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Synthesis and Anticancer activity of Novel Curcumin-Quinolone Hybrids

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Keywords

Curcumin–Quinolone hybrids

MTT Assay

Annexin-V

Cell cycle analysis

Abstract

A number of new curcumin-quinolone hybrids were synthesised from differently substituted 3-formyl-2-quinolones and vanillin and their *in vitro* cytotoxicity was determined on a panel of representative cell lines (A549, MCF7, SKOV3 and H460) using MTT assay. The most potent compound **14,** was analysed for its mode of action using various cell biology experiments. SKOV3 cells treated with compound **14** showed distorted cell morphology under phase contrast imaging and induction of apoptosis was confirmed by Annexin V/PE assay. Further experiments on generation of reactive oxygen species (ROS) and cell cycle analysis revealed that these hybrids induce apoptosis by ROS generation and arrest cell cycle progression in S and G2/M phase.

Curcumin (diferuloylmethane or (1E,6E)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) **18** is currently acclaimed to be one of the most widely researched naturally occurring chemopreventive agent and it has been subjected to several *in vitro, in vivo* and clinical trial studies[1], but it suffers from limitations[2]. In order to circumvent these problems and to promote pharmacological properties, several modifications in aromatic ring and linker chain of curcumin have been attempted and various analogues have been prepared[3]. But synthesis of curcumin hybrids such as curcumin-diaminothiazole hybrid[4] and curcumin-thalidomide hybrid[5] and evaluation of their anti cancer potential has opened up new avenues for research[6]. 3-substituted-2-quinolone moiety is an important structural feature present in numerous compounds with promising anti-cancer activities as they are found to be tyrosine kinase inhibitors [7]. Cytotoxic activity of quinolone derivatives as non nucleosidic MGMT inhibitors have been reported previously[8]. 4-quinolones were synthesised and evaluated as potential antitumor topoisomerase I inhibitors[9]. 2-quinolone based chalcones

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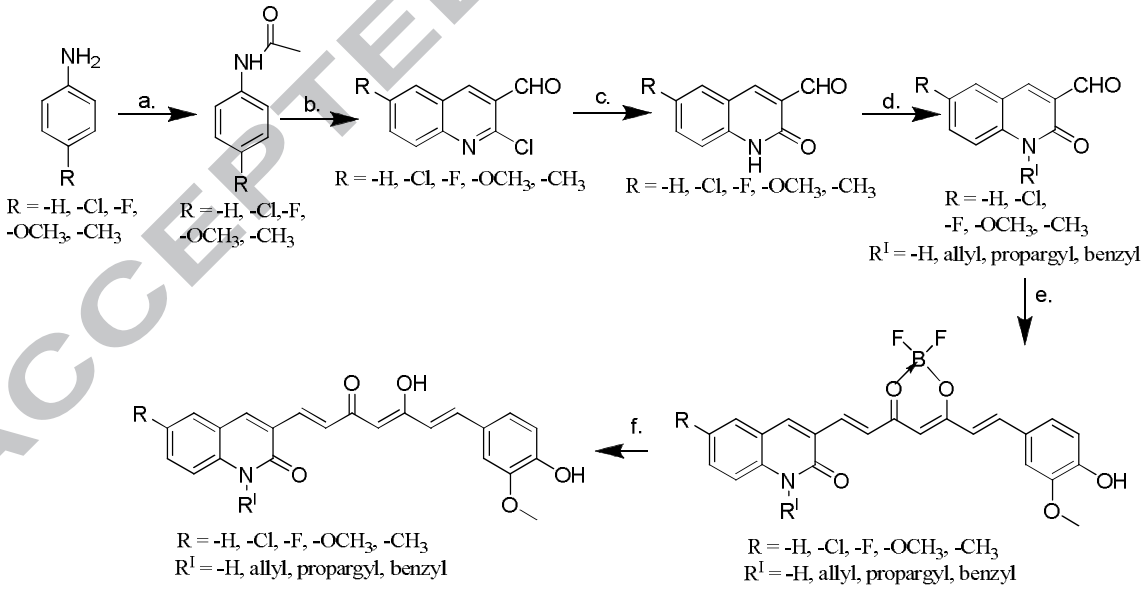
have been proven to display anti-tumour activity[10]. 2-phenyl-4-quinolones were reported to exhibit inhibitory activity on several cell lines[11]. In line of these reports, in this study we have synthesized new curcumin-quinolone hybrids from differently substituted 3-formyl-2-quinolones and vanillin which retain important pharmacophores (α,β-unsaturated ketone moiety and 2-quinolone moiety) to improve potency and selectivity. The proposed hybrids may exhibit the cytotoxicity of curcumin as well as tumour antibiotic effect of 3-formyl-2-quinolones.

