research work on coumarins, we undertake this task of synthesizing coumarin appended oxazines and study their biological properties. Herein we report the synthesis of a series of benzoxazine analogs and their antimicrobial and antioxidant properties.

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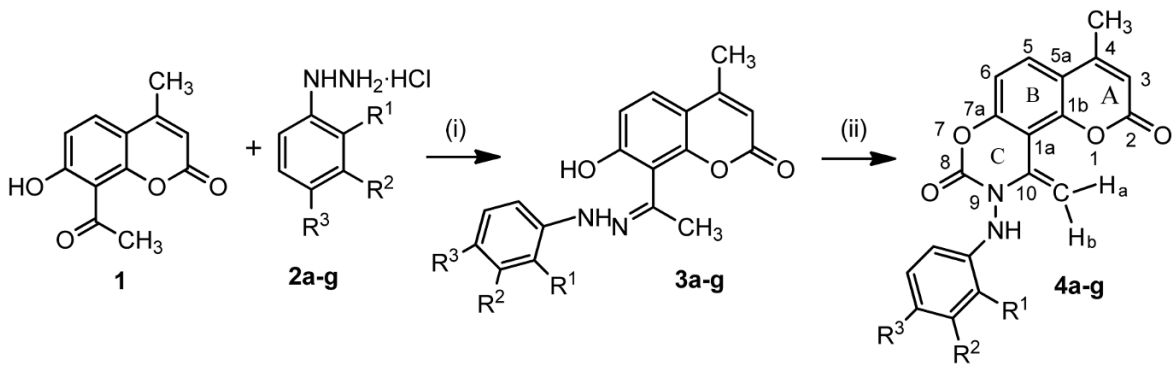

Reagents and conditions: (i). CH_3COOH/C_2H_5OH; 100 °C , 3-4 h.
(ii). $(Cl_3CO)_2CO$, $(C_2H_5)_3N/CH_2Cl_2$; 110-120 °C, N_2 atm, 4-5h.

4 a) R^1=H, R^2=H, R^3=H; b) R^1=OCH_3, R^2=H, R^3=H; c) R^1=CH_3, R^2=H, R^3=H;
d) R^1=H, R^2=H, R^3=Cl; e) R^1=NO_2, R^2=H, R^3=NO_2; f) R^1=CH_3, R^2=H, R^3=CH_3;
g) R^1=H, R^2=CH_3, R^3=H.

Fig. 1. Scheme of the synthesis of oxazines **4a** – **4g**.

2. EXPERIMENTAL

2.1. General

Melting points have been determined by open capillary method and are uncorrected. The IR spectra were recorded in Nujol mulls on Shimadzu 8300 spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Spect 500.1 MHz and Spect 125.7 MHz spectrometers, respectively, using DMSO as solvent and TMS as the internal standard. The chemical shifts are expressed as d, ppm. The mass spectra were obtained on a Shimadzu LCMS-2010A spectrophotometer with CI source. Elemental analysis of compounds was performed on a Thermo Finnigan Flash EA 1112 CHN analyzer.

2.2. Synthesis

The precursor 8-acetyl-7-hydroxy-4-methylcoumarin (**1**) was obtained by the procedure reported previously [12]. In a typical experiment, condensation of compound **1** with substituted phenylhydrazines (**2a** – **2g**) in EtOH in the presence of catalytic amounts of AcOH under reflux conditions produced the corresponding hydrazones (**3a** – **3g**). Treatment of hydrazones with triphosgene in the presence of NEt_3 and CH_2Cl_2 gave oxazines (**4a** – **4g**) depending on the molar ratio of the triphosgene used in the cyclisation [10]. The reaction pathway is illustrated in Fig. 1. The mechanism of formation of 9-(arylamino)-4-methyl-10-methylene-9,10-dihydrochromeno[8, 7-e][1, 3]oxazine-2,8-diones **4a** – **4g** is illustrated by considering compound **4a** in Fig. 2.

2.2.1. General procedure for the synthesis of 7-hydroxy-4-methyl-8-(1-(2-arylhydrazono)ethyl)-2H-chromen-2-ones (3a – 3g). To a solution of 8-acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one (**1**) (0.004 mol) in EtOH was added substituted phenylhydrazine hydrochloride (**2a** – **2g**) (0.004 mol) and 5 mL of acetic acid. The mixture was refluxed on a water bath for 3 – 4 h. After completion of the reaction, the mixture was quenched with ice-cold water; separated solid was filtered and recrystallized with EtOH to obtain target compounds **3a** – **3g**. Compound **3a** is taken as representative to show the data of spectral analysis.

7-Hydroxy-4-methyl-8-(1-(2-phenylhydrazono)ethyl)-2H-chromen-2-one (3a*)*: pale yellow solid; yield, 1.13 g (80%); m. p., 170 – 172°C; IR spectrum in Nujol mull (ν_{max}, cm^{-1}): 3352 (N-H str.), 3215 (O-H str.), 1678 (C=O, str.), 1608 (C=N, str.); ^{1}H NMR (500.1MHz, DMSO-d_6) (δ, ppm): 2.42 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 5.24 (s, 1H, NH), 6.24 (s, 1H, C_3-H), 7.12 (d, J = 4.6 Hz, 1H, C_6-H), 7.38 (d, J = 5.3 Hz, 1H, C_5-H), 7.62-8.12 (m, 5H), 9.12 (s, 1H, OH); ^{13}C NMR (125.7 MHz, DMSO-d_6) (δ, ppm): 19.2 (1C, CH_3), 22.6 (1C, CH_3), 113.3 (1C, C_{5a}), 113.9 (1C, C_3), 114.6 (2C, Ar), 115.5 (1C, C_6), 116.9 (1C, C_8), 121.5 (1C, Ar), 127.2 (1C, C_9), 129.2 (2C, Ar), 138.7 (1C, Ar), 143.6 (1C, C_{1b}), 150.7 (1C, C_4), 157.6 (1C, C_7), 160.2 (1C, C_2), 166.8 (1C, C=N); LCMS *m/z* (I_{rel}, %): 308, 309 (100%) [MH^+]; found (%): C, 70.14; H, 5.15; N, 9.13; $C_{18}H_{16}N_2O_3$; anal. calcd. (%): C, 70.12; H, 5.23; N, 9.09.

2.2.2. General procedure for the synthesis of 9-(arylamino)-4-methyl-10-methylene-9,10-dihydrochromeno-[8,7-e][1,3]oxazine-2,8-diones (4a – 4g). To a stirred solution of hydrazone (**3a:** 1.0 g, 0.0032 mol) and NEt_3 (2 mL) in CH_2Cl_2 (35 mL), solution of 0.5 equivalent (0.48 g, 0.0016 mol) of triphosgene in dichloromethane (10 mL) was added dropwise under N_2 atmosphere. The mixture was refluxed for 4 – 5 h. The progress of reaction was monitored by TLC. After completion of the process, the reaction mass

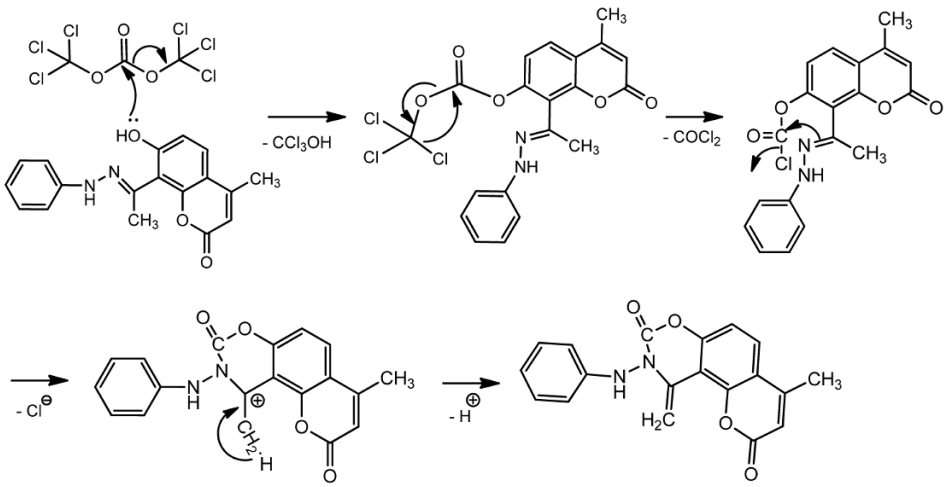

Fig. 2. Proposed mechanism of formation of compound **4a**.

was diluted with 20 mL of CH_2Cl_2 and washed with H_2O; the organic layer was dried over anhydrous Na_2SO_4. Evaporation of solvent *in vacuo* resulted in crude product which was purified by passing through column of silica gel (60 – 120 mesh) using EtOAc – hexane (1:4 v/v) mixture as eluent. The same procedure was used in all cases.

4-Methyl-10-methylene-9-(phenylamino)-9,10-dihydrochromeno[8,7-e][1,3]oxazine-2,8 dione (4a): brown solid; yield, 0.41 g (76 %); m.p. 161 – 163°C; IR (Nujol, ν_{max}, cm^{-1}): 3260 (N-H str.), 1732 (ring C, C=O str.), 1675 (ring A, C=O str.), 1617 (=CH_2 str.); ^{1}H NMR (500.1 MHz, DMSO-d_6) (δ, ppm)*:* 1.990 (s, 3H, CH_3), 4.092 (s, 1H, NH), 4.589 (d, J = 28.2 Hz, 1H, CH_a), 4.827 (d, J = 25.6 Hz, 1H, CH_b), 6.160 (s, 1H, C_3-H), 6.882 – 7.560 (m, 7H, Ar-H); ^{13}C NMR (125.7 MHz, DMSO-d_6) (δ, ppm): 19.60 (1C, CH_3), 108.20 (1C, CH_2), 113.40 (1C, C_3), 113.80 (2C, Ar), 114.80 (1C, C_6), 116.60 (1C, C_{5a}), 120.50 (1C, C_{1a}), 121.96 (1C, Ar), 123.92 (1C, C_5), 130.34 (2C, Ar), 139.40 (1C, C_{10}), 143.20 (1C, C_{1b}), 145.50 (1C, Ar), 148.41 (1C, C_{7a}), 152.42 (1C, C_8), 153.40 (1C, C_4), 160.25 (1C, C_2); LCMS *m/z* (I_{rel}, %): 334, 335 (MH^+, 100), 336 (55), 321 (10), 307 (5), 263 (15), 233 (9), 161 (5); found (%): C, 67.15; H, 4.57; N, 8.66; $C_{19}H_{14}N_2O_4$; anal. calcd. (%): C, 68.26; H, 4.22; N, 8.38.

9-((2-Methoxyphenyl)amino-4-methyl-10-methylene-9,10-dihydrochromeno[8, 7-e][1, 3]oxazine-2,8-dione (4b): brown solid; yield, 0.39 g (66 %); m.p., 182 – 184°C; IR (Nujol, ν_{max}, cm^{-1}): 3272 (N-H str.), 1736 (ring C, C=O str.), 1665 (ring A, C=O str.), 1619 (=CH_2 str.); ^{1}H NMR (500.1 MHz, DMSO-d_6) (δ, ppm): 2.103 (s, 3H, CH_3), 3.850 (s, 3H, OCH_3), 4.212 (s, 1H, NH), 4.725 (d, J = 28.5 Hz, 1H, CH_a), 5.016 (d, J = 25.1 Hz, 1H, CH_b), 6.262 (s, 1H, C_3-H), 6.974-7.695 (m, 6H, Ar-H); ^{13}C NMR (125.7 MHz, DMSO-d_6) (δ, ppm): 19.4 (1C, CH_3), 54.6 (1C, OCH_3), 107.9 (1C, CH_2), 113.6 (1C, Ar), 114.2 (1C, C_3), 114.8 (1C, Ar), 115.6 (1C, C_6), 116.2 (1C, C_{5a}), 120.6 (1C, C_{1a}), 121.5 (1C, Ar), 123.0 (1C, Ar), 124.4 (1C, C_5), 128.5 (1C, Ar), 142.2 (1C, C_{10}), 144.4 (1C, C_{1b}), 145.8 (1C, Ar), 149.8 (1C, C_{7a}), 152.9 (1C, C_8), 153.6 (1C, C_4), 160.0 (1C, C_2); LCMS *m/z* (I_{rel}, %): 364, 365 (MH^+, 100), 352 (50), 350 (22), 243 (12), 161 (5), 123 (14); found (%): C, 65.73; H, 4.11; N, 7.39; $C_{20}H_{16}N_2O_5$; anal. calcd. (%): C, 65.93; H, 4.43; N, 7.69.

4-Methyl-10-methylene-9-(o-tolylamino)-9,10-dihydrochromeno[8,7-e][1,3]oxazine-2,8-dione (4c): brown solid; yield, 0.41 g (73 %); m.p., 142 – 144°C; IR (Nujol, ν_{max}, cm^{-1}): 3260 (N-H str), 1743 (ring C, C=O str), 1682 (ring A, C=O str), 1621 (=CH_2 str); ^{1}H NMR (500.1 MHz, DMSO-d_6) (δ, ppm): 2.156 (s, 3H, CH_3), 2.324 (s, 3H, CH_3), 4.220 (s, 1H, NH), 4.710 (d, J = 29.3 Hz, 1H, CH_a), 4.985 (d, J = 27.6 Hz, 1H, CH_b), 6.942-7.746 (m, 6H, Ar-H); ^{13}C NMR (125.7 MHz, DMSO-d_6) (δ, ppm): 18.2 (1C, CH_3), 19.8 (1C, CH_3), 106.4 (1C, CH_2), 113.5 (1C, Ar), 114.4 (1C, C_3), 114.8 (1C, Ar), 114.2 (1C, C_6), 116.2 (1C, C_{5a}), 120.6 (1C, C_{10a}), 121.5 (1C, Ar), 123.0 (1C, Ar), 124.8 (1C, C_5), 127.7 (1C, Ar), 143.2 (1C, C_{10}), 144.3 (1C, Ar), 145.8 (1C, C_{1b}), 149.7 (1C, C_{7a}), 151.5 (1C, C_8), 152.6 (1C, C_4), 160.4 (1C, C_2); LCMS *m/z* (I_{rel}, %): 348, 349 (MH^+, 100), 312 (45), 273 (21), 161 (5), 123 (14); found (%): C, 68.66; H, 4.21; N, 8.22; $C_{20}H_{16}N_2O_4$; anal. calcd. (%): C, 68.96; H, 4.63; N, 8.04.

9-((4-Chlorophenyl)amino)-4-methyl-10-methylene-9,10-dihydrochromeno[8,7-e] [1,3]oxazine-2,8-dione (4d): pale yellow solid; yield, 0.43 g (73 %); m.p., 194 – 196°C; IR (Nujol, ν_{max}, cm^{-1}): 3268 (N-H str.), 1738 (ring C, C=O str.), 1668 (ring A, C=O str.), 1617 (=CH_2 str.); ^{1}H NMR (500.1 MHz, DMSO-d_6) (δ, ppm): 2.220 (s, 3H, CH_3), 4.214 (s, 1H, NH), 4.722 (d, J = 29.2 Hz, 1H, CH_a), 4.981 (d, J = 26.7 Hz, 1H, CH_b), 6.227 (s, 1H, C_3-H), 7.012-7.893 (m, 6H, Ar-H); ^{13}C NMR (125.7 MHz, DMSO-d_6) (δ, ppm): 19.2 (1C, CH_3), 106.6 (1C, CH_2), 113.2 (2C, Ar), 114.3 (1C, C_3), 114.7 (1C, C_6), 116.5 (1C, C_{5a}), 120.6 (1C, C_{1b}), 124.4 (1C, C_5), 128.2 (1C, Ar), 129.7 (2C, Ar), 143.2 (1C, C_{10}), 145.4

(1C, Ar), 145.8 (1C, C_{1a}), 149.7 (1C, C_{7a}), 151.5 (1C, C_8), 152.6 (1C, C_4), 160.0 (1C, C_2); LCMS *m/z* (I_{rel}, %): 368, 370 [M^+, ^{37}Cl], 368 [M^+, ^{35}Cl], 356 (62), 354 (42), 243 (15), 127 (22), 161 (5); found (%): C, 61.63; H, 3.31; N, 7.32; $C_{19}H_{13}ClN_2O_4$; anal. calcd. (%): C, 61.88; H, 3.55; N, 7.60.

9-((2,4-Dinitrophenyl)amino)-4-methyl-10-methylene-9,10-dihydrochromeno[8,7-e][1,3]oxazine-2,8-dione (4e): brown solid, yield, 0.57 g (84 %); m.p., 186 – 188°C; IR (Nujol, ν_{max}, cm^{-1}): 3302 (N-H str.), 1741 (ring C, C=O str.), 1682 (ring A, C=O str.), 1626 (=CH_2 str.); ^{1}H NMR (500.1 MHz, DMSO-d_6) (δ, ppm): 2.321 (s, 3H, CH_3), 4.543 (s, 1H, NH), 4.678 (d, J = 28.7 Hz, 1H, CH_a), 4.943 (d, J = 26.3 Hz, 1H, CH_b), 6.211 (s, 1H, C_3-H), 6.821-7.789 (m, 5H, Ar-H); ^{13}C NMR (125.7 MHz, DMSO-d_6) (δ, ppm): 19.4 (1C, CH_3), 106.2 (1C, CH_2), 112.8 (1C, C_3), 114.2 (1C, C_6), 115.6 (1C, Ar), 116.1 (1C, C_{5a}), 120.4 (1C, Ar), 121.5 (1C, C_{1a}), 124.4 (1C, C_5), 131.3 (1C, Ar), 134.2 (1C, Ar), 140.3 (1C, Ar), 142.6 (1C, C_{10}), 144.2 (1C, C_{1b}), 147.2 (1C, C_{7a}), 152.5 (1C, C_8), 152.6 (1C, C_4), 154.4 (1C, Ar), 160.0 (1C, C_2); LCMS *m/z* (I_{rel}, %): 424, 425 (MH^+, 100), 379 (65), 310 (26), 161 (7), 123 (13); found (%): C, 53.52; H, 2.55; N, 13.45; $C_{19}H_{12}N_4O_8$; anal. calcd. (%): C, 53.78; H, 2.85; N, 13.20.

