Bioorganic & Medicinal Chemistry Letters 26 (2016) 1342–1347

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Structure activity relationship, cytotoxicity and evaluation of antioxidant activity of curcumin derivatives

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ARTICLE INFO

Article history:
Received 17 June 2015
Revised 16 November 2015
Accepted 4 December 2015
Available online 7 December 2015

Keywords:
Antioxidant activity
Cytotoxicity
Structure–activity relationship
Curcumin derivatives/analogues

ABSTRACT

Series of curcumin derivatives/analogues were designed and efficient method for synthesis thereof is described. All the synthesized compounds have been screened for their cytotoxicity and evaluated their antioxidant activity. Cytotoxicity effect has been evaluated against three cell lines Hep-G2, HCT-116 and QG-56 by MTT assay method. Structure activity relationship has revealed that particularly, compound **3c**, (IC_{50} value **6.25** μM) has shown better cytotoxicity effect against three cell lines. According to results of SAR study, it was found that 4*H*-pyrimido[2,1-*b*]benzothiazole derivatives (**2e** and **2f**), pyrazoles (**3a**, **3b**, **3c** and **3d**) benzylidenes (**4d**) exhibited better antioxidant activity than curcumin. A correlation of structure and activities relationship of these compounds with respect to drug score profiles and other physico-chemical properties of drugs are described and verified experimentally.

Curcuminoids are the major constituents of turmeric (*Curcuma longa* L.), originated from India and Southeast Asia. The powdered rhizome of turmeric is widely used as spice and coloring agent in food by virtue of its yellowish-orange color and pleasant aroma.[1] Yellowish orange color present in turmeric is chemically 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxy phenyl)-(1*E*,6*E*) or curcumin (Fig. 1). Curcumin is a naturally occurring phytochemical which is used for centuries in a variety of pharmaceutical applications.[2,3] Curcumin and its derivatives exhibited many interesting biological activity such as antiviral,[4] anti-inflammatory,[5] antimicrobial,[6] antioxidant,[7] anti-HIV,[8] cancer preventive properties,[9,10] anti-parkinson,[11] anti-Alzheimer's,[12] anti-angiogenesis,[13] free radical scavenging activity,[14] and anticancer.[15]

Various curcumin analogs/derivatives have been designed and synthesized in order to enhance metabolic stability and antiproliferative activity against human cancer cells.[16,17] Recently, many structural modification efforts were carried out by looking at the variations on carbonyl moiety and active methylene group and it was found that some of the active methylene and carbonyl substituted curcumin derivatives/analogues showed better antioxidant activity than curcumin.[14,18–25] One of the most important aspects of curcumin is its effectiveness against various types of cancer with both chemopreventive and chemotherapeutic properties.[26,27]

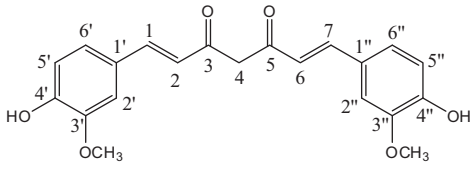

Figure 1. Structure of curcumin used in experiment.

Unfortunately, the potential utility of curcumin is somewhat limited due to poor bioavailability[28] and stability in physiological media.[29] It is believed that the presence of the active methylene group and β-diketone moiety contributes to the instability of curcumin under physiological conditions, poor absorption, and fast metabolism.[30]

Recently, synthetic modifications on carbonyl and active methylene moiety of curcumin has been studied intensively in order to develop the molecules with enhanced properties and stability.[31,32] From these studies it has been shown that compounds synthesized using carbonyl and active methylene moiety of curcumin have enhanced activity and stability in biological medium compared to curcumin.[30–32] However, pyrimido[2,1-*b*]benzothiazole and pyrazole (derived from isoniazide, semicarbazide and thiosemicarbazide) derivatives of curcumin have not been reported so far for their antioxidant activity and cytotoxicity. In an attempt to better understand the curcumin pharmacophore