The synthesis of these curcumin-quinolone hybrids **9-16** and diquinolone **17** are outlined in Schemes **1** and **2** respectively. 3-formyl-2-quinolones **1-6** were prepared from corresponding arylacetamides using Vilsmeier-Haack cyclization as per standard procedure[12]. These quinolones were condensed with feruloyl acetone difluoroboronite complex (prepared from feruloyl acetone[13]and BF_3 etherate) to form difluoroboronite complexes of curcumin-quinolone hybrids which were isolated and characterised by NMR studies. These difluoroboronite complexes required prolonged refluxing in aqueous methanol for decomplexation[14]. In order to reduce the reaction time, the reaction was tried in various other solvents and different experimental conditions. But under microwave irradiation conditions in aqueous methanol, not only the reaction was faster (3-5 minutes) but the yield was also better. For improving the solubility and to facilitate the testing, nitrogen of 3-formyl-2-quinolones was substituted with allyl, propargyl and benzyl groups. Quinolone hybrid **12** with propargyl substituent at nitrogen had better solubility and improved toxicity on all four cell lines when compared to allyl and benzyl substituted quinolone hybrids **10** and **11.** Hence different hybrids were made with N-propargyl quinolone structural unit (**13**-**16**). All these new compounds were characterised by NMR and HR-MS techniques.



Scheme 1. Synthesis of quinolone hybrids (**9-16**): a. $(CH_3CO)_2O$/ CH_3COOH / 30 min / 50-60^{0}C; b. $POCl_3$ / DMF / 80-90^{0}C / 4-16 hrs; c. 4N HCl reflux / 6-8 hrs; d. Allyl bromide or propargyl bromide or benzyl bromide

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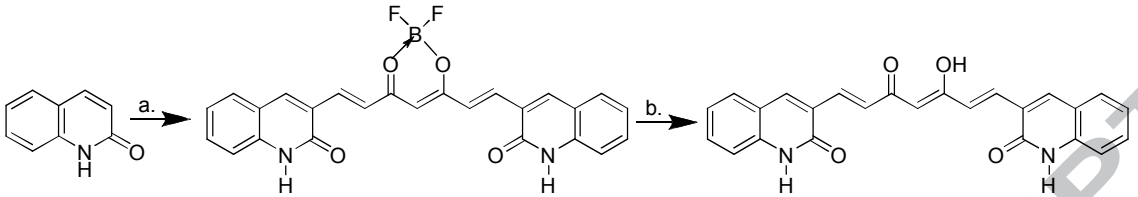
/ DMF / K_2CO_3 / RT / 3-4 hrs; e. Feruloyl acetone difluoroboronite complex / n-$BuNH_2$ / EA / RT / overnight; f. Aq. methanol (MeOH : H_2O 90:10) / Microwave / 3-5 min / 100-150^{0}C



Scheme 2. Synthesis of diquinolone 17. a. Acetylacetone difluoroboronite complex (prepared from acetylacetone and BF_3 etherate) / n-$BuNH_2$ / EA / RT / overnight; b. Aq. methanol (MeOH : H_2O 90:10) / Microwave / 3-5 min / 100-150^{0}C

3-formyl -2-quinolones (Figure **1**) and curcumin-quinolone hybrids (**9-16** in Figure **2**) were screened for cytotoxicity using MTT assay on a panel of representative cell lines and the results are tabulated in Tables **1**&**2** respectively.