9-((2,4-Dimethylphenyl)amino)-4-methyl-10-methylene-9,10-dihydrochromeno[8,7-e][1,3]oxazine-2,8-dione (4f): brown solid, yield, 0.45 g (78 %); m.p., 182 – 184°C; IR (Nujol, ν_{max}, cm^{-1}): 3281 (N-H str.), 1748 (ring C, C=O str.), 1678 (ring A, C=O str.), 1614 (=CH_2 str.); ^{1}H NMR (500.1 MHz, DMSO-d_6) (δ, ppm): 2.220 (s, 3H, CH_3), 2.412 (s, 6H, CH_3), 4.228 (s, 1H, NH), 4.692 (d, J = 30.3 Hz, 1H, CH_a), 4.994 (d, J = 26.2 Hz, 1H, CH_b), 6.236 (s, 1H, C_3-H), 6.982-7.715 (m, 5H, Ar-H); ^{13}C NMR (125.7 MHz, DMSO-d_6) (δ, ppm): 17.3 (1C, CH_3), 19.4 (1C, CH_3), 20.7 (1C, CH_3), 106.4 (1C, CH_2), 112.4 (1C, C_3), 113.0 (1C, Ar), 114.6 (1C, C_6), 116.1 (1C, C_{5a}), 120.2 (1C, C_{1a}), 124.4 (1C, C_5), 125.5 (1C, Ar), 127.6 (1C, Ar), 132.5 (1C, Ar), 137.4 (1C, Ar), 143.0 (1C, C_{10}), 144.4 (1C, C_{1b}), 145.4 (1C, Ar), 148.5 (1C, C_{7a}), 151.6 (1C, C_8), 152.7 (1C, C_4), 160.4 (1C, C_2); LCMS *m/z* (I_{rel}, %): 362, 363 (MH^+, 100), 345 (50), 322 (20), 243 (12), 161 (7), 123 (18); found (%): C, 69.38; H, 4.98; N, 7.70; $C_{21}H_{18}N_2O_4$; anal. calcd. (%): C, 69.60; H, 5.18; N, 8.03.

4-Methyl-10-methylene-9-(m-tolylamino)-9,10-dihydrochromeno[8,7-e][1,3]oxazine-2,8-dione (4g): brown solid; yield, 0.39 g (71 %); m.p., 194 – 196°C; IR (Nujol cm^{-1}) ν_{max}: 3278 (N-H str.), 1741 (ring C, C=O str.), 1688 (ring A, C=O str.), 1621 (=CH_2 str.); ^{1}H NMR (500.1 MHz, DMSO-d_6) (δ, ppm): 2.162 (s, 3H, CH_3), 2.246 (s, 3H, CH_3), 4.238 (s, 1H, NH), 4.813 (d, J = 28.2 Hz, 1H, CH_a), 5.163 (d, J = 27.3 Hz, 1H, CH_b), 6.240 (s, 1H, C_3-H), 6.995-7.729 (m, 6H, Ar-H); ^{13}C NMR (125.7 MHz, DMSO-d_6) (δ, ppm): 19.4 (1C, CH_3), 22.4 (1C, CH_3), 106.8 (1C, CH_2), 109.5 (1C, Ar), 111.9 (1C, Ar), 112.4 (1C, C_3), 114.6 (1C, C_6), 116.8 (1C, C_{5a}), 119.9 (1C, Ar), 120.8 (1C, C_{1a}), 123.7 (1C, C_5), 129.3 (1C, Ar), 139.5 (1C, Ar), 143.6 (1C, C_{10}), 145.8 (1C, C_{1b}), 149.5 (1C, C_{7a}), 15.6 (1C, Ar), 153.5 (1C, C_8), 154.0 (1C, C_4), 160.7 (1C, C_2); found (%): C, 68.62; H, 4.33; N, 7.87; $C_{20}H_{16}N_2O_4$; anal. calcd. (%): C, 68.96; H, 4.63; N, 8.04.

2.3 Biological Screening

Standard chemicals ciprofloxacin (17850-5G-F), amphotericin B (Y0001361) and ascorbic acid (A92902-100G) were purchased from Sigma Aldrich India Ltd.

Preparation of stock solutions: Ciprofloxacin (100 mg) was dissolved in DMSO (3 – 4 drops) and then diluted to 100 mL using doubly distilled water. This solution gives a concentration of 1000 µg/mL. From this stock solution were taken 10, 7.5, 5, 2.5 and 1.25 mL aliquots and each volume was diluted to 100 mL using doubly distilled water. These solutions had concentrations of 100, 75, 50, 25 and 12.5 µg/mL, respectively. Ascorbic acid (100 mg) was dissolved in DMSO (3-4 drops) and then diluted to 100 mL using doubly distilled water. This solution gives a concentration of 1000 µg/mL. From this stock solution were taken 10, 7.5, 5 and 2.5 mL aliquots and each volume was diluted to 100mL using doubly distilled water. These solutions had concentrations of 100, 75, 50 and 25 µg/mL, respectively.

TABLE 1. Minimum Inhibitory Concentrations (MICs, µg mL^{-1}) of Compounds **4a – 4g** against Bacteria Test Species

Compound	R^1	R^2	R^3	*S. aureus*	*S. pyogenes*	*E. coli*	*P. aeruginosa*
4a	H	H	H	25	50	25	25
4b	OCH_3	H	H	12.5	25	12.5	12.5
4c	CH_3	H	H	50	50	25	50
4d	H	H	Cl	25	25	12.5	12.5
4e	NO_2	H	NO_2	50	75	50	50
4f	CH_3	H	CH_3	25	25	12.5	25
4g	H	CH_3	H	75	50	25	50
Ciprofloxacin	–	–	–	25	50	25	12.5

2.3.1. Antimicrobial activity. The synthesized 1,3-oxazines were screened for their antibacterial and antifungal activity by serial dilution method [15]. The experiments were carried out in triplicate and the results were expressed as mean of three determinations (n = 3). Antibacterial studies were performed with species *Staphylococcus aureus, Staphylococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa;* antifungal studies were carried out with *Cryptococcus neoformans, Aspergillus niger, Aspergillus flavus* and *Candila albicans* strains. The standard antibiotics ciprofloxacin and amphotericin B were used as reference drugs against bacteria and fungi species, respectively. The values of minimum inhibiting concentration (MIC) of each synthesized compound against bacteria and fungi species are summarized in Tables 1 and 2, respectively.

2.3.2. DPPH radical scavenging activity. Antioxidants are characterized by their ability to scavenge free radicals. Proton radical scavenging action is an important attribute of antioxidants, which is measured by DPPH scavenging assay. DPPH radical scavenging ability of synthesized compounds **4a – 4g** was studied by reported procedure [16]. According to this, 1 mL of DPPH solution (0.1 mM in 95% methanol) was mixed with various aliquots of test samples (25, 50, 75 and 100 μg mL^{-1}) in methanol. The mixture was shaken vigorously and allowed to stand for 20 min at room temperature. The absorbance was read against blank at 517 nm in an ELICO SL 159 UV-Vis spectrophotometer. The free radical scavenging potential was calculated as percentage (I%) of DPPH decoloration using the following equation:

$$I\% \text{ scavenging} = (A_0 - A_1/A_0) \times 100,$$

where A_0 is the absorbance of the control reaction mixture without test compound and A_1 is the absorbance of mixture with test compound. Tests were carried out in triplicate and the results were expressed as mean I% ± standard deviation and summarized in Table 3.

2.3.3. Nitric oxide radical scavenging assay. Nitric oxide radical scavenging assay of synthesized compounds **4a – 4g** was carried out by reported procedure [17]. Nitric oxide (NO) was generated from sodium nitroprusside in phosphate buffer at physiological pH. The nitric oxide formed reacts with oxygen to produce nitrite ions, which can be estimated by the Griess reagent. Sodium nitroprusside solution (1 mL, 10 mM) and 1.5 mL of phosphate buffer (pH 7.4) were mixed with a test solution (25, 50, 75 and 100 μg mL^{-1}) and incubated at 298 K for 150 min. To this solution, 1 mL of Griess reagent (1% sulfanilamide in 2% phosphoric

TABLE 2. Minimum Inhibitory Concentrations (MICs, μg mL^{-1}) of Compounds **4a – 4g** against Fungi Test Species

Compound	R^1	R^2	R^3	*C. neoformans*	*A. niger*	*A. flavus*	*C. albicans*
4a	H	H	H	75	50	50	50
4b	OCH_3	H	H	25	25	25	12.5
4c	CH_3	H	H	50	25	25	50
4d	H	H	Cl	12.5	25	25	12.5
4e	NO_2	H	NO_2	25	100	100	50
4f	CH_3	H	CH_3	50	25	25	25
4g	H	CH_3	H	50	50	50	50
Amphotericin B	–	–	–	25	50	50	25

TABLE 3. DPPH Radical Scavenging Ability (I, %) of Compounds **4a – 4g** at Various Concentrations

Compound	R^1	R^2	R^3	25 (μg mL^{-1})	50 (μg mL^{-1})	75 (μg mL^{-1})	100 (μg mL^{-1})
4a	H	H	H	27.787 ± 0.58	37.128 ± 0.37	40.146 ± 0.55	49.981 ± 0.33
4b	OCH_3	H	H	32.978 ± 0.23	42.003 ± 0.40	46.549 ± 0.42	50.234 ± 0.55
4c	CH_3	H	H	28.531 ± 0.65	38.472 ± 0.93	40.245 ± 0.25	49.161 ± 0.36
4d	H	H	Cl	26.600 ± 0.11	34.794 ± 0.26	39.216 ± 0.41	49.417 ± 0.34
4e	NO_2	H	NO_2	24.135 ± 0.24	32.593 ± 0.11	36.527 ± 0.22	48.417 ± 0.32
4f	CH_3	H	CH_3	30.047 ± 0.45	08.945 ± 0.67	41.087 ± 0.49	49.300 ± 0.24
4g	H	CH_3	H	28.060 ± 0.36	36.157 ± 0.28	40.772 ± 0.62	48.494 ± 0.24
Ascorbic acid	–	–	–	27.194 ± 0.29	36.186 ± 0.51	40.904 ± 0.56	49.655 ± 0.62

acid and 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride) was added and allowed to stand for 3 min, after which the absorbance of chromatophore was read at 546 nm; ascorbic acid was used as standard. The experiments were carried out at four different concentration in triplicates and the results were expressed as mean *I*% ± standard deviation and summarized in Table 4.

2.3.4. Hydroxyl radical scavenging assay. Hydroxyl radical scavenging assay of synthesized compounds **4a – 4g** was carried out the reported procedure [18]. The experiment was performed by mixing 0.1 mL of phosphate buffer; 0.2 mL of 2-deoxyribose, test solution (25, 50, 75 and 100 μg mL^{-1}), 0.1 mL of H_2O_2 (10 mM), 0.1 mL of ascorbic acid (1 mM), 0.1 mL of EDTA and 0.01 mL of $FeCl_3$ (100 mM). The mixture was incubated at 310 K for 60 min and then the reaction was terminated by adding 1 mL of cold 2.8% trichloroacetic acid. The reaction product was measured upon adding 1 mL of 1% thiobarbituric acid (TBA) solution (1 g in 100 mL of 0.05 N NaOH) on boiling water bath for 15 min. The product is formed by degraded deoxyribose on heating with TBA that yields a pink colored chromogen, which confirms the formation of OH· radical. The addition of oxazine compound with the reaction mixture separates hydroxyl radicals from deoxyribose and prevents their degradation. The absorbance was measured at 535 nm. Ascorbic acid was used as the positive control. Decreased absorbance of the reaction mixture indicates increased hydroxyl radical scavenging activity. The experiment was carried out in triplicate and the results were expressed as mean *I*% ± standard deviation and summarized in Table 5.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The proposed structures of synthesized compounds **4a – 4g** were confirmed by ^{1}H NMR, mass spectra, and elemental analysis. 8-Acetyl-7-hydroxy-4-methylcoumarin (**1**) on treatment with substituted phenyl hydrazines **2a – 2g** yielded corresponding hydrazones **3a – 3g**, which was confirmed by the absence of C=O absorption band of $COCH_3$ (present in compound **1)** and the appearance of absorption bands for C=N and N-H groups at about 1608 and 3352 cm^{-1}, respectively. In ^{1}H NMR spectra, the appearance of $–NH_2$ peak supports the formation of hydrazones. Further, all the substituted hydrazones on treatment with triphosgene as a cyclising agent give target compounds **4a – 4g**. In the IR spectra, compounds **4a – 4g** showed strong absorption bands at frequencies within 1665 – 1682 cm^{-1} and 1732 – 1748 cm^{-1} for C=O stretching of ring A and ring C, respectively. A broad absorption band observed at 3260 – 3302 cm^{-1} was due to N-H stretching and the absorption band observed at 1614 – 1626 cm^{-1} was due to $=CH_2$ stretching. These observations suggest the intramolecular cyclization of hydrazones **3a – 3g** to oxazines **4a – 4g**.

In ^{1}H NMR spectra, compounds **4a – 4g** showed a singlet for one proton at δ = 4.092 – 4.543 ppm, which was was assigned to N-H proton. A two-proton signal of $=CH_2$ function exhibited diatropic nature and appeared as two doublets for one proton each at δ = 4.589 – 4.813 ppm and δ = 4.827 – 5.163 ppm. Further, the absence of signal due to phenolic –OH proton of its precursor confirms formation of the product.

In ^{13}C NMR spectra, a signal observed in the region of δ = 166.8 ppm due to C=N group present in compound **3a** disappeared in compounds **4a – 4g**. Further, compounds **4a – 4g** showed a consistent pattern of signals due to C_8-carbon, which appear in the region of δ = 151.5 – 153.5 ppm and indicate the presence of keto group and the conversion of CH_3 in compound **3a** to CH_2 in oxazines. This was clearly manifested by the appearance of peaks in the region of δ = 106.4 – 108.2 ppm rather than at 22.6 ppm. These additional signals give evidence for the formation of oxazine.

Finally, compounds **4a – 4g** showed significantly stable (MH^+) mass peak with a relative abundance ranging up to 62%, and gave satisfactory CHN analysis data consistent with the calculated values.

TABLE 4. Antioxidant Activity (*I*, %) of Compounds **4a – 4g** by Nitric Oxide Radical Scavenging Assay

Compound	R^1	R^2	R^3	25 (μg mL^{-1})	50 (μg mL^{-1})	75 (μg mL^{-1})	100 (μg mL^{-1})
4a	H	H	H	09.531 ± 0.21	18.651 ± 0.13	26.709 ± 0.18	30.027 ± 0.36
4b	OCH_3	H	H	16.868 ± 0.07	22.104 ± 0.11	37.720 ± 0.29	39.484 ± 0.25
4c	CH_3	H	H	14.461 ± 0.17	19.321 ± 0.17	36.438 ± 0.32	40.054 ± 0.18
4d	H	H	Cl	10.779 ± 0.26	16.600 ± 0.43	34.531 ± 0.28	37.686 ± 0.42
4e	NO_2	H	NO_2	10.220 ± 0.23	17.348 ± 0.46	32.573 ± 0.34	36.639 ± 0.15
4f	CH_3	H	CH_3	14.604 ± 0.27	20.139 ± 0.61	37.476 ± 0.41	39.434 ± 0.15
4g	H	CH_3	H	12.263 ± 0.36	18.387 ± 0.29	36.034 ± 0.59	38.891 ± 0.43
Ascorbic acid	–	–	–	11.585 ± 0.43	19.391 ± 0.33	34.837 ± 0.22	38.782 ± 0.40

3.2. Biology

The synthesized 1,3-oxazine derivatives were evaluated *in vitro* for their antibacterial activity against various microorganisms. These compounds exhibited a wide range of antibacterial activity against test microbes. The results are summarized in Tables 1 and 2. Compound **4b with** methoxy substituent showed excellent activity against all organisms tested. Compounds **4d** and **4f** containing chloro and dimethyl substituents inhibited to greater extent the growth of *S. pyogenes* and *E. coli*. Nitro substitution present in compound **4e** retarded the inhibitory effect of these compounds against test organisms, which may be due to electron withdrawing nature of this functional group. Compounds **4c** and **4g** containing CH_3 substituents exhibited moderate activity against *S. pyogenes* and *E. coli*. The absence of substitution in compound **4a** resulted in moderate activity against all test microorganisms.

Compounds **4b** and **4d** having methoxy and chloro substituents, respectively, demonstrated excellent antifungal activity by inhibiting spore germination of all test microorganisms, whereas compounds **4c** and **4f** containing methyl substituents displayed activity against *A. niger* and *A. flavus*. However, compounds **4g** and **4a** having methyl and no substitution, respectively, showed moderate activity against *A. niger* and *A. flavus.* Compound **4e** with nitro substituent exhibited lower activity against *A. niger*, *A. flavus* and *C. albicans*.