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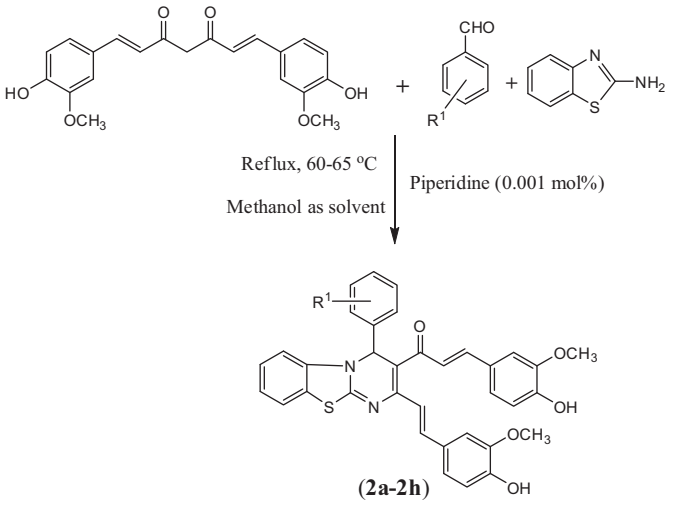
http://dx.doi.org/10.1016/j.bmcl.2015.12.013
0960-894X/

and to improve its pharmacodynamic profile, we designed molecules retaining the *E*,*E*-1,7-diarylhepta-1,6-diene-3,5-dione backbone and synthesized curcumin analogues/derivatives on carbonyl and active methylene moiety of curcumin. Further, synthesized compounds have been evaluated for their cytotoxicity against human cancer lines (hepato carcinoma, colon carcinoma and lung carcinoma) using standard MTT assay method and antioxidant activity by adopting DPPH,[33] superoxide[34] and nitric oxide radical[35] scavenging activity evaluation.

4*H*-Pyrimido[2,1-*b*]benzothiazole derivatives of curcumin (**2a**–**2h**) were synthesized with good yield as outlined in Scheme 1 by condensation of curcumin (5 mmol), aldehydes (5 mmol) and 2-aminobenzothiazole (5 mmol) in the presence of piperidine using conventional heating (Table 1). The structures of 4*H*-pyrimido[2,1-*b*]benzothiazole derivatives of curcumin were confirmed by IR, NMR, Mass spectra and elemental analysis.

Methodology for the synthesis of pyrazole derivative (**3a**–**3d**), involves reaction of curcumin (5 mmol), and hydrazines (5 mmol) in acetic acid at 60–65 °C temperature (Scheme 2, see Supporting information). Under the above optimized conditions, benzylidene derivatives (**4a**–**4d**) of curcumin were synthesized (Scheme 3) using curcumin (5 mmol) and substituted aromatic aldehydes (5 mmol); see Supporting information.

Various aromatic aldehydes containing electron-withdrawing and electron-donating substituents at *ortho*, *meta* or *para* positions show equal ease toward the product formation (Table 1). There was no significant effect of electron donating and electron withdrawing substituents on yield and reaction time.



Scheme 1. Synthesis of 4*H*-pyrimido[1,2-*b*]benzothiazole derivatives of curcumin (**2a**–**2h**).

Table 1
One-pot synthesis of 4*H*-pyrimido[2,1-*b*]benzothiazole, pyrazole and benzylidene derivatives of curcumin

Entry	R^1, R^2, R^3	Product	Time (h)/yield (%)	Mp (°C)
1	H	**2a**	16/81	97–98
2	2-OH	**2b**	16/81	155–156
3	4-Cl	**2c**	18/89	112–113
4	4-NO_2	**2d**	18/86	85–86
5	4-OH-3-OCH_3	**2e**	18/81	80–81
6	4-OH	**2f**	20/89	101–102
7	4-CH_3	**2g**	20/84	108–109
8	2,6-Cl_2	**2h**	20/85	90–92
9	–$CONH_2$	**3a**	6/87	204–206
10	–$CSNH_2$	**3b**	7/81	100–101
11	–COC_5H_4N	**3c**	7/83	105–106
12	–$C_6H_3(NO_2)_2$	**3d**	6/86	118–119
13	H	**4a**	12/87	119–120
14	4-$N(CH_3)_2$	**4b**	13/86	104–106
15	2-OH	**4c**	12/89	92–94
16	4-OH-3-OCH_3	**4d**	14/81	98–100