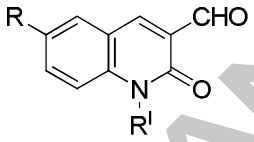


Figure 1. 3-formyl-2-quinolones

Table 1. List of parent quinolones synthesised and their IC_{50} values

Entry No.	Compound No.	R	R^I	IC_{50} Values (μM)			
				A549	MCF-7	SKOV3	H460
1.	**1**	-H	-H	>100	>100	>100	>100
2.	**2**	-H	$-CH_2-CH=CH_2$	>100	>100	>100	>100
3.	**3**	-H	$-CH_2-C_6H_5$	>100	31.9±1.19	>100	>100
4.	**4**	-H	$-CH_2-C\equiv C-H$	>100	>100	>100	>100
5.	**5**	-Cl	$-CH_2-C\equiv C-H$	>100	>100	>100	>100
6.	**6**	-F	$-CH_2-C\equiv C-H$	>100	>100	>100	>100
7.	**7**	$-OCH_3$	$-CH_2-C\equiv C-H$	>100	>100	>100	>100
8.	**8**	$-CH_3$	$-CH_2-C\equiv C-H$	>100	>100	>100	>100

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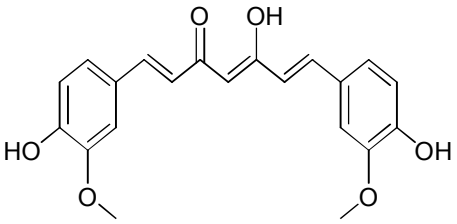
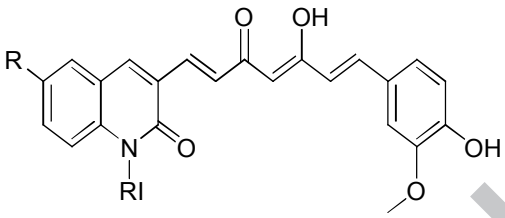
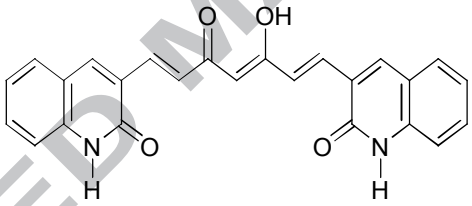


Curcumin
18



Curcumin-Quinolone hybrid
9-16



Diquinolone
17

Figure 2. Curcumin (**18**), Curcumin-Quinolone hybrids (**9-16**) and Diquinolone (**17**)

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Table 2. List of curcumin-quinolone hybrids (**9-16**), diquinolone (**17**) and curcumin (**18**) synthesized and their IC_{50} values

Entry No.	Compound No.	R	R^1	IC_{50} Values (μM)			
				A549	MCF-7	SKOV3	H460
1.	**9**	-H	-H	*	*	*	*
2.	**10**	-H	$-CH_2-CH=CH_2$	31.3±2.18	23.5±2.69	26.0±2.60	**
3.	**11**	-H	$-CH_2-C_6H_5$	*	*	*	*
4.	**12**	-H	$-CH_2-C\equiv C-H$	21.3±1.51	15±1.56	19±2.38	25.4±1.5
5.	**13**	$-OCH_3$	$-CH_2-C\equiv C-H$	20.62±1.18	>100	14.04±2.97	38.14±0.81
6.	**14**	$-CH_3$	$-CH_2-C\equiv C-H$	23.9±2.5	36.2±1.99	12.8±0.21	21.75±0.55
7.	**15**	-F	$-CH_2-C\equiv C-H$	40.45±2.9	25.0±2.3	>100	44.3±1.45
8.	**16**	-Cl	$-CH_2-C\equiv C-H$	**	**	**	**
9.	**17**	-	-	69.1±1.62	31.5±1.2	34.0±2.3	63.3±1.25
10.	**18**	-	-	60.2±2.6	32.2±1.90	43.63±0.63	82.5 ±0.5

*Not active upto 10 μM and beyond that it is not soluble
** Not active upto 30 μM and beyond that it is not soluble

These results indicate dose dependant decrease in cell viability and the hybrids are more potent than 3-formyl-2-quinolones and curcumin. The IC_{50} of each compound was calculated using semi log curve fitting with regression analysis. Among the curcumin-quinolone hybrids, compound **14** was found to show maximum activity against SKOV3 cells with the least observed IC_{50} value of 12.8 μM and hence taken up for further biological experiments. At IC_{50} concentration, the compound was not toxic to normal fibroblast cell line NIH3T3 with cell viability of 74.5%.

To observe the effect on cell morphology, SKOV3 cells were treated with 12.8 μM (IC_{50} value) of **14** for 24 hours. Phase contrast microscopy images of treated cells are shown in Figure **3**. Cell shrinkage and development of bubble like blebs on the membrane were observed. Cells with ruptured cell membranes were also found.