A freshly prepared DPPH solution shows a deep purple color with an absorption maximum at 517 nm. A change from the purple color to yellow indicates decrease in the absorbance. This is because antioxidant molecules reduce DPPH free radicals through donation of hydrogen atom. Hence, instantaneous or concomitant decrease in absorbance indicates the more potent antioxidant activity of a compound tested. The results of DPPH radical scavenging assay of the synthesized compounds **4a – 4g** are summarized in Table 3. These data indicate that all the synthesized compounds possess moderate to good activity due to their H-donating capacity. Compounds **4b** and **4f** having OCH_3 and 2, 4-CH_3 substituents, respectively, showed stronger DPPH scavenging activity than other compounds. Compounds **4c**, **4g,** and **4a** having methyl and no substitution show moderate activity; the chloro and nitro substituted compounds **4d** and **4e** shown lower activity as compared to that of standard ascorbic acid.

Nitric oxide plays a significant role in inflammatory processes. In the immunological system, it fights against tumor cells and infectious agents. During inflammatory reactions, nitric oxide is produced by the inducible enzyme nitric oxide synthase in cells like macrophages and renal cells after stimulation by lipopolysaccharides. NO react with oxygen or superoxide anion radical to form even stronger oxidant peroxynitrite [13]. The results of nitric oxide radical scavenging ability assay of compounds **4a – 4g** are summarized in Table 4. From these data, it was concluded that compounds **4f**, **4g** and **4b** having CH_3 and OCH_3 substituents in the phenyl ring exhibited stronger activity in scavenging $^{\bullet}NO$ radicals, while the remaining compounds **4a**, **4d,** and **4e** showed lower activity as compared to that of standard drug.

The hydroxyl radical is a highly reactive free radical formed in biological systems, which is able to damage biomolecules occurring in living cells. The hydroxyl radical has the ability to break DNA and cause strand breakage, thus contributing to carcinogenesis, mutagenesis, and cytotoxicity [14]. The results of hydroxyl radical scavenging ability assay of compounds **4a – 4g** are summarized in **Table-5**. From these data, it has been found that compounds **4a – 4g** possess variable (strong to weak) hydroxyl radical scavenging activity. Among the series studied, compound **4c**, **4f**, **4g,** and **4b** exhibited remarkable capacity for scavenging hydroxyl radical, which was significantly higher than that of standard BHA, whereas compounds **4a**, **4d,** and **4e** possess weak activity against hydroxyl radical.

CONCLUSION

In conclusion, we have demonstrated the synthesis of new coumarin based 1, 3-benzoxazine derivatives and evalu-

TABLE 5. Antioxidant Activity (*I*, %) of Compounds **4a – 4g** by Hydroxyl Radical Scavenging Assay

Entry	R^1	R^2	R^3	25 (μg mL^{-1})	50 (μg mL^{-1})	75 (μg mL^{-1})	100 (μg mL^{-1})
4a	H	H	H	07.670 ± 0.46	11.609 ± 0.17	25.170 ± 0.35	31.560 ± 0.22
4b	OCH_3	H	H	14.459 ± 0.74	20.382 ± 0.61	30.040 ± 0.56	36.699 ± 0.20
4c	CH_3	H	H	12.589 ± 0.48	19.101 ± 0.44	28.874 ± 0.57	35.817 ± 0.41
4d	H	H	Cl	04.686 ± 0.29	09.861 ± 0.90	22.719 ± 0.34	26.837 ± 0.66
4e	NO_2	H	NO_2	07.081 ± 0.76	12.613 ± 0.32	24.609 ± 0.83	30.504 ± 0.89
4f	CH_3	H	CH_3	14.207 ± 0.27	20.630 ± 0.28	29.439 ± 0.36	36.463 ± 0.27
4g	H	CH_3	H	11.930 ± 0.61	18.613 ± 0.35	27.260 ± 0.17	34.898 ± 0.47
Ascorbic acid	–	–	–	09.325 ± 0.39	14.113 ± 0.61	27.353 ± 0.59	32.621 ± 0.56

ated their antimicrobial and antioxidant activity. The synthesized compounds exhibited moderate to good antibacterial and antifungal activity against some of the test organisms. The obtained results revealed that compounds with methoxy and chloro substituents in the phenyl ring exhibited maximum antimicrobial activity. The new 1,3-oxazine derivatives exhibited free radical scavenging activity when tested on different models. Among these, methoxy and methyl substituents in the phenyl ring manifested profound antioxidant properties in all assays. It is commonly recognized that free radicals are responsible for numerous diseases. The obtained results prove that the free radical scavenging by 1,3-oxazines with OCH_3 and CH_3 substituents is evidence of potent antioxidant activity that can be used for treating several diseases. Triphosgene used for the synthesis of title compounds holds a benefit to other reagents, such as phosgene and diphosgene, in being a safe and easy to handle solid.

ACKNOWLEDGEMENTS

The authors are grateful to the University Grants Commission, New Delhi, for financial support through major research project grant (F: 42-230/2013 (SR) dated March 25, 2013). One of the authors, Renuka N, is grateful to the UGC for awarding NON-NET Fellowship (Order No. DV9/192/NON-NETFS/2013-14, dated November 11, 2013).

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Data article

Synthesis, crystal and molecular structure, Hirshfeld surface analysis of diethyl 2-(4-methylbenzylidene)malonate

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ARTICLE INFO

Article history:
Received 6 May 2016
Revised 10 June 2016
Accepted 12 June 2016
Available online 23 June 2016

Keywords:
Condensation
Malonate
Piperidine
X-ray diffraction
Molecular structure

ABSTRACT

Diethyl 2-(4-methylbenzylidene)malonate was synthesized by Knoevenagel condensation reaction of 4-methylbenzaldehyde and diethyl malonate in the presence of catalytic amount of piperidine and trifluoroacetic acid in benzene under reflux conditions. The product obtained was characterized by ^{1}H NMR, Mass spectroscopy and by X-ray diffraction studies. The title compound $C_{15}H_{18}O_4$ crystallizes in the monoclinic crystal system with space group C_2/c and unit cell parameters, $a = 18.210(4)$ Å, $b = 8.316(2)$ Å, $c = 20.130(4)$ Å, $\beta = 109.0(2)°$ and $Z = 8$. The crystal and molecular structure of the title compound is stabilized by C–H ···O hydrogen bond interactions.

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http://dx.doi.org/10.1016/j.cdc.2016.06.003
2405-8300/

Specifications table	
Subject area	*Organic chemistry, X-ray crystallography*
Compound	Diethyl 2-(4-methylbenzylidene)malonate
Data category	*^{1}H NMR, mass spectra, crystallographic data*
Data acquisition format	*CIF for crystallography*
Data type	*Analyzed*
Procedure	The compound $C_{15}H_{18}O_4$, Diethyl 2-(4-methylbenzylidene)malonate was synthesized and colorless rectangular block shaped crystals of the compound were obtained by slow evaporation technique. A single crystal of dimension 0.30 × 0.35 × 0.42 mm of the title compound was selected and X-ray intensity data were collected with χ fixed at 54° and φ, from 0° to 360° at a scan width of 0.5°, exposure time of 3 s and a sample to detector distance of 50.0 mm at 293 K.
Data accessibility	*CCDC **1477439**, URL:* https://summary.ccdc.cam.ac.uk/structure-summary-form

1. Rationale

Design and developing a procedure for the transformation of simple molecules in to a bioactive molecule with different functionalities is a worthwhile contribution in organic synthesis. Among the simple molecules α, β-unsaturated carbonyl compounds were proven as versatile intermediates in organic synthesis for the construction of various classes of bioactive molecules. The Knoevenagel condensation was most commonly employed method for preparation of α, β-unsaturated carbonyl compounds; it involves the reaction of aldehydes or ketones with active methylene compounds in the presence of bases [1,2]. Unsaturated systems such as alkenes were extensively used as intermediates in the synthesis of bioactive molecules such as pyarans [3], isoxazolines [4], pyrazolines [5,6], benzothiazepines [7–9] and cyclopropyl esters [10].

In view of wide applications associated with unsaturated system such as alkenyl derivatives, we herein report the synthesis, crystal growth, spectral analysis and single crystal X-ray diffraction studies of diethyl 2-(4-methylbenzylidene)malonate.

2. Procedure

2.1. Materials and methods

To the solution of diethyl malonate, **2** (0.0166 mol) in dry benzene (20 ml), piperidine (0.0016 mol) and trifluoroacetic acid (0.0016 mol) were added; the mixture was stirred at room temperature for 15 min. After this, a solution of 4-methylbenzaldehyde, **1** (0.0016 mol) in dry benzene was added. The mixture was refluxed on a water bath for 6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice cold water. The solid separated was filtered, washed with ice cold water and crystallized from ethyl acetate to obtain diethyl 2-(4-methylbenzylidene)malonate, **3** as colorless rectangular block like crystals in 85% yield, m.p. 47–50 °C. The schematic diagram of the reaction is depicted in Fig. 1.

Spectral data: ^{1}H NMR spectra was recorded on Agilent-NMR 400 MHz spectrophotometer in $CDCl_3$. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrophotometer TOF mode. ^{1}H NMR ($CDCl_3$): δ 1.28 (t, 6H, CH_3), 2.28 (s, 3H, CH_3), 4.17 (q, 4H,

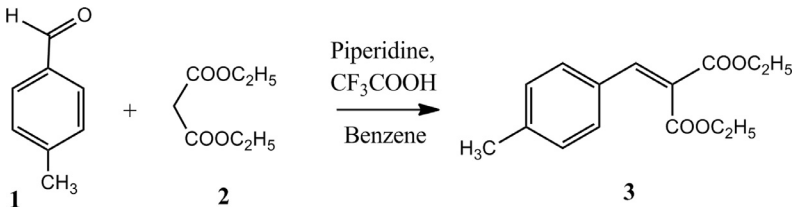

Fig. 1. Schematic diagram of the synthesis of diethyl 2-(4-methylbenzylidene)malonate.

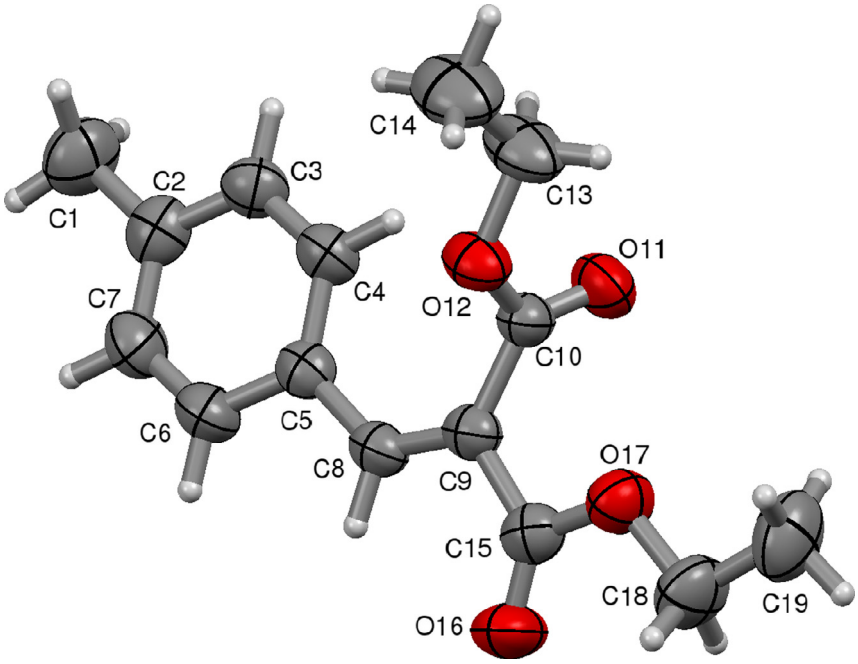

Fig. 2. *ORTEP* of the molecule with numbering scheme for non hydrogen atoms drawn at 50% probability level.

Table 1
Crystal data and structure refinement details.

CCDC number	CCDC **1477439**
Empirical formula	$C_{15}H_{18}O_4$
Formula weight	262.29 g mol^{-1}
Temperature	293 K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, C_2/c
Unit cell dimensions	a = 18.21(4) Å
	b = 8.32(2) Å
	c = 20.13(4) Å
	β = 109.0(2)°
Volume	2882.0(1) Å ^{3}
Z, calculated density	8, 1.209 Mg/m^3
Absorption coefficient	0.087 mm^{-1}
F_{000}	1120
Crystal size	0.30 × 0.35 × 0.42 mm
Theta ranges for data collection	3.1°–24.7°
Limiting indices	$-21 \le h \le 14$, $-9 \le k \le 7$, $-16 \le l \le 23$
Absorption correction	Multi-scan, T_{min}= 0.964, T_{max} = 0.974
Reflections collected/unique	3757 / 2420 (R_{int} = 0.094)
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2420/ 0 / 177
Goodness-of-fit on F^2	1.30
Final R indices [I > 2sigma(I)]	$R1$ = 0.0767, w$R2$ = 0.2191
R indices (all data)	$R1$ = 0.1077, w$R2$ = 0.2453
Largest diff. peak and hole	0.26 and −0.22 e. Å^{-3}

Table 2
Bond lengths and angles (Å, °).

C1–C2	1.501(6)	C9–C15	1.480(5)
C2–C7	1.361(5)	C10–O11	1.187(4)
C2–C3	1.364(5)	C10–O12	1.311(4)
C3–C4	1.373(5)	O12–C13	1.452(5)
C4–C5	1.380(5)	C13–C14	1.423(6)
C5–C6	1.384(5)	C15–O16	1.182(4)
C5–C8	1.454(5)	C15–O17	1.325(4)
C6–C7	1.367(6)	O17–C18	1.447(5)
C8–C9	1.320(5)	C18–C19	1.469(6)
C9–C10	1.474(5)		
C7–C2–C3	117.6(3)	C8–C9–C15	118.7(3)
C7–C2–C1	121.4(4)	C10–C9–C15	115.6(3)
C3–C2–C1	121.0(3)	O11–C10–O12	123.8(3)
C2–C3–C4	122.2(3)	O11–C10–C9	124.0(3)
C3–C4–C5	120.4(3)	O12–C10–C9	112.2(3)
C6–C5–C4	116.9(3)	C10–O12–C13	115.5(3)
C6–C5–C8	117.9(3)	C14–C13–O12	109.2(4)
C4–C5–C8	125.1(3)	O16–C15–O17	124.0(3)
C7–C6–C5	121.6(3)	O16–C15–C9	125.1(3)
C2–C7–C6	121.3(3)	O17–C15–C9	110.9(3)
C9–C8–C5	131.6(3)	C15–O17–C18	116.9(3)
C8–C9–C10	125.6(3)	O17–C18–C19	107.2(4)

Table 3
Torsion angles (°).

C7–C2–C3–C4	−0.2(5)	C8–C9–C10–O11	−101.7(4)
C1–C2–C3–C4	−179.4(3)	C15–C9–C10–O11	75.6(4)
C2–C3–C4–C5	0.7(5)	C8–C9–C10–O12	77.9(4)
C3–C4–C5–C6	−0.8(5)	C15–C9–C10–O12	−104.8(3)
C3–C4–C5–C8	177.4(3)	O11–C10–O12–C13	0.4(5)
C4–C5–C6–C7	0.5(5)	C9–C10–O12–C13	−179.3(3)
C8–C5–C6–C7	−177.9(3)	C10–O12–C13–C14	174.4(4)
C3–C2–C7–C6	−0.2(5)	C8–C9–C15–O16	6.0(5)

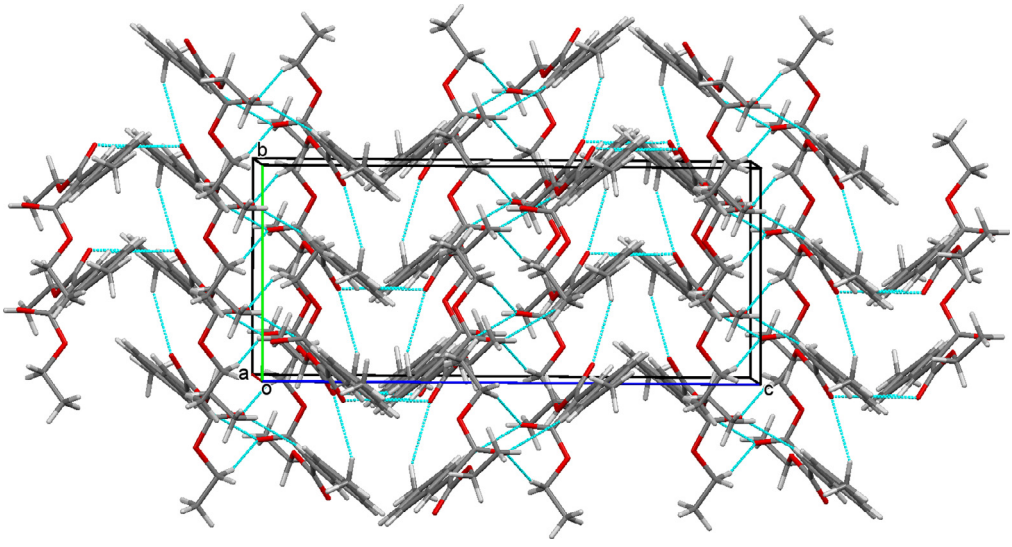

Fig. 3. The packing of molecules when viewed down along *a* axis. The dotted lines indicate C–H···O hydrogen bond interactions.

OCH_2), 7.19 (d, 2H, Ar–H), 7.57 (d, 2H, Ar–H), 8.21 (s, 1H, CH=C). MS (*m/z*) for $C_{15}H_{18}O_4$: 262 (M+, 100).