The in vitro cytotoxicity of the synthesized curcumin derivatives (**2a**–**2h**, **3a**–**3d**, **4a**–**4d**) were evaluated by MTT assay method[36,37] using three selected human tumor cell lines Hep-G2, HCT-116 and QG-56. Inhibitory activities (IC_{50}) are being presented in μM concentrations of the synthesized curcumin derivatives as shown in Table 2. As we can see among pyrimido benzothiazole derivatives of curcumin (**2a**–**2h**), derivatives **2c**, **2d** and **2e** showed good activity with 25 μM IC_{50} against HCT-116, Hep-G2 and QG-56, respectively. Introduction of methyl substituent at *para* position (**2g**) showed much better activity against two cell lines, that is, HCT-116 and QG-56 with 25 μM IC_{50}. Results of cytotoxicity activity have been demonstrated that electron withdrawing and electron releasing groups at *para* position enhanced the activity. As far as compounds **3c** (6.25 μM), **3d** (6.25 μM) and **2f** (12.5 μM) are concerned, they showed better activity than curcumin against all of the tested cancer cell lines, indicating a wide anticancer spectrum. Particularly, cytotoxicity of isoniazid moiety containing pyrazole (**3c**), has sharply increased against Hep-G2, HCT-116 (6.25 μM). Anticancer activity of derivatives **3c** might be due to isoniazid moiety because isoniazid itself shown anticancer activity which increased the overall activity by linking with curcumin molecule, because of derivatives **3c** is a hybrid of isoniazid and curcumin.

Compound **3d** showed very potent activity against Hep-G2 (6.25 μM) is over eight-folds higher than curcumin (50 μM), while weak activity toward the other cell lines, indicating selective inhibition effect. However, the positive control, adriamycin demonstrated the IC_{50} in the range of <2.5–5.0 μM. In other hand, derivatives **3a** exhibited four folds cytotoxicity against HCT-116 and QG-56 than curcumin. As far as other derivatives are concerned, they showed comparable cytotoxicity activity to curcumin. Interestingly, compound **3c** showed potent activity against both Hep-G2 and HCT-116 cancer cell lines, it may be due to isoniazid moiety in curcumin derivatives.

Structure activity relationship has revealed that pyrazole having isoniazid moiety has showed better influence on cytotoxicity. Isoniazid derived pyrazole derivative of curcumin **3c** showed the potent activity against all tested cancer cell lines, indicating a wide anticancer spectrum while other pyrazole derivatives showed selective cytotoxic activity (4–10 folds better than curcumin). Among benzylidene derivatives of curcumin, only *para* methoxy derived (**4d**) derivative showed much better activity against QG-56 (12.5 μM) in series. Furthermore, *para* hydroxyl derivatives (**2f**, 12.5 μM) of 4*H*-pyrimido[2,1-*b*]benzothiazole had the 2–8 folds better activity than curcumin while active methylene linked compounds (**4a**–**4d**, range between 12.5 and 100 μM) showed weakest activity. It seem to have no influence on the cytotoxicity, as no significant difference of activity was found in benzylidene derivatives which have not possess pyrazole moieties in their structure like pyrazole derivatives (**3a**–**3d**). Overall structure activity relationship studies demonstrated that pyrazole moiety is significant for cytotoxicity activity.

It is believed that curcumin moiety has responsible for antioxidant effect as biological activity. In order to investigate whether target compounds maintain the antioxidant activity, synthesized curcumin derivatives were evaluated for their antioxidant activity by $DPPH^{\cdot}$, superoxide and nitric oxide radical scavenging activity evaluation methods. The present study identified the structure activity relationship in curcumin derivatives by DPPH method, which underscores the important chemical feature for this class of molecules to increased antioxidant activity. All of the tested compounds showed moderate to strong free radical scavenging