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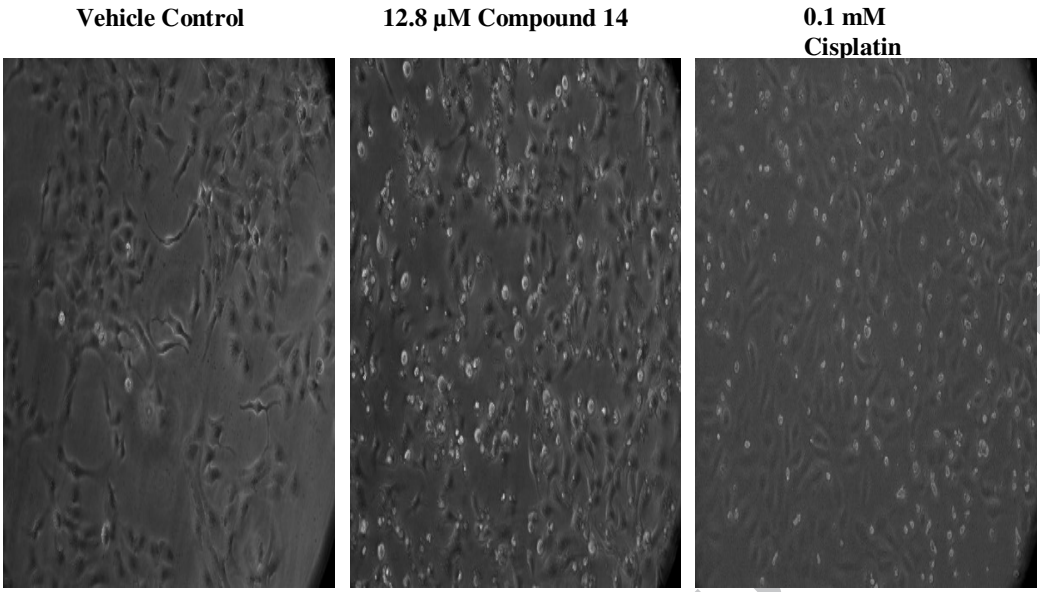

Figure 3. Morphology of SKOV3 cells treated with vehicle control, compound **14** at IC_{50} and 0.1mM Cisplatin treated cells as positive control

As the morphological changes observed were associated with apoptosis, Annexin V/PE staining was carried out to confirm apoptosis as the mode of cell death. SKOV3 cells treated with compound **14** were stained with Annexin V PE/7 AAD and quantified by flow cytometry. Figure **4A** shows the distribution of live, early & late apoptotic and dead cells on treatment with 12.8µM compound **14** and compared with the vehicle control. It was therein observed that 12.8µM of compound **14** significantly induced apoptosis in SKOV3 cells in 24 hours. Apoptosis analysis revealed that the percentage of late apoptotic cells was found to be 62.5% in compound **14** treated cells as compared to 9.0% in vehicle control. While the percentage of early apoptotic cells reduced from 6.8% to 2.5%, the percentage of late apoptotic cells was found to increase from 9.0% to 62.5%. In total, there were 72.3% of dead cells in compound **14** treated cells while it was only 16.5% in vehicle control. Thus, we could observe that compound **14** strongly induced apoptosis at its IC_{50} concentration on SKOV3 cells.

A comparison of the distribution of cells in various stages of apoptosis when treated with 12.8µM of compound **14** and vehicle control is shown in Figure **4B**.

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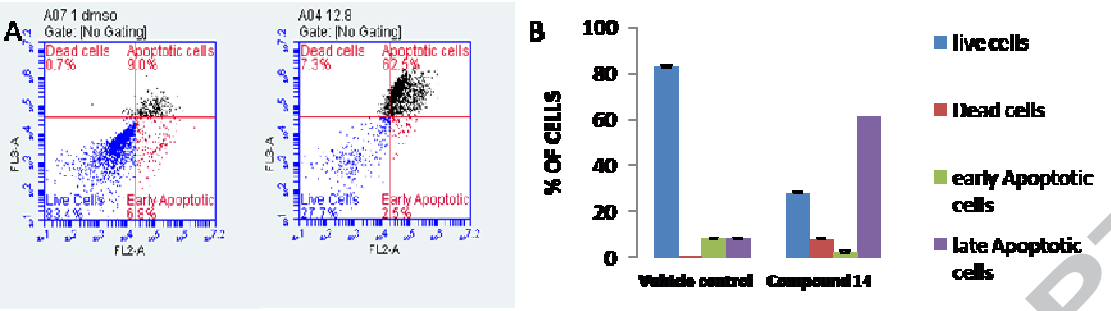

Figure 4. Compound **14** treated SKOV3 cells in various stages of apoptosis (**A and B)**

To examine how compound **14** triggers apoptosis, generation of ROS and the effects of compound **14** on cell cycle machinery were investigated.