2.1.1. X-ray intensity data collection procedure

A colorless rectangular block shaped single crystal of dimension 0.30 × 0.35 × 0.42 mm of the title compound was selected and X-ray intensity was collected with χ fixed at 54° and φ, from 0° to 360°, scan width at 0.5°, exposure time of 3 s and the sample to detector distance of 50.0 mm at a temperature 293 K on Rigaku XtaLAB Mini X-ray diffractometer operating at 50 kV and 12 mA with MoK_α radiation of wavelength $\lambda = 0.71073$ Å. A complete data set was processed by *CRYSTAL CLEAR* [11]. The structure was solved by direct methods and refined by full-matrix least squares method on F^2 using *SHELX* [12]. After several cycles of refinement, the final difference Fourier map showed peaks of no chemical significance and the residue is saturated to 0.0767. The geometrical calculations were carried out using *PLATON* [13]. The molecular and packing diagrams were generated using *MERCURY* [14].

Table 4
Hydrogen bond geometry (Å, °).

D–H···*A*	*D*–H	H···*A*	*D*···*A*	*D*–H···*A*
C13–H13B···O11$^{(i)}$	0.97	2.57	3.515 (10)	165
C4–H4···O11$^{(i)}$	0.93	2.67	3.376 (8)	134
C1–H1C···O16$^{(ii)}$	0.96	2.64	3.527 (10)	153

Symmetry code: (i) ½ −*x*, ½ −*y*, 1−*z*; (ii) ½ +*x*, ½ +*y*, *z*.

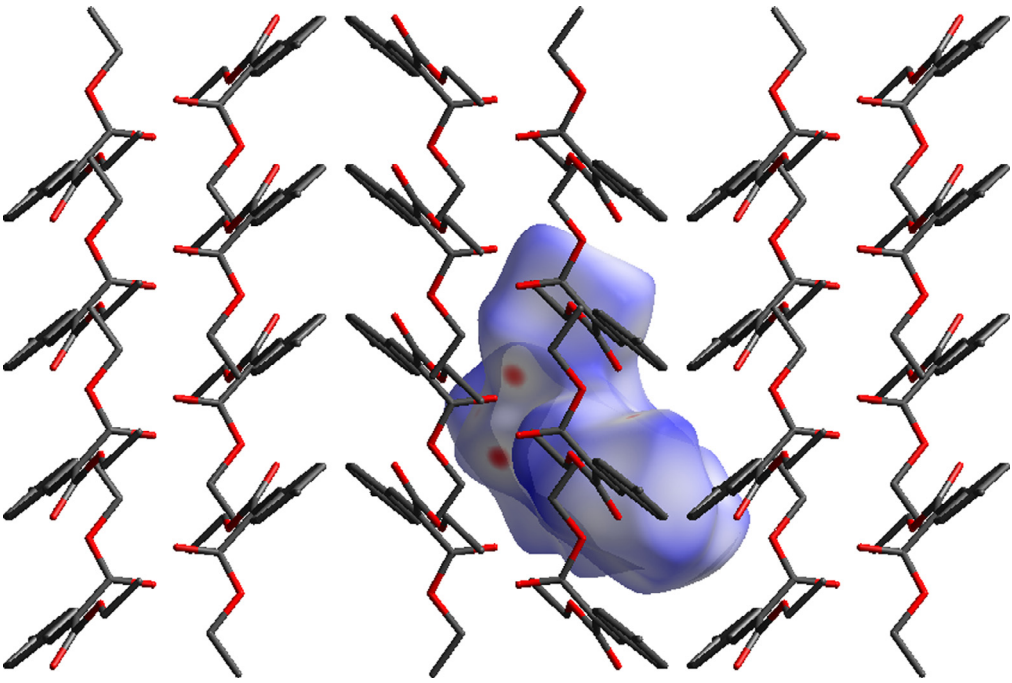

Fig. 4. d_{norm} mapped on the Hirshfeld surface for visualizing the intercontacts of the molecule. Color scale between −0.050 au (blue) and 1.100 au (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Data, value and validation

Single crystal X-ray diffraction studies revealed that the compound diethyl 2-(4-methylbenzylidene)malonate is crystallized in the monoclinic crystal system with the space group C_2/c and with unit cell parameters, $a = 18.210(4)$ Å, $b = 8.316(2)$ Å, $c = 20.130(4)$ Å; $\beta = 109.0(2)^\circ$ and $V = 2882.0(1)$ Å^3. The *ORTEP* of the molecule with displacement ellipsoids drawn at 50% probability level is shown in Fig. 2, which was obtained by a FORTRAN plot program [15].

The crystal data and structure refinement details are given in Table 1. The bond lengths and bond angles are given in Table 2. The molecules are packed layer-by-layer to form a staircase network, leading to a three dimensional supramolecule structures as shown in Fig. 3. The torsion angles are listed in Table 3.

In a molecule, diethyl 2-(4-methylbenzylidene)malonate, a torsion angle of −101.7(4)° about C8–C9–C10–O11 indicates that the segment C10–O11 is in an *–Anti-Clinal* conformation with the mean plane described by C1/C2/C3/C4/C5/C6/C7/C8 of the 4-methylbenzylidene moiety. The torsion angle 6.0(5)° of C8–C9–C15–O16 shows that the segment C15–O16 is in a *+ Syn-Periplanar* conformation with respect to the mean plane described by C1/C2/C3/C4/C5/C6/C7/C8 of 4-methylbenzylidene moiety. The dihedral angles 79.77(2)° and 14.37(2)° suggested that the plane

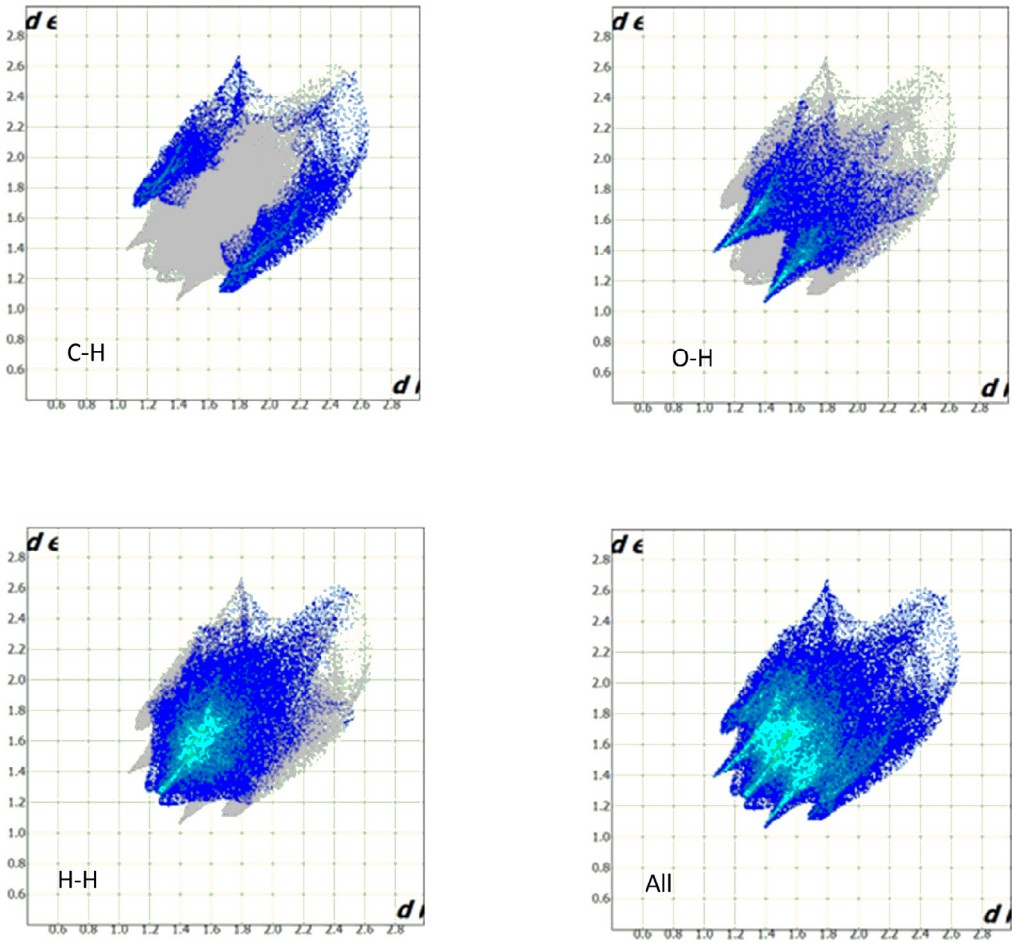

Fig. 5. Fingerprint plots of the molecule.

of the benzene ring C2/C3/C4/C5/C6/C7 lies in an equatorial orientation with respect to the mean plane of C9/C10/O11/O12/C13, and lies in an axial orientation with respect to the mean plane of C5/C8/C9/C15/O16/O17/C18 of malonate moiety.

The molecules also exhibit *Cg*···*Cg* interaction; *Cg1*···*Cg1* (*Cg1* is the centroid of the ring C2/C3/C4/C5/C6/C7) with a *Cg*–*Cg* distance of 4.923(1) Å, the dihedral angle between the mean planes of the benzene rings of the neighboring diethyl 2-(4-methylbenzylidene)malonate molecules being 80.15(2)°, $\beta = 7.9°$, $\gamma = 72.4°$, a perpendicular distance of *Cg1* on ring C2/C3/C4/C5/C6/C7 of the adjacent molecule = − 1.488(1) Å and a perpendicular distance of *Cg1* on ring C2/C3/C4/C5/C6/C7 of the parent molecule = 4.876(1) Å with a symmetry code ½ −*x*, ½+*y*, ½ −*z*. The crystal and molecular structure is stabilized by the C–H···O intermolecular hydrogen bond interactions summarised in Table 4, and were found similar to the crystal structure of dimethyl 2-(4-ethylbenzylidene)malonate [16].

The Hirshfeld surface analysis [17–18] was carried in order to visualize the intercontacts in the molecular structure using *CRYSTAL EXPLORER* [19]. The Hirshfeld surface volume and surface area were 353.36 Å^3 and 333.42 Å^2 respectively. The Hirshfeld surface for the molecule diethyl 2-(4-methylbenzylidene)malonate is shown in Fig. 4 and has been mapped over a d_{norm} range of −0.1–1.75 Å.

Hydrogen bond interactions were visualized with the help of dark red spots on the Hirshfeld surface, as a result of hydrogen bond acceptors C13–H13B···O11, C4–H4···O11 and C1–H1C···O16. The combination of d_e and d_i in the form of two-dimensional fingerprint plot [20] gives the summary of intermolecular contacts in the crystal lattice. The fingerprint plots of a molecule diethyl 2-(4-methylbenzylidene)malonate are shown in Fig. 5.

The H···H inter contacts appear as a pair of close blunt spikes of light sky-blue colour in the region 1.15 Å < (*de* + *di*) < 1.25 Å and O···H inter contacts as a pair of very sharp spikes in the range 1.05 Å < (*de* + *di*) < 1.40 Å of the full fingerprint plots. The fingerprint plots can be separated to highlight the inter contacts of a particular pair of atoms. The contributions of C···H, O···H and H···H inter contacts to the Hirshfeld surface are 19%, 26% and 55% respectively.

Acknowledgments

The authors thank the National single crystal X-ray diffractometer facility, Department of Studies in Physics, University of Mysore, Mysuru-570 006, India, for providing the X-ray intensity data.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cdc.2016.06.003.

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Bioorganic & Medicinal Chemistry Letters 23 (2013) 6406–6409

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Bioorganic & Medicinal Chemistry Letters

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Synthesis and biological evaluation of novel formyl-pyrazoles bearing coumarin moiety as potent antimicrobial and antioxidant agents

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ARTICLE INFO

Article history:
Received 6 July 2013
Revised 14 September 2013
Accepted 18 September 2013
Available online 26 September 2013

Keywords:
Antibacterial
Antifungal
Antioxidant
Heterocycles
MIC

ABSTRACT

A series of coumarin appended formyl-pyrazoles **14**–**18** were synthesized by a simple and accessible approach. The reaction of 8-acetyl-4-methyl-7-hydroxy coumarin **3** and phenyl hydrazine hydrochlorides **4**–**8** produces the intermediate compounds 8-acetyl-4-methyl-7-hydroxy coumarin hydrazones **9**–**13**. The reaction of compounds **9**–**13** and DMF in the presence of $POCl_3$ yielded formyl-pyrazoles bearing coumarin moiety **14**–**18** in good yield. The synthesized new compounds **14**–**18** and the intermediates 8-acetyl-4-methyl-7-hydroxy coumarin hydrazones **9**–**13** prepared were screened in vitro for their antibacterial, antifungal antioxidant activities. The compounds **12** and **17** having chloro substitution exhibited promising antifungal and antibacterial activity against the different organisms tested. The compound **17** showed remarkable DPPH radical scavenging ability.

Coumarins are chemically known as 2*H*-1-benzopyran-2-ones and were first identified in 1820s as an oxygen heterocycle. Alternariol is chemically a 3,7,9-trihydroxy-1-methyl-6*H*-benzo[*c*] chromen-6-one, a toxic metabolite of *Alternaria* fungi and is an important contaminant in cereals and fruits exhibiting antifungal and phytotoxic activity.[1] Coumarins are widely distributed in plants, for example, umbelliferone (7-hydroxy coumarin) was found in *Apiaceae*, osthole (7-methoxy-8-(3-methylbut-2-en-1-yl)coumarin) was found in *Cnidium monnieri* and scoparone (6,7-dimethoxy coumarin) was found in *Artemisia scoparia*.[2] In the recent years coumarins have attracted great attention because of their synthetic utility as building blocks for the synthesis of biologically potent molecules. Coumarin derivatives exhibit enormous amount of biological activities such as antioxidant, antimicrobial, anti-HIV, antibiotic, anticancer, muscle relaxant, anti-inflammatory and anti-coagulant activity.[3] Further they are widely used as perfumes, additives in food, chemical, laser dyes, optical brightening agents and cosmetics.

The discovery of antipyretic action of a pyrazole derivative in man by Knorr in 1884 created interest in the researchers. Pyrazoles represent a key motif in heterocyclic chemistry and occupy a prime place in medicinal chemistry due to their competence to exhibit a wide range of pharmacological activities such as antimicrobial,[4] anticancer,[5] anti-inflammatory,[6] anticonvulsant,[7] antipyretic activities.[8] Pyrazoles having a functional group like aldehyde or carboxylate C-4 position have shown promising antimicrobial properties.[9] Pyrazole incorporated with coumarin was synthesized and observed in the enhancement of pharmacological effect.[10] When one biodynamic heterocyclic system was coupled with another heterocyclic system, enhanced biological activity was observed.

With this in view, this project was undertaken to synthesize a series of new pyrazoles bearing coumarin nucleus. We herein report the synthesis of series of novel heterocyclic scaffolds formyl-pyrazoles bearing coumarin nucleus and in vitro screening of the synthesized compounds for their antibacterial, antifungal and antioxidant activities.

In this work we intended to introduce the pyrazole fragment to the coumarin skeleton in order to build a novel family of bioactive molecules. Thus a series of pyrazole derivatives **14**–**18** were synthesized starting from 4-methyl-7-hydroxy coumarin **1**. Pechmann reaction has been widely used for the synthesis of coumarins because of its preparative simplicity and inexpensive starting material.[11] 4-Methyl-7-hydroxy coumarin **1** was prepared by the resorcinol with ethyl acetoacetate in the presence of sulfuric acid as a catalyst.[12]

4-Methyl-7-hydroxy coumarin **1** was converted to an ester **2** with acetic anhydride by a standard procedure; then the ester was subjected to Fries rearrangement at 140–160 °C to get 8-acetyl-4-methyl-7-hydroxy coumarin **3**, the product characteristics are in good agreement with the reported results.[13] The condensation of the compound **3** with substituted phenylhydrazines **4**–**8** in ethyl alcohol and a catalytic amount of acetic acid at

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http://dx.doi.org/10.1016/j.bmcl.2013.09.053

water bath reflux conditions, produced the corresponding hydrazones **9–13**.[14]

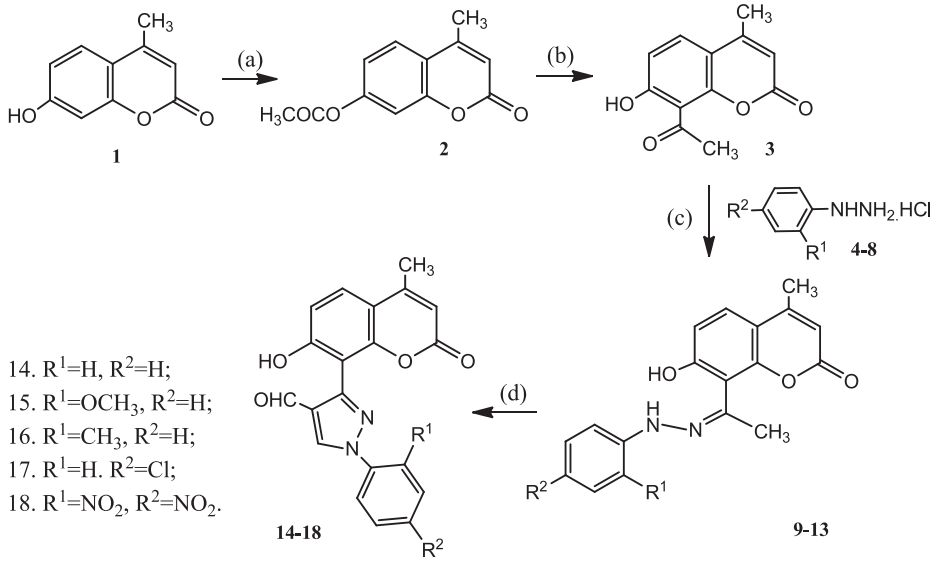
The hydrazones **9–13** (0.0032 mol) were added to the Vilsmeier-Haack reagent prepared by drop-wise addition of $POCl_3$ (1.2 mL) in ice cooled DMF (10 mL). The mixture was stirred at 60–65 °C for 6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice cold water, neutralized with $NaHCO_3$, the solid separated was filtered, washed with water and recrystallized from ethanol to obtain target molecules 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehydes **14–18** in good yield.[15] The reaction pathway is illustrated in Scheme 1.

