EXECUTIVE SUMMARY OF FINAL REPORT OF THE WORK DONE ON THE PROJECT SANCTIONED UNDER MINOR RESEARCH PROJECT BY THE UNIVERSITY GRANT COMMISSION

THE PRINCIPAL INVESTIGATOR: Dr SHARADA A C, Associate Professor, Department of Biochemistry, Yuvaraja's College, University of Mysore, Mysuru-570005.

TENURE OF THE PROJECT: 02 Years, 01.10.2015 - 01.10.2017.

TOTAL GRANT ALLOCATED: Two Lakhs

TITLE OF THE PROJECT: "A Study on Synthesis and biological evaluation of curcumin analogues for anticancer and antioxidant properties".

UGC APPROVAL LETTER NO. AND DATE: MRP(S)-0149/12-13/KAMY002/UGC-SWRO dated, 29-03-2013

Introduction:

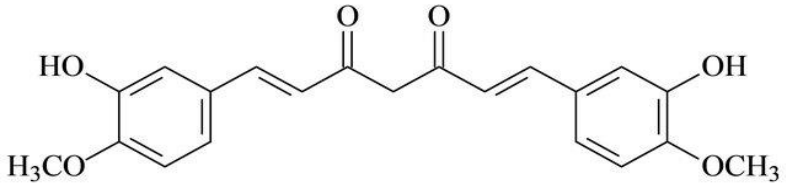
Curcumin is a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa*. Curcumin has been identified as the major active constituent in turmeric and pharmacologically, curcumin has been found to be safe even at very high doses in human clinical trials. It has been widely used for various treatments due to its medicinal properties. Synthetic chemical modifications of curcumin have been studied & various curcumin analogues are synthesized to improve the pharmacological profile of natural product. In this context, we synthesized a series of curcumin analogues (2a & 2b) and studied for antioxidant and anticancer properties.

Objectives of the project:

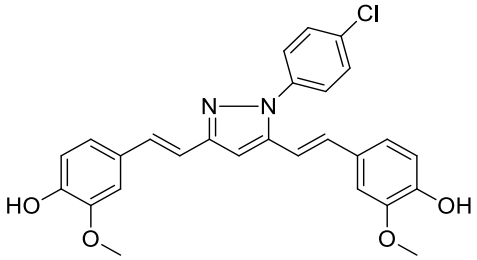
1. Synthesis and chemical characterization of a series of curcumin analogues.
2. Evaluation of biological activity of these analogues.
    a) Antioxidant activity.
3. Evaluation of biological activity of curcumin analogues.
    b) Anticancer activity.

Part A: Synthesis and characterization of curcumin pyrazole derivatives.

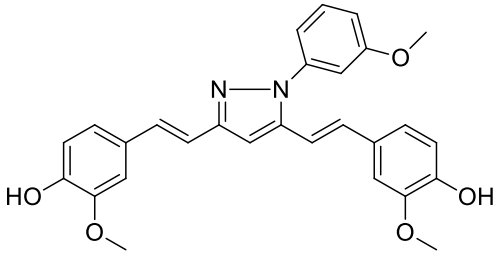
A series of curcumin pyrazoles (2a & 2b) were synthesized and the structures of the curcumin analogues were determined by ^{1}H and ^{13}C NMR spectroscopic techniques. The purity of the compounds was confirmed by LC-MS.



Curcumin
(1,7-bis(4-hydroxy-3- methoxyphenyl)-1,6-heptadiene-3,5-dione).



4,4'-(1E,1'E)-2,2'-(1-(4-chlorophenyl)-1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol) (2a)



*4,4'-(1E,1'E)-2,2'-(1-(**3**-methoxyphenyl)-1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol) (2b)*

Evaluation of biological activity of curcumin analogues (2a & 2b)

The curcumin and its pyrazole series (2a & 2b) along with standard drugs were assessed for *in vitro* antioxidant and anticancer studies. Antioxidant activity was assessed by DPPH free radical scavenging assay, superoxide anion scavenging assay and nitric oxide scavenging assay.

Anticancer activity was assessed by using MTT and trypan blue assay on three different cell lines, cervical carcinoma (HeLa), breast carcinoma (MCF-7) and leukemic cells (K562).