Studies have shown that generation of ROS after treatment with cytotoxic agents trigger apoptosis[15]. The cytotoxicity of curcumin has also been attributed to generation of ROS, where in approximately 1.5 fold increase in ROS generation was reported in Rh30 cells within 1-2 hours of treatment with 10µM of curcumin[16]. In the present study, we analyzed generation of ROS through flow cytometry assay using the dye DCFDA (dichloro fluorescein diacetate). When cells were treated at IC_{50} concentration, there was an increase in ROS production. As seen in Figure **5**, 61.5% ROS generation was observed in cells treated with 12.8µM of compound **14** after 3 hours when compared to 20.9% in vehicle control (approximately 3 fold). This confirms the generation of ROS as a mechanism of action for this compound.

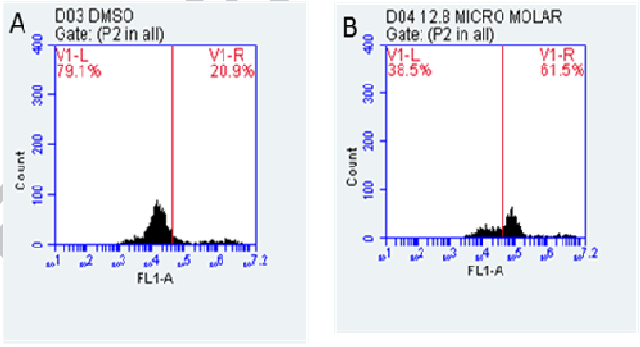

Figure 5. Percentage of ROS generated in SKOV3 cells treated with vehicle control (A) and IC_{50} concentration of compound **14** (B)

Another mode by which apoptosis can be induced is by causing cell cycle arrest[17]. To determine the effect of **14** on SKOV3 cell cycle, cell cycle analysis was performed by hypo propidium iodide method. On treatment with 12.8µM of **14**, there was an increase in the number of cells in the S phase (19.49%) and G2/M phase (25.0%) of the cell cycle as well as a significant reduction in number of cells in the G0/G1 phase (54.43%) as compared to

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vehicle control which showed 11.4% of cells in S phase and 15.43% of cells in G2/M phase and 72.52% of cells in G0/G1 phase of the cell cycle (Figure **6A**). This indicates that compound **14** induces cell cycle arrest in the S phase and G2/M phase rather than G0/G1 phase. These findings are in concordance with reported data wherein curcumin was found to arrest cells in the G2/M phase from 12% in control to 33% at 50µM concentration in HT-29 cells and from 17% in control to 32% at 50µM concentration in HCT-15 cells[18] as well as from 9% in control to 28% at 10µM concentration in MCF-7 cells[19] in 24 hours. A comparison of the distribution of cells in various stages is shown in Figure **6B.** All experimental results were found to be statistically significant by Students t test with $p<0.05$.

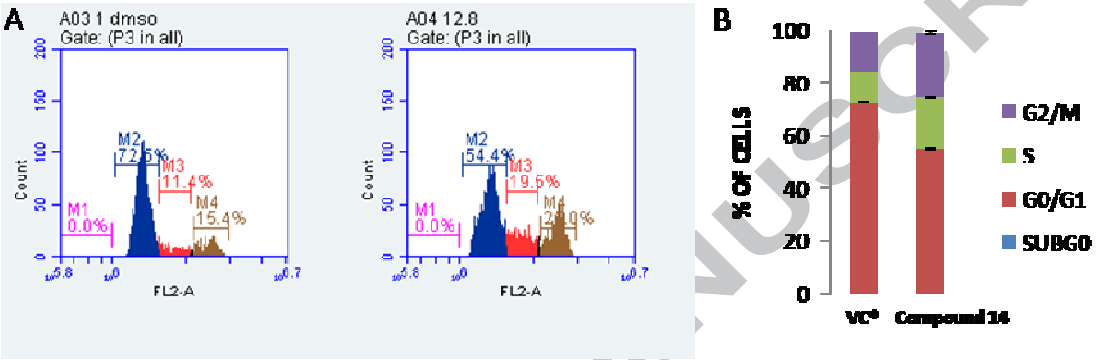

Figure 6. A) Distribution of SKOV3 cells in various stages of the cell cycle on treatment with vehicle control and IC_{50} concentration of compound **14**. Region M2 represents G0/G1 phase, region M3 represents the S phase and region M4 represents G2/M phase. B) Graphical representation of cell cycle analysis on SKOV3 cells treated with compound **14.** *VC= Vehicle control