Designed series of molecules **14–18** were characterized by spectral and CHN analysis[15] before being evaluated for in vitro antimicrobial and antioxidant activities. For instance, all new compounds showed a sharp and strong absorption band in the region 1660–1671 cm^{-1} in the IR spectrum, which is due to C=O str. of newly formed –CHO functional group. ^1H NMR spectrum of the compounds showed singlet in the region δ 10.70–10.82 ppm. These results supported the presence of –CHO functional group in the products. Furthermore, all compounds showed signals due to aromatic, substituent protons in the expected region. MS results and elemental analysis data confirmed the formation of these compounds.

Minimum inhibitory concentrations (MICs) of the synthesized compounds **9–18** against different bacterial and fungal strains were determined by broth dilution technique. Gram-negative bacteria species *Escherichia coli*, *Pseudomonas aeruginosa*, Gram-positive bacteria species *Staphylococcus aureus*, *Streptococcus pyogenes* were used as bacterial strains and *Cryptococcus neoformans*, *Aspergillus nigar*, *Aspergillus flavus*, *Candila albicans* were used as fungal strains. The antibiotics Ciprofloxacin and Amphotericin B were used as standard drugs against bacteria and fungi species respectively. The experiments were carried out in triplicate; the results were taken as a mean of three determinations. The results of MIC's of the synthesized compounds against bacteria species are summarized in Table 1; and against fungal species in Table 2.

All the synthesized compounds hydrazones and formyl-pyrazoles exerted a wide range of modest in vitro antibacterial activity against all the tested organisms. However, the compound **9** failed to inhibit the growth of *S. pyogenes* and *E. coli* even at a higher concentration of 200 μg/mL. Similarly, compound **10** failed to inhibit *P. aeruginosa*; **13** failed to inhibit *E. coli* and *P. aeruginosa*; and **14** failed to inhibit *S. aureus* and *S. pyogenes* organisms.

The presence of chloro substitution in compounds **12**, **17** influenced these molecules to exhibit inhibition to greater extent against the organisms tested. Nitro substitution present in compounds **13**, **18** retarded the inhibitory effect of these compounds against the organism tested, which may be due to electron withdrawing nature of this functional group. The presence of electron donating groups at ortho position or no substitution resulted with moderate activity.



Scheme 1. Synthesis of compounds **9–13** and **14–18**. Reagents and conditions: (a)Ac_2O; (b) Anh. $AlCl_3$, 140–160 °C, reflux, 2 h; (c) CH_3 COOH, C_2H_5OH; (d) DMF, $POCl_3$, 60–65 °C, 5–6 h.

Table 1
MIC's of the test compounds (**9–18**) against bacteria species

Compound	Minimum inhibitory concentration (MIC's) in μg/mL			
	Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*
09	40	–	–	25
10	30	60	50	–
11	40	50	75	100
12	20	50	50	25
13	50	75	–	–
14	–	–	30	50
15	30	50	75	25
16	30	50	40	50
17	15	35	30	15
18	40	75	40	20
Ciprofloxacin	25	50	25	12.5

Table 2
MIC's of the test compounds **9–18** against fungi species

Compound	Minimum inhibitory concentration (MIC) in μg/mL			
	Cryptococcus neoformans	Aspergillus niger	Aspergillus flavus	Candila albicans
09	50	75	75	50
10	40	50	40	30
11	40	75	–	–
12	25	60	50	30
13	–	–	–	–
14	50	100	–	–
15	25	50	25	50
16	30	60	–	–
17	15	40	60	25
18	–	–	–	–
Amphotericin B	25	50	50	25

Table 3
DPPH Radical Scavenging ability of the compounds **14–18** relative to the standard antioxidant BHT

Test Samples	% Radical Scavenging activity*				
	10 (μg/mL)	20 (μg/mL)	30 (μg/mL)	40 (μg/mL)	50 (μg/mL)
14	39.36 ± 0.81	42.12 ± 0.78	46.42 ± 0.85	50.12 ± 0.88	57.76 ± 0.94
15	11.10 ± 0.89	11.98 ± 0.89	13.33 ± 0.92	15.63 ± 0.86	17.36 ± 0.98
16	12.80 ± 0.81	13.81 ± 1.01	14.11 ± 0.68	15.12 ± 0.83	17.10 ± 0.74
17	48.86 ± 0.98	53.33 ± 0.78	58.15 ± 0.78	63.89 ± 0.98	69.35 ± 0.98
18	34.67 ± 0.71	41.93 ± 1.00	47.12 ± 1.00	51.07 ± 0.82	56.02 ± 1.00
Control	at a 0 μg/mL concentration 0.00 ± 0.00				

* Results are expressed as mean ± standard deviation ($n = 3$).

All the synthesized compounds **9–18** exerted a moderate to good in vitro antibacterial activity against all the tested organisms, except **13**, **18** which failed to exhibit inhibition against all the organisms. However, the compounds **11**, **14**, **16** failed to inhibit the growth of *A. flavus* and *C. albicans* even at a higher concentration of 200 μg/mL. The compounds **12**, **17** having chloro substitution exhibited remarkable activity against all the organisms tested, while the remaining compounds exhibited moderate activity.

The antioxidant activity of synthesized compounds **14–18** was carried out by DPPH radical scavenging assay using butylated hydroxyl toluene (BHT) as standard antioxidant.[16] The experiments were carried out at five different concentrations in triplicates; the results are expressed as mean ± standard deviation (SD) and were summarized in Table 3.

The compounds **14–18** showed promising DPPH free radical scavenging ability, but of lesser activity compared with the standard antioxidant. The results reveal that, all exhibited poor radical scavenging ability at lower concentrations. However, the gradual increase in the activity in all the cases was observed with increase in the concentrations of the test compounds. Among the compounds tested, the compound **17** having chloro substituent in the aromatic ring exhibited promising radical scavenging ability. The compounds **14** and **18** having no and two nitro substituents in the aromatic ring showed moderate radical scavenging abilities; while **15** and **16** having methoxy and methyl substituent respectively in the aromatic ring showed poor radical scavenging abilities in comparison with the standard antioxidant BHT. The presence of electron donating methoxy and methyl groups; stereo chemical factors due to *ortho* substitution might be the cause for lesser activity associated with the compounds **15** and **16**.

The reactions described represent a simple access to the synthesis of coumarin based pyrazoles derivatives of potential interest from the readily available materials. The antibacterial, antifungal and antioxidant properties of the synthesized compounds show promising activity. However, the structure–activity relationship remains of interest. From the results of biological activity, it is concluded that, these molecules can be designed as potential drugs with a slight modification in the structure of the molecules.

Acknowledgment

The authors are grateful to the University Grants Commission, New Delhi for financial assistance, Dr. B.N. Mylarappa, Rangos Research Center, University of Pittsburgh, PA 15201, USA, for help in biological activity studies.

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14. [Crystallized from ethyl alcohol, **9**: yield 80%, mp 170–172 °C; 10: yield 78%, mp 98–100 °C; 11: yield 62%, mp 138–140 °C; 12: yield 74%; 13: yield 70%, mp 116–118 °C].
15. *[3-(7-Hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde **14**:* Obtained as light yellow solid in 75% yield, mp 220–222 °C. IR (Nujol): 1660, 1745, 3210 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.43 (s, 3H), 6.20 (s, 1H),

7.03 (d, 1H), 7.41 (d, 1H), 7.68–8.02 (m, 5H), 9.32 (s, 1H), 9.70 (s, 1H), 10.72 (s, 1H). MS (*m*/*z*): 347 (M+1), 319, 271, 250, 177 (base peak). Anal. Calcd for $C_{20}H_{14}N_2O_4$: C, 69.36; H, 4.07; N, 8.09; Found: C, 69.36; H, 4.15; N, 8.15.

3-(7-Hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-(2-methoxy-phenyl)-1H-pyrazole-4-carbaldehyde ***15****:* Obtained as orange solid in 78% yield; mp 108–110 °C. IR (Nujol): 1671, 1740, 3228 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.49 (s, 3H), 3.46 (s, 3H), 6.26 (s, 1H), 7.09 (d, 1H), 7.40 (d, 1H), 7.52–7.83 (m, 4H), 9.36 (s, 1H), 9.70 (s, 1H), 10.75 (s, 1H). MS (*m*/*z*): 361 (M+1), 333, 271, 255, 177 (base peak). Anal. Calcd for $C_{21}H_{16}N_2O_5$: C, 67.02; H, 4.28; N, 7.44; Found: C, 67.08; H, 4.23; N, 7.35.

3-(7-Hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1-(2-methylphenyl)-1H-pyrazole-4-carbaldehyde ***16****:* Obtained as light orange solid in 64% yield; mp 206–208 °C. IR (Nujol): 1662, 1760, 3218 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.12 (s, 3H), 2.48 (s, 3H), 6.22 (s, 1H), 7.08 (d, 1H), 7.46 (d, 1H), 7.52–7.66 (m, 4H), 9.38 (s, 1H), 9.79 (s, 1H), 10.80 (s, 1H). Anal. Calcd for $C_{21}H_{16}N_2O_4$: C, 69.99; H, 4.48; N, 7.77; Found: C, 69.83; H, 4.28; N, 7.43.

1-(4-Chloro-phenyl)-3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1H-pyrazole-4-carbaldehyde ***17****:* Obtained as light orange solid in 60% yield. IR (Nujol): 1668, 1752, 3220 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.44 (s, 3H), 6.18 (s, 1H), 7.03 (d, 1H), 7.43 (d, 1H), 7.56 (m, 4H), 9.35 (s, 1H), 9.71 (s, 1H), 10.77 (s, 1H). MS (*m*/*z*): 382 [M^+, ^{37}Cl], 380 [M^+, ^{35}Cl, 352, 270, 255, 177 (base peak). Anal. Calcd for $C_{20}H_{13}ClN_2O_4$: C, 63.08; H, 3.44; N, 7.36; Found: C, 63.03; H, 3.44; N, 7.26.

1-(2,4-Dinitro-phenyl)-3-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)-1H-pyrazole-4-carbaldehyde ***18****:* Obtained as brown solid in 71% yield; mp 130–132 °C. IR (Nujol): 1665, 1748, 3215 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.46 (s, 3H), 6.13 (s, 1H), 7.23 (d, 1H), 7.42 (d, 1H), 7.82 (d, 1H), 8.34 (d, 1H), 8.98 (s, 1H), 9.33 (s, 1H), 9.76 (s, 1H), 10.81 (s, 1H). Anal. Calcd for $C_{20}H_{12}N_4O_8$: C, 55.05; H, 2.77; N, 12.84; Found: C, 55.12; H, 2.68; N, 12.96.]

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Chemical Data Collections 5–6 (2016) 36–45

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Synthesis, crystal and molecular structure of ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate: Studies on antioxidant, antimicrobial activities and molecular docking

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ARTICLE INFO

Article history:
Received 2 July 2016
Revised 29 September 2016
Accepted 11 October 2016
Available online 25 October 2016

Keywords:
Antimicrobial
Antioxidant
Packing
Single crystal
Spectral studies
X-ray diffraction

ABSTRACT

Ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate was synthesized by Knoevenagel condensation reaction of 4-chlorobenzaldehyde and ethyl acetoacetate in the presence of catalytic amount of piperidine and trifluoroacetic acid in benzene under reflux conditions. The structure proof of the synthesised molecule was obtained by spectral studies and was confirmed by X-ray diffraction studies. The title compound crystallizes in the monoclinic crystal system under the space group P21/n. The structure adopts a *Z* conformation about the C=C bond. The synthesised compound was evaluated in vitro for its antimicrobial and antioxidant susceptibilities.

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http://dx.doi.org/10.1016/j.cdc.2016.10.002
2405-8300/

Specifications table

Subject area	Chemical physics
Compound	Ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate
Data category	Synthesis, ^{1}H NMR, mass spectra, crystallographic data
Data acquisition format	CIF for crystallography
Data type	Analyzed
Procedure	The compound $C_{13}H_{13}ClO_3$, Ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate was synthesized and yellow rectangular shaped crystals of the compound were obtained by slow evaporation technique. A single crystal of dimension $0.29 \times 0.25 \times 0.23$ mm of the title compound was selected and X-ray intensity data were collected with a 2θ value fixed 46.6° and φ, from 0° to 90° at a scan width of 0.5°, exposure time of 2 s and a sample to detector distance of 45.10 mm at temperature 296 K.
Data accessibility	CCDC **1489216** URL: HYPERLINK " https://www.ccdc.cam.ac.uk/conts/retrieving.html

1. Rationale

α,β-Unsaturated carbonyl compounds were synthesised usually by Knoevenagel condensation of aromatic aldehydes with active methylene compounds, these were proved to be versatile intermediates in organic synthesis for the construction of wide range of bioactive molecules. The exploitation of these simple molecules for the synthesis of pharmaceutically important heterocycles is a worthwhile contribution in the field of heterocycles. Among the various methods developed for the synthesis of α, β-unsaturated ketones, the condensation reaction of aldehydes with active methylene compounds in the presence of bases was more commonly employed [1,2]. Condensation of the *p*-chlorobenzaldehyde and ethyl acetoacetate in benzene with a catalytic amount of piperidine and trifluoro acetic acid offers chlorobenzylidine chalcones, which acts as good Michael-addition acceptors and can be used directly in the Diels–Alder reaction and are of wide synthetic utilities in pharmaceutical industries [3]. An enzyme Lipase lipoprotein (LPL) isolated from *A. niger* proved to be the effective catalyst for the Knoevenagel condensation of aromatic aldehydes and active methylene compounds in a green protocol, it improves reaction efficiency that includes, the conditions, solvent, enzyme loading and temperature [4].

The stereochemical assignment for the α, β-unsaturated ketones were made on the basis of DPFGSE-NOE (Double Pulse Field Gradient Spin Echo NOE) experiments [5]. These compounds have been extensively used as precursors in the synthesis of biologically active molecules. Reaction of α-benzotriozyl α,β-unsaturated ketones with hydroxylamine and monosubstitted hydrazines produced 2,5-disubstituted isoxazoles and 1,3,5-triaryl-4-alkylpyrazolines with high regioselectivity [6]. α,β-Unsaturated ketones with varied electron withdrawing groups in the active methylene compounds moiety were employed in the synthesis of substituted thiazepines [7–9], isoxazoles [10], pyrazoles [11], cyclopropyl esters [12]. In view of synthetic utilities and biological activities associated with α, β-unsaturated ketones, we herein report the synthesis, spectral analysis, single crystal X-ray diffraction studies, antioxidant and antimicrobial activities and molecular docking studies of ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate.

2. Procedure

2.1. Materials and methods

To the solution of ethyl acetoacetate, **2** (0.016 mol) in dry benzene (15 ml), piperidine (0.0016 mol) and trifluoro acetic acid (0.0016 mol) were added; the mixture was stirred for 15 minutes. After this, 4-chlorobenzaldehyde, **1** (0.016 mol) was added and then the mixture was refluxed on a water bath for 5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice cold water. The solid separated was filtered, washed with ice cold water and crystallized from ethyl acetate to obtain target molecule ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate, **3** as yellow rectangular slab like crystals in 82% yield, m.p. 85–86 °C. The synthetic pathway of the reaction is depicted in Fig. 1.

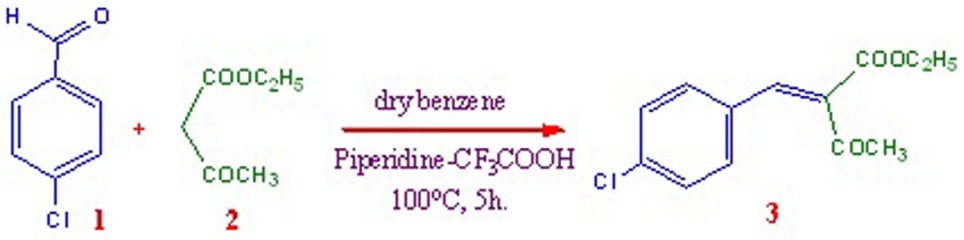

Fig. 1. Reaction pathway for the synthesis of ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate.

Spectral data: ^{1}H NMR spectra was recorded on Agilent-NMR 400 MHz spectrometer in $CDCl_3$ with TMS as an internal standard. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrometer TOF mode. ^{1}HNMR ($CDCl_3$): δ 1.252–1.291 (t, 3H, CH_3), 2.400 (s, 3H, $COCH_3$), 4.2892–4.345 (q, 2H, OCH_2), 7.250–7.351 (dd, 2H, Ar-H), 7.357–7.371 (dd, 2H, Ar-H), 7.496 (s, 1H, CH=C) (Fig. S1). MS (m/z) for $C_{13}H_{13}ClO_3$: 254 (M+, ^{37}Cl, 32), 252 (M+, ^{35}Cl, 100) (Fig. S2).