Summary of the findings:

Antioxidant activity:

The free radical scavenging ability of curcumin and its pyrazoles (2a & 2b) along with ascorbic acid were analyzed by the DPPH method. The free radical scavenging activity of each compound at a concentration range of 25-100 µM was evaluated by measuring the change of absorbance formed by the reduction of DPPH. Curcumin, ascorbic acid, 2a & 2b showed an effective quenching with 78.23, 86.23, 88.58 & 78.12% inhibition respectively at 100 µM concentration. Compound 2a was found to be potent as ascorbic acid in quenching DPPH free radicals followed by curcumin and 2b respectively.

The nitric oxide scavenging activity of each compound at a concentration range of 25-100 µM was assessed. Curcumin, ascorbic acid, 2a & 2b showed 74.13, 93.59, 86.11 & 76.23% inhibition respectively at 100 µM concentration. Compound 2a exhibited more potency in scavenging nitric oxide radical than curcumin and compound 2b.

The superoxide scavenging activity of each compound at a concentration range of 25-100 µM was assessed. Curcumin, ascorbic acid, 2a & 2b showed an effective inhibition of 76.51, 81.74, 75.89 & 70.79% respectively. Compound 2a and 2b exhibited prominent activity and compound 2a was found to be potent as curcumin in scavenging superoxide anion free radicals.

Anticancer activity:

All compounds were evaluated for *in-vitro* cytotoxicity on three different cancer cell lines, MCF-7, HeLa and K562 cells by MTT assay and trypan blue dye exclusion assay. Paclitaxel, avastin and tamoxifen drugs were used as positive control and curcumin was taken as a standard reference. The results of cytotoxicity studies of curcumin and its analogues (2a & 2b) were recorded at different concentrations (10, 20 and 40 µM respectively) and at different time intervals (48 & 72 h respectively).

The data obtained by MTT assay showed that curcumin, compound 2a and 2b have considerable inhibitory effects on the growth of cancer cell lines after 48 and 72 h of treatment. Compound

2a was found to be more effective and showed significant growth inhibitory effects against all the three cancer cell lines. Compound 2a was potent as curcumin and showed 47.9% of viable cells at 40 µM concentration after 72 h of treatment against HeLa cells and comparable effect was observed with curcumin (47.7% of viable cells) where as compound 2b showed 51.36% viable cells at the same condition. K-562 cells were more sensitive among the cell lines tested for the treatment and compound 2a, 2b and curcumin treated cells showed 22.85, 39.42 and 30.75% viable cells respectively at 40 µM concentration after 72 h of treatment. Curcumin was found to be effective against MCF-7 cells and showed 37.49% of cell survival at 40 µM concentration after 72 h of treatment than compound 2a (43.18% of viable cells). Compound 2b showed 57% of cell survival at 40 µM concentration after 72 h of treatment.

Further, in the trypan blue assay on MCF-7 cells after 48 h exposure, compound 2a showed highest inhibition of proliferation (69.27%) followed by curcumin and compound 2b (68.7 & 65.8% respectively). In HeLA cells, the growth suppressive effect was found to be 53.16, 48.58 and 46.89% respectively for compound 2a, curcumin and compound 3b after 48 h exposure. K-562 cells were inhibited prominently by compound 2a after 48 h exposure and the inhibition was found to be 81.54%. The growth suppressive effect for compound 2b and curcumin was found to be 71.72 and 77.93% respectively after 48 h exposure. Thus compound 2a was found to be more efficient in inhibiting cell growth in the tested cell lines than 2b and curcumin in both MTT and trypan blue assays.

Conclusion: From the studies we investigated that the compound 2a has promising in-vitro anticancer and antioxidant efficiency and was found to be more effective than the natural curcumin. The synthetic analogue of curcumin, compound *4,4'-(1E,1'E)-2,2'-(1-(4-chlorophenyl)-1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)* (2a) could be a promising inexpensive drug effective at non-toxic doses and necessitate further *in-vitro* and *in-vivo* investigations.

Date: 08.02.2018 Principal Investigator The Principal