In summary, compound **14** was found to be the most potent amongst the curcumin-quinolone hybrids tested, inducing morphological changes like cell shrinkage and formation of blebs in SKOV3 cells. ROS generation, S and G2/M phase arrest were found to be the mechanisms of action of compound **14** on SKOV3 cells triggering apoptotic cell death. In the present study, diquinolone **17** with seven carbon linker showed better cytotoxicity than diquinolone with five carbon linker reported in the literature[10] which can be attributed to the presence of β-diketone moiety. The hybrids possessing one vanillin and one quinolone moiety linked by a seven carbon chain showed better activity than both curcumin and diquinolone supporting the concept of synthesising hybrids with active pharmacophores.

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References

1. Ji, JL.; Huang, XF.; Zhu, HL. *Anticancer Agents Med Chem.* **2012**, 12(3), 210
2. Anand, P.; Thomas, SG.; Kunnumakkara, AB.; Sundaram, C.; Harikumar, KB.; Sung, B.; Tharakan, ST.; Misra, K.; Priyadarsini, IK.; Rajasekharan, KN.; Aggarwal, BB. *Biochem. Pharmacol.* **2008**, 76(11), 1590
3. Mosley, CA.; Liotta, DC.; Snyder, JP. *Adv. Exp. Med. Biol.* **2007**, 595, 77
4. Juneja, M.; Vanam, U.; Paranthaman, S.; Bharathan, A.; Keerthi, VS.; Reena, JK.; Rajaram, R.; Rajasekharan, KN.; Karunagaran, D. *Eur. J. Med. Chem.* **2013,** 63, 474
5. Liu, K.; Zhang, D.; Chojnacki, J.; Du, Y.; Fu, H.; Grant, S.; Zhang, *S. Org. Biomol. Chem.* **2013**, 11(29), 4757
6. Teiten, MH.; Dicato, M.; Diederich, M. *Molecules*. **2014**, 19(12), 20839
7. Renhowe, PA.; Pecchi, S.; Shafer, CM.; Machajewski, TD.; Jazan, EM.; Taylor, C.; Antonios-McCrea, W.; McBride, CM.; Frazier, K.; Wiesmann, M.; Lapointe, GR.; Feucht, PH., Warne, RL.; Heise, CC.; Menezes, D.; Aardalen, K.; Ye, H.; He, M.; Le, V.; Vora, J.; Jansen, JM.; Wernette-Hammond, ME., Harris, AL. *J. Med. Chem.* **2009**, 52(2), 278
8. Ruiz, FM.; Gil-Redondo, R.; Morreale, A.; Ortiz, AR.; Fabrega, C.; Bravo, J. *J. Chem. Inf. Model.* **2008**, 48(4), 844
9. Shou, KJ.; Li, J.; Jin, Y.; Lv, YW. *Chem. Pharm. Bull.* **2013**, 61(6), 631
10. Abonia, R.; Insuasty, D.; Castillo, J.; Insuasty, B.; Quiroga, J.; Nogueras, M.; Cobo. J. *Eur. J. Med. Chem.* **2012**, 57, 29
11. Chou, LC.; Tsai, MT.; Hsu, MH.; Wang, SH.; Way, TD.; Huang, CH.; Lin, HY.; Qian, K.; Dong, Y.; Lee, KH.; Huang, LJ.; Kuo, SC. *J. Med. Chem.* **2010**, 53(22), 8047
12. Srivastava, A.; Singh, RM. *Indian. J. Chem*., Sec B. **2005**, 44B, 1868
13. Wichitnithad, W.; Nimmannit, U.; Wacharasindhu, S.; Rojsitthisak, P. *Molecules*. **2011**, 16(2), 1888
14. Rao, EV.; Sudheer, P. *Indian. J. Pharm. Sci*. **2011**, 73(3), 262
15. Circu, ML.; Aw, TY. *Free Radical Biol. Med.* **2010,** 48(6), 749
16. Han, X.; Xu, B.; Beevers, CS .; Odaka, Y.; Chen, L.; Liu, L.; Luo, Y.; Zhou, H.; Chen ,W.; Shen, T.; Huang, S. *Carcinogenesis*. **2012**, 33(4), 868
17. DiPaola, RS*. Clin. Cancer Res*. **2002**, 8, 3512
18. Hanif, R.; Qiao, L.; Shiff, SJ.; Rigas, B. *J. Lab. Clin. Med.* **1997**, 130(6), 576
19. Simon, A.; Allais, DP.; Duroux, JL.; Basly, JP.; Durand-Fontanier, S.; Delage, C. *Cancer Lett.* **1998**, 129(1), 111

