Bioorganic & Medicinal Chemistry Letters 25 (2015) 3601–3605

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and anticancer activity of novel curcumin–quinolone hybrids

Saiharish Raghavan [a], Prasath Manogaran [b], Krishna Kumari Gadepalli Narasimha [a,*], Balasubramanian Kalpattu Kuppusami [a,†], Palanivelu Mariyappan [a], Anjana Gopalakrishnan [b], Ganesh Venkatraman [b,*]

[a] Department of Medicinal Chemistry, Sri Ramachandra University, Chennai 600 116, India
[b] Department of Human Genetics, Sri Ramachandra University, Chennai 600 116, India

ARTICLE INFO

Article history:
Received 8 March 2015
Revised 4 June 2015
Accepted 19 June 2015
Available online 26 June 2015

Keywords:
Curcumin–quinolone hybrids
MTT assay
Annexin-V
Cell cycle analysis

ABSTRACT

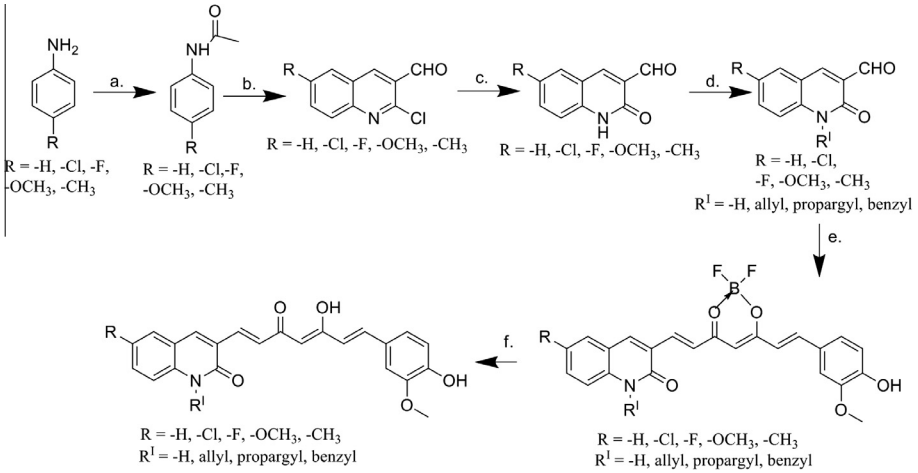
A number of new curcumin–quinolone hybrids were synthesised from differently substituted 3-formyl-2-quinolones and vanillin and their in vitro cytotoxicity was determined on a panel of representative cell lines (A549, MCF7, SKOV3 and H460) using MTT assay. The most potent compound **14**, was analysed 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 (1*E*,6*E*)-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 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.

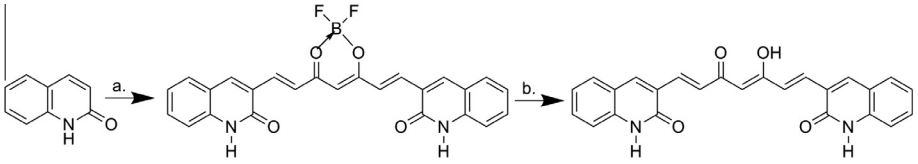
The synthesis of these curcumin–quinolone hybrids **9**–**16** and diquinolone analogue of curcumin **17** are outlined in Schemes 1 and 2 respectively. 3-Formyl-2-quinolones **1**–**8** were prepared from corresponding arylacetamides using Vilsmeier–Haack cyclization as per standard procedure.[12] These 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

* Corresponding authors. Tel.: +91 9444080916 (G.N.K.K.), +91 24765512x237 (G.V.).
E-mail addresses: drkrishna2011@gmail.com (K.K. Gadepalli Narasimha), ganeshv@sriramachandra.edu.in (G. Venkatraman).
† Present address: Department of Biotechnology, IIT-Madras, Chennai 600 036, India.

http://dx.doi.org/10.1016/j.bmcl.2015.06.068
0960-894X/



Scheme 1. Synthesis of curcumin-quinolone hybrids (**9**–**16**). Reagents and conditions: (a) $(CH_3CO)_2O/CH_3COOH$ 30 min, 50–60 °C; (b) $POCl_3$/DMF 80–90 °C, 4–16 h; (c) 4 N HCl reflux, 6–8 h; (d) allyl bromide or propargyl bromide or benzyl bromide, DMF, K_2CO_3, rt, 3–4 h; (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 °C.



Scheme 2. Synthesis of diquinolone analogue of curcumin **17**. Reagents and conditions: (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 °C.

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 spectroscopy and HR-MS techniques.

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

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.

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 12.8 μM compound **14** and compared with the vehicle control. It was therein observed that 12.8 μM 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

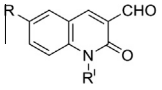


Figure 1. 3-formyl-2-quinolones.

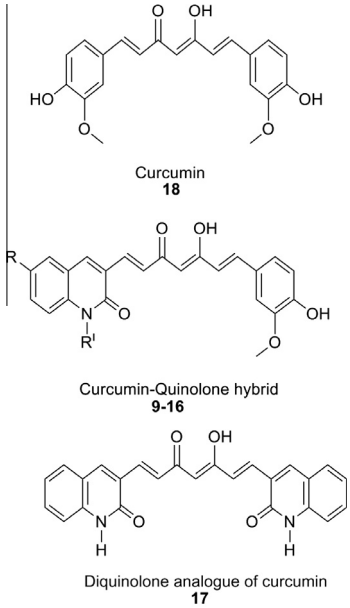


Figure 2. Curcumin (**18**), Curcumin-quinolone hybrids (**9**–**16**) and diquinolone analogue of curcumin (**17**).

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