2.2. X-ray intensity data collection

A pale yellow coloured rectangle shaped single crystal of dimensions 0.29 × 0.25 × 0.23 mm of the title compound was chosen for an X-ray diffraction study. The X-ray intensity data were collected at a temperature of 296 K on a Bruker Proteum2 CCD diffractometer equipped with an X-ray generator operating at 45 kV and 10 mA, using CuK_α radiation of wavelength 1.54178 Å. Data were collected for 24 frames per set with different settings of φ (0° and 90°), keeping the scan width of 0.5°, exposure time of 2 s, the sample to detector distance of 45.10 mm and 2θ value at 46.6°. A complete data set was processed using *SAINT PLUS* [13]. The structure was solved by direct methods and refined by full-matrix least squares method on F^2 using *SHELXS* and *SHELXL* programs [14]. All the non-hydrogen atoms were revealed in the first difference Fourier map itself. All the hydrogen atoms were positioned geometrically (C–H = 0.93 Å, O–H = 0.82 Å) and refined using a riding model with $U_{iso}(H) = 1.2\ U_{eq}$ and 1.5 U_{eq} (O). After ten cycles of refinement, the final difference Fourier map showed peaks of no chemical significance and the residuals saturated to 0.0370. The geometrical calculations were carried out using the program *PLATON* [15]. The molecular and packing diagrams were generated using the software *MERCURY* [16].

2.3. Antioxidant activity

Antioxidant property of the synthesized ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate was carried out by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [17] using ascorbic acid as standard. Compound dissolved in methanol (0–50 µg/ml; 0–5 µg/ml for ascorbic acid) in 200 µl aliquot was mixed with 100 mM tris-HCl buffer (800 µl, pH 7.4) and then added 1 ml of 500 µM DPPH in ethanol (final concentration of 250 µM). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The experiments were performed in triplicates; the results are expressed as mean ± standard deviation (SD).

2.4. Antimicrobial activity

Minimum inhibitory concentrations (MICs) were determined by serial dilution technique [18]. The nutrient broth, which contain logarithmic serially two-fold diluted amount of test compound ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate and controls were inoculated with approximately 5×10^5 c.f.u of actively dividing bacteria cells. The bacterial cultures were incubated for 24 h at 37 °C and fungi cultures were incubated for 48 h at 37 °C, the growth was monitored visually. The lowest concentration required to arrest the growth of bacteria and fungi was regarded as minimum inhibitory concentration

(MIC). The synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Bacillus substilis, Staphylococus aureus;* and antifungal activity against *Aspergillus niger, Aspergillus flavus, C. albicans*. The antibiotics streptomycin and nystatin were used as standard drugs against bacteria and fungi species respectively. The experiments were carried out in triplicate; the results were taken as a mean of three determinations.

2.5. Molecular docking and ADME predictions

The co-ordinates of superoxide dismutase (SOD) and AmpC β-lactamase (AmpC β) were obtained from the Brookhaven Protein Data Bank, whose PDB ids are 1CB4 [19] and 1KE4 [20] respectively. Ligand were drawn using Maestro 2D sketcher and energy minimize was computed by OPLS 2005. Proteins were prepared by retrieving into Maestro 9.3 platform (Schrödinger, Inc.). Protein structure was corrected, by using Prime software module of Schrödinger to correct the missing loops and in the protein. Water molecules from SOD and AmpC β were removed beyond 5 Å from the hetero atom respectively. Water molecules which are through to be important in aiding the interaction between the receptor were optimized during protein pepwizard. Automated, necessary bonds, bond orders, hybridization, explicit hydrogens and charges were assigned. OPLS 2005 force field was applied to the protein to restrained minimization and RMSD of 0.30 Å was set to converge heavy atoms during the pre-processing of protein before starting docking. Using Extra-precision (XP) docking and scoring each compound were docking into the receptor grid of radii 20 Å and the docking calculation were judge based on the Glide score, ADME results and Glide energy. QikProp, the prediction program was used to calculate ADME properties of all the ligand and molecular visualization was done under Maestro 9.3.

3. Data, value and validation

In ^{1}H NMR spectra, compound showed a triplet at δ 1.252–1.291 ppm. for an ester CH_3 protons, a singlet at δ 2.400 ppm for $COCH_3$ protons, a quartet at δ 4.2892–4.345 ppm for an ester OCH_2 protons. Due to para substitution effect, four aromatic protons absorbed as two doublets of doublet for two protons each at δ 7.250–7.351 and 7.357–7.371 ppm. A singlet absorbed at δ 7.496 ppm was assigned to benzylidene =CH proton. In Mass spectra, compound showed a M+ peak at m/e 254 with a relative abundance of 32% corresponding to isotope ^{37}Cl, and a base peak at m/z 252 corresponding to molecular mass with ^{35}Cl. The mass spectral data obtained was in agreement with the reported data [21].

The title compound crystallizes in the monoclinic crystal system under the space group P21/n. The bond lengths and bond angles are in good agreement with the standard values. The structure adopts a *Z* conformation about the C=C bond. The pendant ethyl chain has an extended conformation as indicated by the torsion angle value of 172.53(12)° for C11/O1/C12/C13. The carbonyl groups of both the chains are twisted out of the plane of the phenyl ring indicated by the torsion angle values of 100.3(2)° and −178.19(12)° respectively for O2/C11/C8/C7 and O3/C9/C8/C7. The *ORTEP* of the molecule with thermal ellipsoids drawn at 50% probability level is shown in Fig. 2, which was obtained by a FORTRAN plot program [22]. No classic hydrogen bonds were observed in the structure. The packing of the molecules when viewed down the *c* axis indicates that the molecules exhibit layered stacking as shown in Fig. 3.

The details of crystal data and structure refinement are given in Table 1. The bond lengths are given in Table 2. The selected bond angles are listed in Table 3.

The results of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability of the compound ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate are summarised in Table 4. The results of MICs of the synthesized compound ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate tested against different bacterium and fungi organisms were tabulated in Table 5.

The results of the studies indicated that, relative to the standard antioxidant ascorbic acid, the synthesised compound ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate exhibited poorer radical scavenging ability at lower concentrations up to 30 µg/ml. However, it showed promising activities at 40 µg/ml

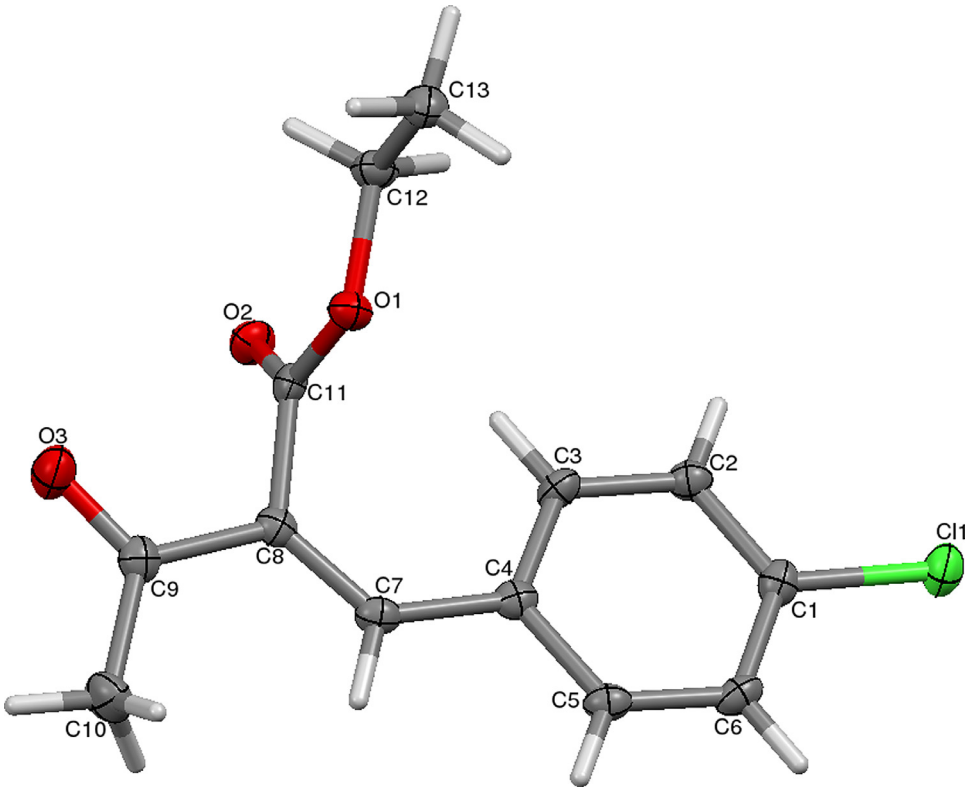

Fig. 2. *ORTEP* of the molecule with displacement ellipsoids drawn at 50% probability level.

Table 1
Crystal data and structure refinement details.

Parameter	Value
CCDC deposit No.	CCDC 1489216
Empirical formula	$C_{13}H_{13}ClO_3$
Formula weight	252.68
Temperature	293(2) K
Wavelength	1.54178 Å
Crystal system, space group	Monoclinic, $P2_1/n$
Unit cell dimensions	$a = 9.9233(4)$ Å, $b = 7.6299(3)$ Å, $c = 16.0932(6)$ Å $\beta = 99.2980(10)^o$
Volume	1202.47(8) Å^3
Z, Density(calculated)	4, 1.396 Mg/m^3
F_{000}	528
Crystal size	0.29 × 0.25 × 0.23 mm
θ range for data collection	5.57° to 64.30°
Limiting indices	$-10 \le h \le 11$, $-8 \le k \le 7$, $-18 \le l \le 18$
Reflections collected/unique	9074/ 1977 [R(int) = 0.0403]
Absorption correction	Multi-scan
Refinement method	Full matrix least-squares on F ^{2}
Data / restraints / parameters	1977 / 0 / 156
Goodness-of-fit on F 2	1.092
Final [$I > 2\sigma(I)$]	$R1 = 0.0370$, $wR2 = 0.0987$
R indices (all data)	$R1 = 0.0381$, $wR2 = 0.0998$
Largest diff. peak and hole	0.359 and −0.439 e. Å^{-3}

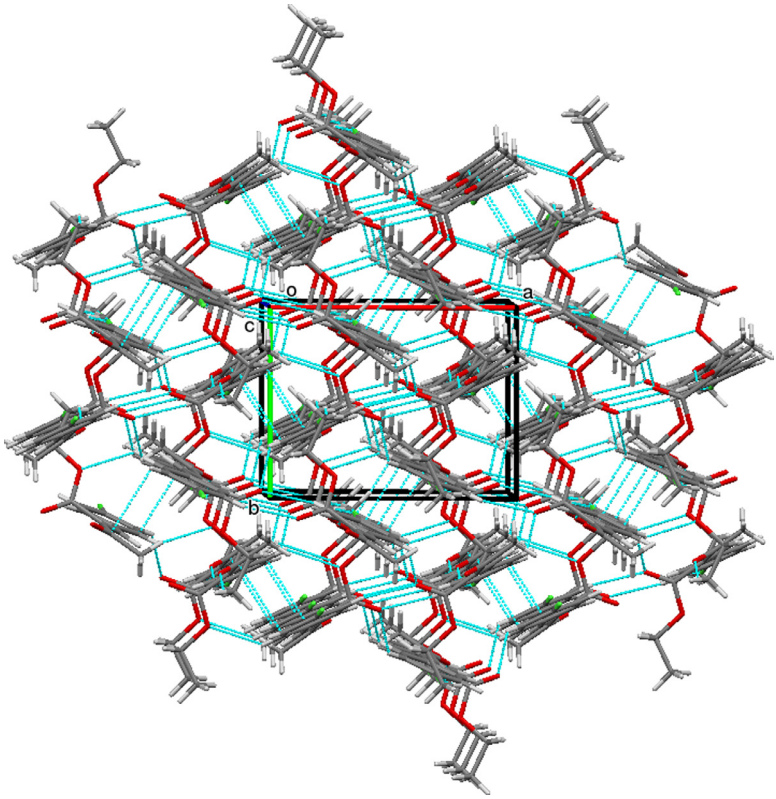

Fig. 3. The packing of molecules when viewed down the *c* axis.

Table 2
Bond lengths (Å).

Atoms	Length	Atoms	Length
Cl1-C1	1.7406(16)	C4-C5	1.402(2)
O1-C11	1.333(2)	C4-C7	1.471(2)
O1-C12	1.4591(19)	C7-C8	1.345(2)
O3-C9	1.222(2)	C8-C9	1.492(2)
O2-C11	1.2056(19)	C8-C11	1.507(2)
C1-C2	1.385(2)	C12-C13	1.502(2)
C1-C6	1.389(2)	C5-C6	1.385(2)
C2-C3	1.383(2)	C9-C10	1.501(2)
C3-C4	1.405(2)		

and concentrations. Thus, it was concluded that, that compound poses better antioxidant susceptibility at higher concentrations (50 µg/ml). Preliminary investigations on the antimicrobial abilities of the synthesised compound ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate showed that, the compound exhibited promising activity against bacterium *B. Substilis;* found moderately active against *S. aureus,* and *E. Coli* organisms. The compound exhibited moderate antifungal susceptibilities against the tested fungi species *A. Niger, A. flavus* and *C. albicans*.

Molecular docking of the ligand (**3**) result was similar with *in vitro* studies and the ligand satisfies both the docking in Table 6, and ADME drug like criteria in Table 7. Ligand of parental nucleus, imparted a specific geometrical space orientation around the Cu-Zn binding loop, which is curial for SOD to enhance its activity on oxidative stress induced by ROS [23]. Binding modes of ligand (**3**)

Table 3
Selected bond angles (°).

Atoms	Length	Atoms	Length
C11-O1-C12	116.69(12)	C7-C8-C11	123.16(14)
C2-C1-C6	121.41(15)	C9-C8-C11	112.91(13)
C2-C1-Cl1	118.75(13)	O2-C11-O1	125.05(14)
C6-C1-Cl1	119.84(12)	O2-C11-C8	124.74(15)
C3-C2-C1	119.47(15)	O1-C11-C8	110.21(12)
C2-C3-C4	120.83(15)	O1-C12-C13	106.22(12)
C5-C4-C3	118.05(14)	C6-C5-C4	121.60(14)
C5-C4-C7	117.94(13)	C5-C6-C1	118.62(14)
C3-C4-C7	123.99(14)	O3-C9-C8	118.79(14)
C8-C7-C4	129.77(14)	O3-C9-C10	121.24(14)
C7-C8-C9	123.93(14)	C8-C9-C10	119.97(14)

Table 4
DPPH radical scavenging ability ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate.

Compound	Percentage radical scavenging activity with respect to standard ascorbic acid (measured at different concentrations)*				
	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
3	18.76 ± 0.94	26.23 ± 0.88	45.20 ± 0.71	54.66 ± 0.98	56.17 ± 0.82

Control at concentration 0 µg/ml 0.00 ± 0.00.
* Values are expressed as mean ± standard deviation ($n = 3$); ascorbic acid was used as standard

Table 5
Antimicrobial activity of ethyl 2-(4-chlorobenzylidene)−3-oxobutanoate tested against bacteria and fungi species.

Compound	Minimum inhibitory concentration (MIC) (μg/ml)*					
	S. aureus	*B. substilis*	*E. coli*	*A. niger*	*A. flavus*	*C. albicans*
3	30	20	40	30	40	35
Streptomycin	20	30	20	–	–	–
Nystatin	–	–	–	30	20	20

* Values are expressed as mean of the three determinations ($n = 3$).

Table 6
Docking scores of the synthesized ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate (**3**) against Cu-ZnSOD and AmpC β-lactamase. Glide scores and average van der Waal (E_{evdw}) and Glide energies (kcal/mol) as obtained through Glide docking.

Protein	Ligand	RMS derivative-OPLS-2005	Glide score (kcal/mol)	Glide energy (kcal/mol)	XP HBond
1CB4	**3**	0.010	−2.39	−23.79	−0.65
	Ascorbic acid	0.050	−2.14	−30.56	−0.47
1KE4	**3**	0.002	−6.01	−27.10	−0.70

showed the better pose with docking and glide score, which have been determined and those that retain interactions with Ala138, Glu131.Gly59, Pro60,Arg141, His46, His118, Ser140 and Thr135 residing at Cu-Zn loop of the SOD (Fig. 4). ROS and microbial infection are very closely related [24], hence we checked the potency of ligand for antibacterial activity. β-Lactamases are the most resistance to β-lactam antibiotics and are an increasing menace to public health. Ligand (**3**) binds deeply into active site of AmpC β-lactamase, suggesting very tight binding hereby inhibiting the accessibility of the enzyme to act on substrate (Fig. 5), indicating that it possess both antioxidant and antibacterial potency. QikProp, the prediction program was used to calculate ADME properties consisting of principal descriptors and physiochemical properties. Qikprop modules provide the ranges of molecular predicting properties for comparing the properties of a particular molecule with those of 95% of known drugs

Table 7
Computer aided ADME screening of the synthesized compound ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate (**3**).

