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Synthesis and preliminary evaluation of curcumin analogues as cytotoxic agents

Qin Zhang, Ying Zhong, Lin-Na Yan, Xun Sun, Tao Gong, Zhi-Rong Zhang *

Key Laboratory of Drug Targeting and Drug Delivery Systems, Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu 610041, PR China

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ABSTRACT

A series of curcumin analogues with different substituents at the 4-position of the phenyl group were synthesized and screened for in vitro cytotoxicity against a panel of human cancer cell lines. Several novel curcumin analogues, especially **32** and **34**, exhibited selective and potent cytotoxic activity against human epidermoid carcinoma cell line A-431 and human glioblastoma cell line U-251, implying their specific potential in the chemoprevention and chemotherapy of skin cancer and glioma. The preliminary SAR information extracted from the results suggested that introduction of appropriate substituents to the 4′-positions could be a promising approach for the development of new cytotoxic curcumin analogues with special selectivity for A-431 and U-251 cell lines.

Curcumin [diferuloyl methane; 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (Fig. 1), a naturally occurring phenolic diarylheptanoid and major bioactive constituent from the rhizome of *Curcuma longa*, has been reported to possess various biological properties including anticancer,[1–3] chemopreventive,[4] anti-inflammatory[5] and antioxidant effects.[2] It has entered phase I and II clinical trials for cancer chemoprevention and chemotherapy.[6–10] Curcumin exerts remarkable cytotoxic activity and apoptosis induction upon a variety of cancer cell lines by modulating the activities of numerous transcription factors, growth regulators, adhesion molecules, apoptotic genes and cellular signaling molecules.[3,4] Due to its anticancer properties, lack of adverse effects and absence of systemic toxicity,[6] curcumin could be an ideal candidate for chemotherapy. However, its in vivo application has been limited for its low potency, poor selectivity and unsatisfactory pharmacokinetics,[11] which necessitate search for new curcumin analogues with a similar safety profile, but improved pharmacological properties.

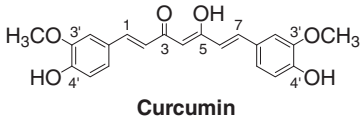
Curcumin has been employed as a promising lead compound for structural modification to develop novel anticancer agents by medicinal chemists worldwide.[12–20] Structure–activity relationship (SAR) studies so far have revealed that the enolic β-diketone moiety and the aromatic rings of curcumin (Fig. 1) may be essential for its biological activities, which prompted researchers to focus on substantial chemical modification in the two parts.

In the previously published studies, the substituent on the 4′-position of curcumin has been proven to be an important pharmacophore playing a pivotal role in various biological activities including anti-inflammatory activity, cytotoxicity and anti-androgenic activity.[12–14,21,22] According to the literature, an appropriate modification at 4′-position could cause enhanced potency and selectivity. The outstanding representative of 4′-substituted analogues was ASC-J9 which exhibited enhanced anti-androgenic activity and cytotoxicity against prostate cancer cell lines.[14,22–25]

Curcumin has been reported to undergo extensive phase I and phase II metabolism via oxidation, reduction, glucuronidation and sulfation in vitro and in vivo.[26–29] This metabolic instability may partly account for its dissatisfied pharmacokinetics. Since the glucuronidation and sulfation occur on the 4′-OH groups of curcumin,[27,28] and shielding the 4′-OH groups could increase its stability,[30] we hypothesized that appropriate modification at 4′-position would also be beneficial for improving its pharmacokinetic properties.

We used a variety of functional groups to replace the 4′-OH groups of curcumin in this study attempting to develop potent and selective cytotoxic anticancer agents. However, to our best knowledge, there is no systematic rationale for 4′-position modification to overcome the above-mentioned shortcomings of curcumin. Therefore, another purpose of this study was to correlate the observed biological activity to the physicochemical properties of the substituents to gain more specific insight into the SAR of 4′-substituted curcumin analogues.

Since the synthesis of a large number of analogues for evaluation was not economically feasible, some representative substituents were selected in our study. Heteroaromatic rings are



Curcumin

Figure 1. Structure of curcumin (keto–enol form).