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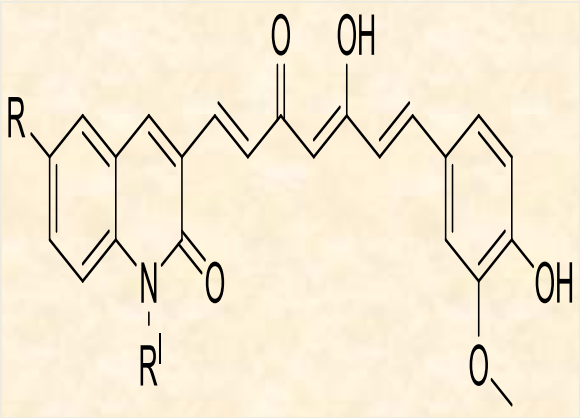
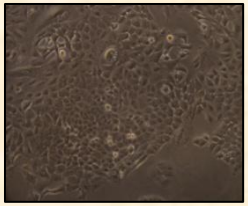


Curcumin- Quinolone hybrid



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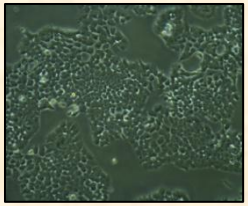

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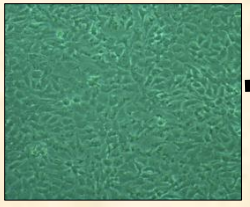

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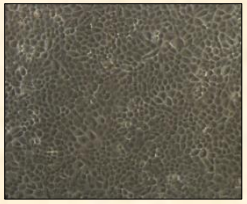

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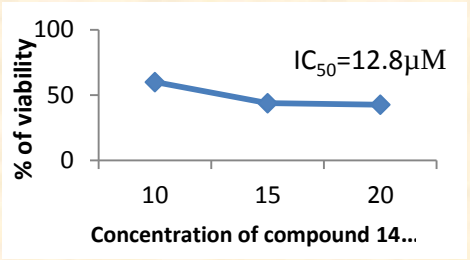
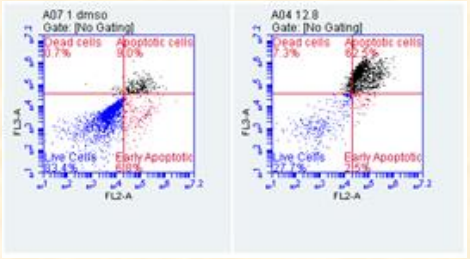
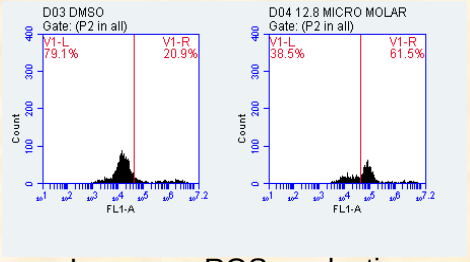


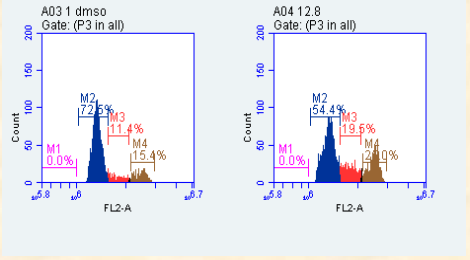
Cytotoxicity to cancer cells



Induces apoptotic cell death



Increases ROS production



Arrests cell cycle at S and G2/M phase