Ligand	Mol MW	QPlogHERG	QPPCaco	QPlogBB	QPlogKp	a*	b*	c*	d*
3	252.7	−4.66	1347	−0.41	−2.23	−0.18	100	1686	0
Range 95% of drugs	130.0 to 725.0	< −5	< 25 poor, > 500 great	−3.0 to 1.2	−8.0 to −1.0	−1.5 to 1.5	> 80% is high	< 25 poor, > 500 great	0–4

a*- QPlogKhsa, b*- % Human oral absorption, c*- QPPMDCK, d*- Volition of Lipinski's rule

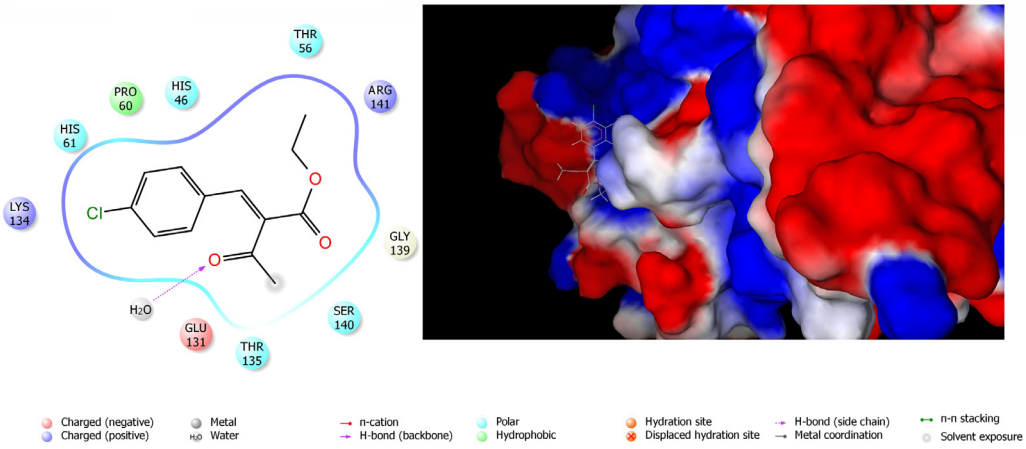

Fig. 4. Putative binding pose of compound ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate with SOD enzyme (PDB ID: 1CB4).

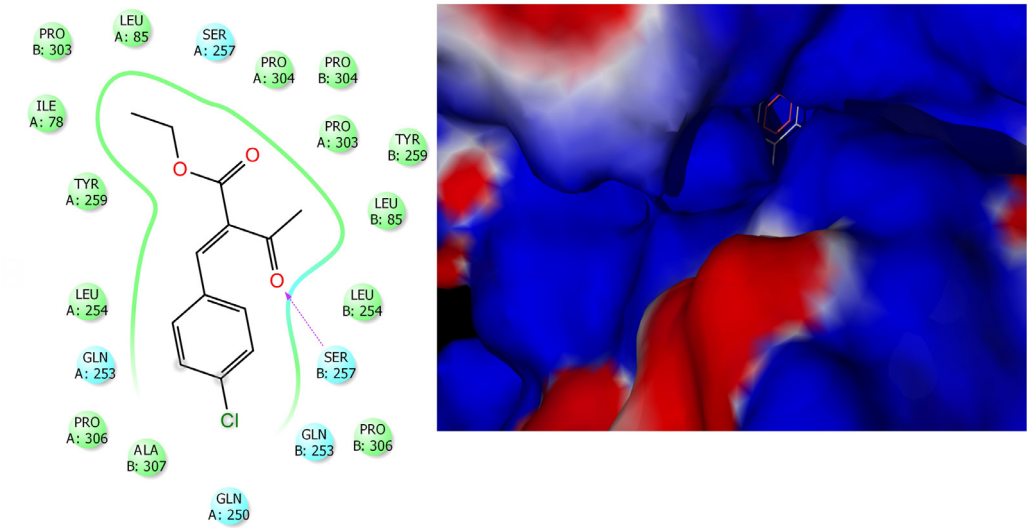

Fig. 5. Putative binding pose of compound ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate with AmpC β-lactamase (PDB ID: 1KE4).

(Table 7) [25]. The ligand obeys the Lipinski's rules: molecular weight below 500 Da, hydrogen bond donor (less than five) and acceptor (less than ten). QPlogPo/w (octanol/water partition coefficient) for the ligand is less than five [25]. The ligand satisfy the values of partition coefficient of octanol/gas (QPlogPoct), water/gas (QPlogPw) and brain/blood (QPlogBB), Skin permeability (QPlogKp), aqueous solubility (QPlogS) and predicted for ligand within the permissible range.

4. Conclusion

The ADME result indicates that all these molecules possess pharmaceutical properties in the range of 95% of drugs. It may be concluded from docking and ADME studies of synthesised compound might act as a good antioxidant and antibacterial compound with satisfactory ADME properties and are the suitable candidates to be carried forward as a potential lead targeting various diseases affected by free radicals.

Acknowledgements

The authors are grateful to the Institution of Excellence, Vijnana Bhavana, University of Mysore, India, for providing the single-crystal X-ray diffractometer facility.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi: 10.1016/j.cdc.2016.10.002.

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Chemical Data Collections 5–6 (2016) 68–78

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Data article

Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate: Synthesis, crystal structure and antimicrobial activities

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ARTICLE INFO

Article history:
Received 27 July 2016
Revised 29 October 2016
Accepted 6 November 2016
Available online 12 November 2016

Keywords:
Antimicrobial
Packing
Single crystal
Spectral studies
X-ray diffraction

ABSTRACT

Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate was synthesized by Knoevenagel condensation reaction of 4-fluorobenzaldehyde and ethyl acetoacetate in the presence of catalytic amount of piperidine and trifluoroacetic acid in benzene under reflux conditions. The structure of the synthesized molecule was obtained by spectral studies and was confirmed by X-ray diffraction studies. The title compound crystallizes in the monoclinic crystal system under the space group P21/n. The structure adopts a *Z* conformation about the C=C bond. The synthesized new molecule was evaluated *in vitro* for its antifungal and antimicrobial susceptibilities.

Specifications Table

Subject area	Chemical physics
Compound	Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate
Data category	Synthesis, ^{1}H NMR, mass spectra, crystallographic data
Data acquisition format	CIF for crystallography

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http://dx.doi.org/10.1016/j.cdc.2016.11.003
2405-8300/

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Data type	Analyzed
Procedure	The compound $C_{13}H_{13}FO_3$, ethyl 2-(4-fluoorobenzylidene)-3-oxobutanoate was synthesized and yellow rectangular block shaped crystals of the compound were obtained by slow evaporation technique. A single crystal of dimension $0.38 \times 0.44 \times 0.54$ mm of the title compound was selected and X-ray intensity data were collected with χ fixed at 54° and φ, from 0° to 360° at a scan width of 0.5°, exposure time of 3 s and a sample to detect distance of 50.0 mm at 293 K.
Data accessibility	CCDC 1491046 URL: https://www.ccdc.cam.ac.uk/conts/retrieving.html

1. Rationale

Knoevenagel condensation was a versatile tool for the synthesis of α, β-unsaturated carbonyl compounds [1], which involve the base catalysed reaction of aldehydes and reactive methylene compounds. Exploration of the simple molecules such as α, β-unsaturated carbonyl compounds in to bioactive compounds is a worthwhile contribution in the field of medicinal chemistry. α, β-unsaturated ketones were extensively used as a synthetic scaffolds in the construction of biologically potent molecules such as such as benzothiazepines [2,3], isoxazoles [4], pyrazoles [5], cyclopropyl esters [6], thiadiazoles [7], lignans [8] etc. In view of wide synthetic utilities associated with α, β-unsaturated carbonyl compounds, we herein report the synthesis, spectral analysis, single crystal X-ray diffraction studies, and antimicrobial activity studies of ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate.

2. Procedure

2.1. Synthesis of the title compound

To the solution of ethyl acetoacetate, **2** (0.016 mol) in dry benzene (15 ml), piperidine (0.0016 mol) and trifluoro acetic acid (0.0016 mol) were added; the mixture was stirred for 15 min. After this, 4-fluorobenzaldehyde, **1** (0.016 mol) was added and then the mixture was refluxed on a water bath for 5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice cold water. The solid separated was filtered, washed with ice cold water and crystallized from ethyl acetate to obtain the target molecule Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate, **3** as yellow rectangular slab like crystals in 76% yield, m.p. 131 °C. The schematic representation of the reaction is given in Fig 1.

2.2. Spectral data

^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrometer respectively. The solvent $CDCl_3$ with TMS as an internal standard was used to record the spectra. The

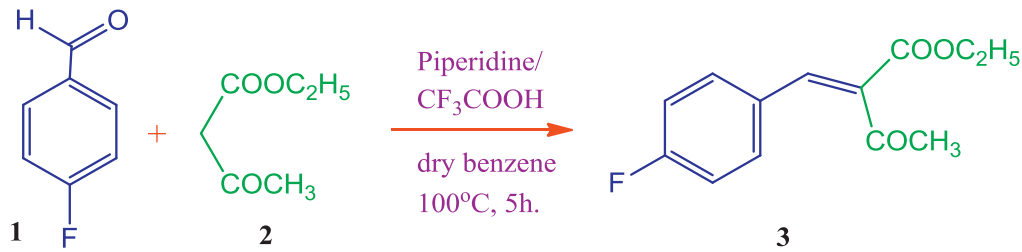

Fig. 1. Reaction pathway for the synthesis of Ethyl 2-(4-fluobenzylidene)-3 oxobutanoate.

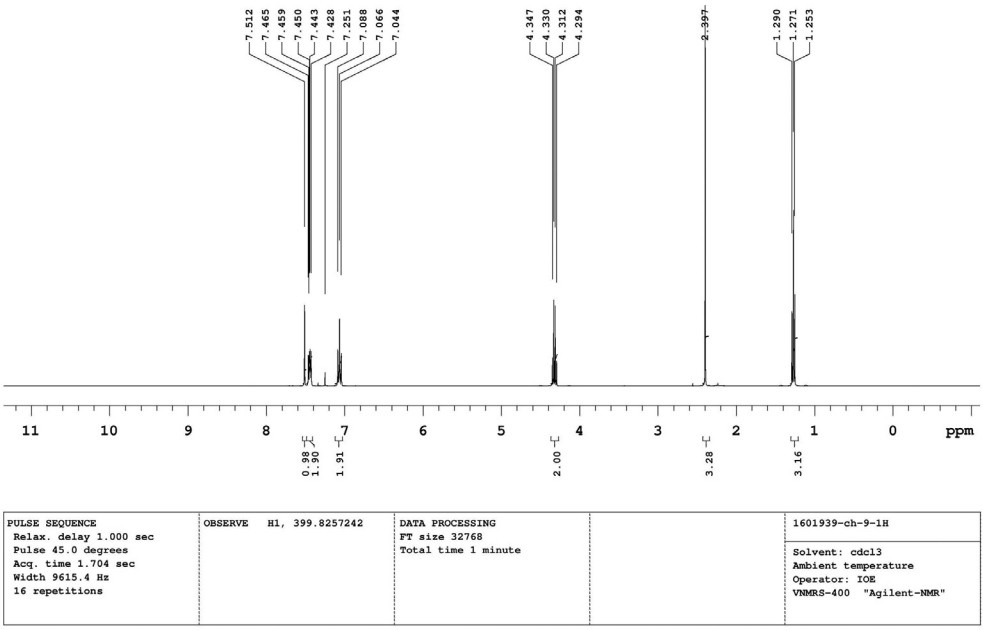

Fig. 2. ^{1}H NMR spectra of Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate.

chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrometer TOF mode. ^{1}H NMR ($CDCl_3$): δ 1.253–1.290 (t, 3H, CH_3), 2.397 (s, 3H, $COCH_3$), 4.294–4.357 (q, 2H, OCH_2), 7.044–7.251 (dd, 2H, Ar-H), 7.428–7.465 (dd, 2H, Ar-H), 7.512 (s, 1H, CH=C) (Fig 2).

^{13}C NMR ($CDCl_3$): δ 13.85 (1C, CH_3), 26.56 (1C, CH_3), 61.75 (1C, CH_2), 115.95 (1C, Ar-C), 116.17 (1C, Ar-C), 129.16 (1C, Ar-C), 131.62 (1C, Ar-C), 131.70 (1C, Ar-C), 139.86 (1C, =C), 162.29 (1C, C=), 165.21 (1C, Ar-C), 167.66 (1C, COO), 194.21 (1C, C=O) (Fig 3). MS (m/z) for $C_{13}H_{13}FO_3$: 236 (M+, 100).

2.3. X-ray intensity data collection and structure refinement

A yellow coloured rectangular block shaped single crystal of dimension $0.38 \times 0.44 \times 0.54$ mm of the title compound was chosen and X-ray intensity were collected with χ fixed at 54° and φ, from 0° to 360°, scan width at 0.5°, exposure time of 3 s and the sample to detector distance of 50.0 mm at a temperature 293 K on Rigaku XtaLAB Mini Single crystal X-ray diffractometer operating at 50 kV and 12 mA with MoK_α radiation of wavelength $\lambda = 0.71073$ Å. A complete data set was processed by CrystalClear [9]. All frames could be indexed by the primitive monoclinic lattice. The structure was solved by direct methods with R_{int} and R_{sigma} values of 0.057 and 0.046 respectively, and refined by full-matrix least squares method on F^2 using SHELX [10]. After several cycles of refinement, the final difference Fourier map showed peaks of no chemical significance and the residual is saturated to 0.0527. The geometrical calculations were carried out using PLATON [11]. The molecular and packing diagrams were generated using MERCURY [12].

2.4. Antimicrobial activity

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique [13]. The nutrient broth, which contain logarithmic serially two-fold diluted amount of test compound ethyl 2-(4-methylbenzylidene)-3-oxobutanoate and controls were inoculated with approximately 5×10^5 c.f.u of actively dividing bacteria cells. The bacterial cultures were incubated for 24 h at 37 °C and fungi

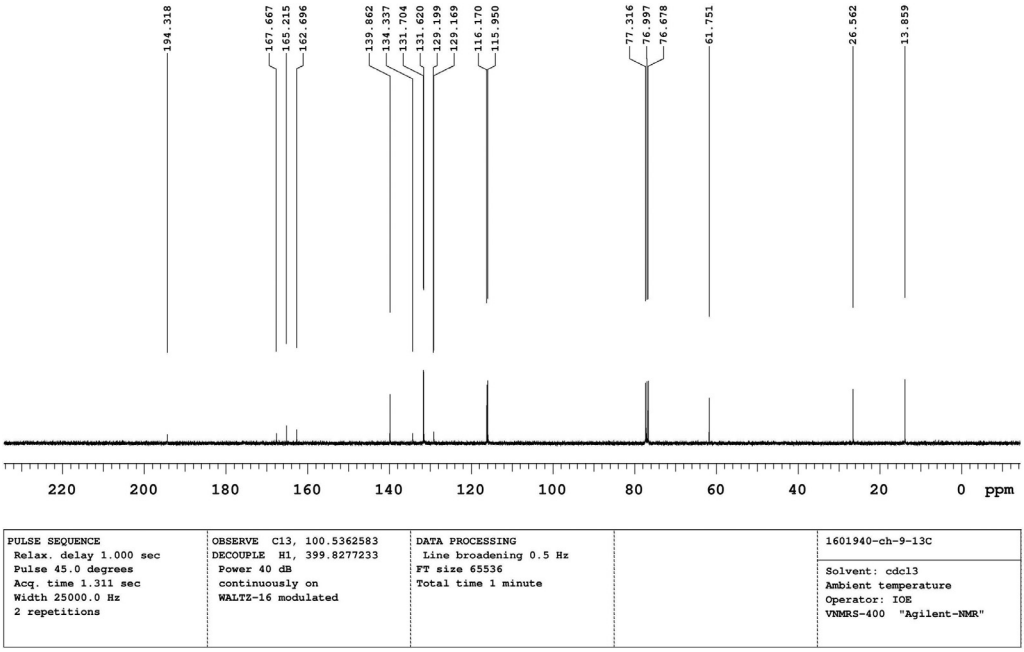

Fig. 3. ^{13}C NMR spectra of Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate.

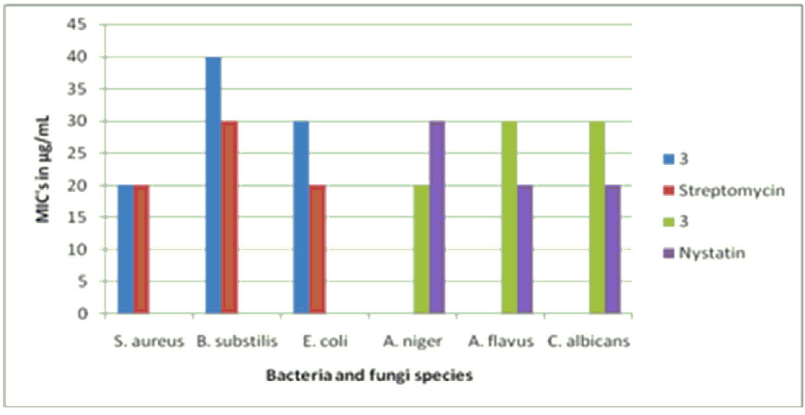

Fig. 4. Antimicrobial activity of Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate tested against bacteria and fungi species.

cultures were incubated for 48 h at 37 °C, the growth was monitored visually. The lowest concentration required to arrest the growth of bacteria and fungi was regarded as minimum inhibitory concentration (MIC). The synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Bacillus substilis, Staphylococcus aureus.* and antifungal activity against *Aspergillus niger, Aspergillus flavus, C. albicans*. The antibiotics streptomycin and nystatin were used as standard drugs against bacteria and fungi species respectively. The experiments were carried out in triplicate; the results were taken as a mean of three determinations. The results of MICs of the synthesized compound ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate tested against different bacterium and fungi organisms were depicted in Fig 4. Values are expressed as mean of three determinations ($n = 3$).

Table 1
The details of crystal data, data collection and refinement.

CCDC Number	CCDC 1491046
Crystal data	
Formula	$C_{13}H_{13F}O_3$
Formula weight	236.23 g mol^{-1}
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions	$a = 9.916$ (12) Å $b = 7.769$ (8) Å $c = 15.744$ (19) Å B = 99.8 (5)°
Volume	1195.1 (2) Å^3
Z, calculated density	4, 1.313 Mg/m^3
Absorption coefficient	0.103 mm^{-1}
F_{000}	496
Crystal size	0.38 × 0.44 × 0.54 mm
Data collection	
Temperature	293 K
Radiation, wavelength	MoK$_\alpha$, 0.71073 Å
Theta ranges for data collection	3.3 to 27.5°
Limiting indices	$-12 \le h \le 12$, $-8 \le k \le 9$, $-20 \le l \le 9$
Total reflections, unique, R_{int}	4361, 2671, 0.057
Refinement	
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2671/ 0 / 156
Goodness-of-fit on F^2	1.08
Final R indices [I>2sigma(I)]	$R1 = 0.0527$, $wR2 = 0.1489$
R indices (all data)	$R1 = 0.0676$, $wR2 = 0.1655$
Max. and average shift/error	0.00, 0.00
Largest diff. peak and hole	0.25 and −0.19 e. Å^{-3}

From the preliminary studies, it was observed that the compound ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate possess moderate activity against *S. aureus,* and remarkable activities against *B. Substilis* and *E. Coli* cells in comparison with the standard drug streptomycin, while, in comparison with the standard nystatin, ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate showed lesser activity against the fungi *A. niger;* and promising activity against *A. flavus* and *C. albicans* organisms.

3. Data, value and validation

In ^{1}H NMR spectra, title compound showed a triplet at δ 1.253–1.290 ppm. for an ester CH_3 protons, a singlet at δ 2.397 ppm. for $COCH_3$ protons, and a quartet at δ 4.294–4.357 ppm. for an ester OCH_2 protons. Due to para substitution effect, four aromatic protons absorbed as two doublets of doublet for two protons each at δ 7.044–7.251 and 7.428–7.465 ppm. A singlet absorbed at δ 7.512 ppm was assigned to benzylidene =CH proton. In ^{13}C NMR spectra, the signals absorbed at δ 194.21 and 167.66 ppm were assigned to C=O and COO carbons, where as the signals absorbed at δ 13.85, 26.56, 61.75, 139.86, and 162.29 ppm were assigned to ester CH_3, CH_3, OCH_2 =C, and C= carbon atoms. Six aromatic carbons absorbed at δ 115.95, 116.17, 129.16, 131.62, 131.70, and 165.21 ppm. In Mass spectra, compound showed M+ peak at m/e 236 corresponding to molecular mass as base peak.

3.1. Crystal structure description

The title compound Ethyl 2-(4-fluorobenzylidene)-3-oxobutanoate crystallizes in the monoclinic crystal system under the space group $P2_1/n$. The details of crystal data, data collection and structure refinement are given in Table 1.

The literature reveals that the compound (*Z*)-Ethyl 2-benzylidene-3-oxobutanoate adopts a Z conformation about the C=C double bond, and showed weak intermolecular C–H···O hydrogen bonds

Table 2
Selected bond lengths and bond angles (Å, °).

F1—C2	1.3578 (19)	C10—O11	1.2036 (17)
C5—C8	1.467 (2)	C10—O12	1.3305 (18)
C9—C15	1.490 (2)	O12—C13	1.4550 (18)
C9—C10	1.5018 (19)	C15—O17	1.211 (2)
F1—C2—C3	118.3 (2)	C15—C9—C10	113.3 (1)
F1—C2—C7	118.6 (2)	O11—C10—O12	124.6 (1)
C3—C2—C7	123.1 (2)	O11—C10—C9	124.4 (1)
C4—C5—C8	124.3 (1)	O12—C10—C9	111.0 (1)
C7—C6—C5	121.7 (1)	C10—O12—C13	116.5 (1)
C9—C8—C5	130.2 (1)	O12—C13—C14	107.2 (1)
C8—C9—C15	123.6 (1)	O17—C15—C9	118.8 (2)
C8—C9—C10	123.1 (1)	O17—C15—C16	121.1 (2)

Table 3
Hydrogen bond geometry (Å, °).

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*	Symmetry
C7—H7···O11	0.93	2.62	3.357 (2)	137	$-1/2+x,\ -1/2-y,\ -1/2+z$
C4—H4···O11	0.93	2.60	3.411 (2)	147	

with phenyl –CH atoms functioning as donors and the carbonyl O atom of an ester group as acceptor [14]. The selected bond lengths and bond angles are listed in Table 2. The bond lengths and angles are in close agreement with those reported for the molecular structure of the compound (*Z*)-Ethyl 2-benzylidene-3-oxobutanoate.

The compound Ethyl 2-(4-methylbenzylidene)-3-oxobutanoate crystallizes in the triclinic P-1 space group with two molecules in an asymmetric unit having closely comparable geometries and assumes a *Z* conformation about the C=C bond. The phenyl rings in both the molecules are essentially planar with the mean plane of the part of the oxobutanoate unit. The molecules exhibit layered stacking [15, 16] and the compound (Z)-Ethyl 2-(3-nitrobenzylidene)-3-oxobutanoate adopts a Z conformation at the C=C double bond. The ethoxy atoms of the ethyl ester group are disordered over two orientations in a 3:2 ratio and showed weak intermolecular C—H···O hydrogen bonds [17]. The compound Ethyl 2-(4-chlorobenzylidene)-3-oxobutanoate crystallizes in the monoclinic crystal system under the space group $P2_1/n$. The structure adopts a *Z* conformation about the C=C bond. The pendant ethyl chain has an extended conformation. The carbonyl groups of both the chains are twisted out of the phenyl ring plane.

The Oak Ridge Thermal Ellipsoid Plot (ORTEP) which was obtained by a FORTRAN plot program [18] for the title molecule Ethyl 2-(4-fluobenzylidene)-3-oxobutanoate, drawn at 50% probability level is shown in Fig 5. The structure adopts a *Z* conformation about the C=C bond. The pendant ethoxy group has an extended conformation as indicated by the torsion angle value of −173.5(1)° for C10/O12/C13/C14. The carbonyl groups of both the chains are twisted out of the mean plane of the phenyl ring indicated by the torsion angle values of −100.3 (2)° and 178.8 (2)° respectively for C8/C9/C10/O11 and C8/C9/C15/O17.

The molecules are chained through C—H···O hydrogen bond interactions as listed in Table 3, since the carbon C7 atom in the molecule at (*x, y, z*) of fluorophenyl moiety acts as donor to the oxygen O11 atom at $(-1/2+x,\ -1/2-y,\ -1/2+z)$ of the neighbouring molecule, unfolding the fact that supra molecular construction may be achieved even with weak C—H···O hydrogen bond interactions. The 3D supra molecular layered stacking of the molecules viewed along *b* axis is shown in Fig. 6.

3.2. Hirshfeld surface analysis

The shorter contacts in the molecular structure were visualized by carrying out the Hirshfeld surface [19,20] analysis by the computational methods implemented in *Crystal Explorer* [21]. The Hirshfeld surface of the title molecule of volume 292.94 Å^3, area 280.49 Å^2, mapped over a d_{norm} range of −0.05 to 1.3 Å and shown as transparent to allow the visualize all the atoms of the molecule is shown in Fig. 7.

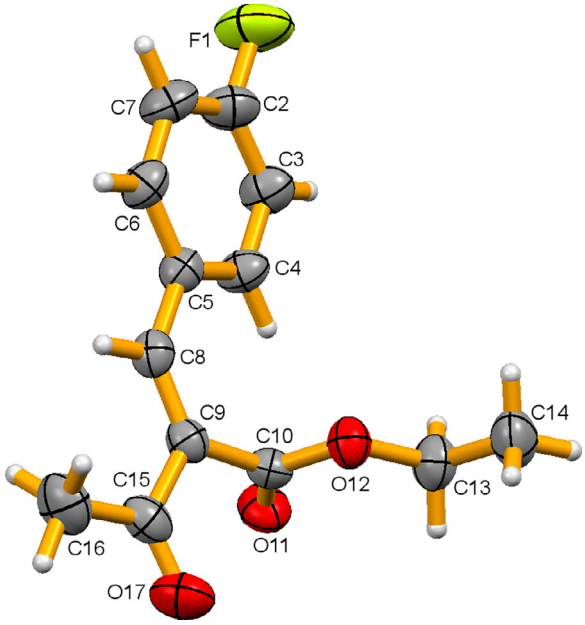

Fig. 5. *ORTEP* of the molecule with displacement ellipsoids drawn at 50% probability level.

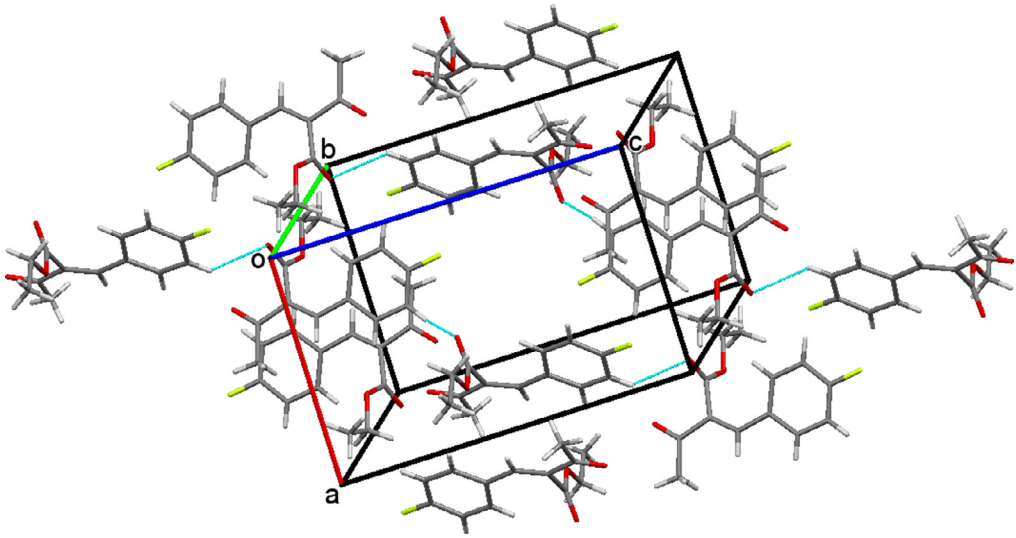

Fig. 6. Three dimensional supra molecular layered stacking of the molecules viewed down along *b* axis. The dotted lines represent C—H···O hydrogen bond interactions.

The hydrogen bond interactions discussed in X-ray diffraction studies can be visualized through dark red spots obtained on the Hirshfeld surface, as a result of hydrogen bond acceptors of C7—H7···O11 as shown in Fig. 8.

The combination of d_e and d_i in the form of two-dimensional fingerprint plot [22] gives the summary of intermolecular contacts in the crystal lattice. Fingerprint plots for the title molecule and inter molecular close contacts are shown in Fig. 9a and b respectively.

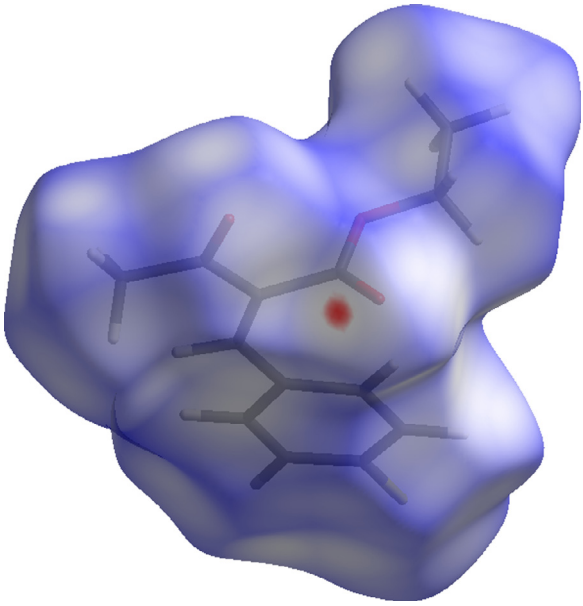

Fig. 7. d_{norm} mapped on the Hirshfeld surface of the molecule.

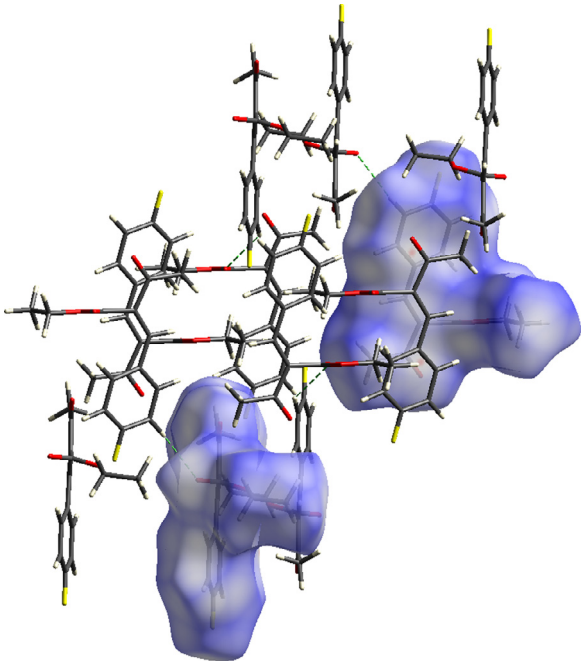

Fig. 8. The visualization of C–H···O hydrogen interactions through the Hirshfeld surface.

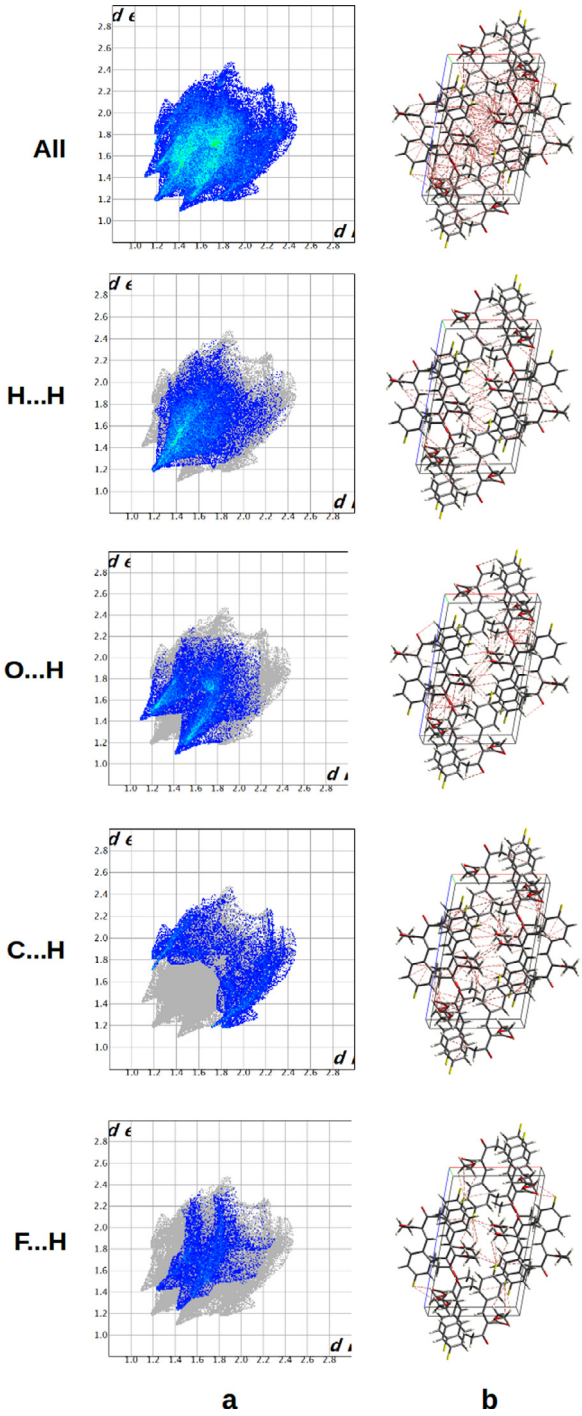

Fig. 9. (a) Fingerprint plots of the molecule. (b) Inter molecular close contacts.

Table 4
X···Y close contacts (Å).

X[a]	Y[b]	X···Y distance shorter than
H	H	3.27
O	H	3.40
C	H	3.40
F	H	3.40

[a] An atom of a parent molecule.
[b] An atom of a neighbouring molecule.

The H···H short contacts appear as a pair of very sharp spikes of sky blue color, almost of same length and merged with one another in the region 1.18 Å < (*de* + *di*) < 1.20 Å The O···H close contacts were seen as two distinct spikes of dark blue color in the region 1.13 Å < (*de* + *di*) < 1.4 Å The C···H inter molecular contacts become noticeable as two sharp, wide and slightly curved spikes of sky-blue color in the region 1.20 Å < (*de* + *di*) < 1.70 Å The F···H inter molecular contacts become visible as a pair of two blunt and distinct spikes of blue color in the region 1.22 Å < (*de* + *di*) < 1.41 Å of the full fingerprint plots.

The inter molecular close contacts, H···H (36%), O···H (28%), C···H (18%) and F···H (14%) at distances shorter than 3.4 Å, which play a vital role in the formation of Hirshfeld surface is depicted in Table 4. The close contacts F···C and C···C, each contributed less (2%) in the stabilization of crystal and molecular structure.

Acknowledgements

The authors are grateful to the Department of Studies in Physics, University of Mysore, Mysuru, India, for providing the National single crystal X-ray diffractometer facility.

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