* Corresponding author. Tel.: +86 28 85501566; fax: +86 28 85501615.
E-mail address: zrzzl@vip.sina.com (Z.-R. Zhang).

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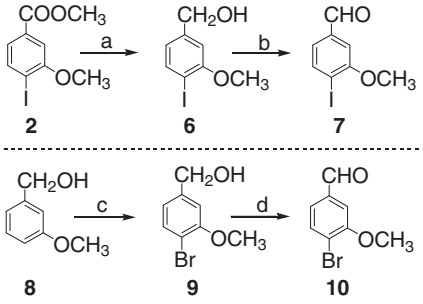
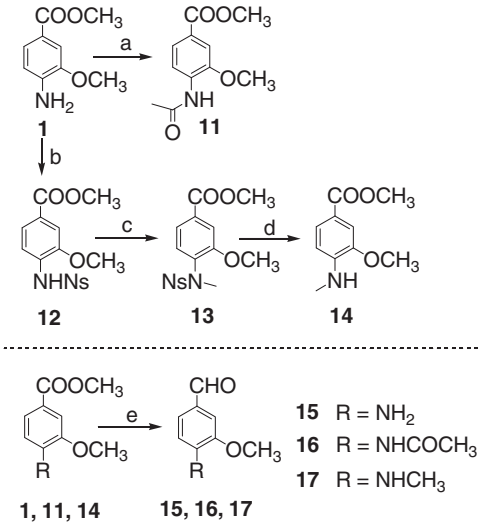
commonly found in synthetic bioactive small molecules that exert specific biological effects. Their hydrophobic nature, flat shape, lack of flexibility, combined with the hydrogen bonding potential of their heteroatoms, might provide some target selectivity for the curcumin analogues.[31] Therefore, several common heteroaromatic rings, such as indole, imidazole, pyrazole and benzoimidazole, were used to replace the 4′-OH groups. Certain 4′-halogen substituted (4′-iodo and 4′-bromo) benzoate precursors would be used as the building blocks of these 4′-heterocycle substituted analogues (**25**–**28**). Since halogen atoms are relative metabolic inertia and could serve as hydrophobic substituents with halogen-bonding capacity,[32–34] the above-mentioned 4′-halogen precursors were also converted into corresponding 4′-halogen substituted curcumin analogues (**29**–**30**). Bioisosteric replacements of functional groups have been reported to be able to enhance the potential for the successful development of new chemotherapeutic agents.[35,36] Thus, the amino group, a well-known bioisosteric replacement for the hydroxyl group, was employed in our study to replace the 4′-OH groups. Interestingly, the 4′-bromo (**30**) and 4′-NH_2 analogues (**31**) both exhibited distinguished cell line selectivity, suggesting that halogen/hydrogen bonding played an important role in the molecular recognition and binding of curcumin derivatives. To further explore the SAR, two substituted amino groups that have the potential to act as hydrogen bond donors and/or acceptors were grafted to the 4′-positions.[34,35] Compared with the amino group, the more hydrophobic methylamino group in **33** would serve as a poorer hydrogen bond donor due to the electron-donating effect of the methyl group. However, for the polar acetylamino group in **32**, the amide proton would serve as a better hydrogen bond donor than amino proton owing to the electron-withdrawing effect of acetyl group, and the carbonyl oxygen of acetyl group would serve as a good hydrogen-bond acceptor. Reversal of the amide bond would change the location and orientation of the hydrogen bond, which might lead to enhanced target selectivity and biological half-life more adequate for therapeutic use.[35,36] Thus, 4′-retro-amide curcumin analogue (**34**) was synthesized and evaluated for in vitro cytotoxicity against a panel of human cancer cell lines, together with other curcumin analogues.

The synthesis of the desired curcumin analogues (**25**–**34**) was accomplished according to following procedures: (1) synthesis of benzaldehyde precursors **5a–5d**, **7**, **10**, **15–17** and **23** (Scheme 1–4); (2) condensation of the benzaldehyde precursors with 2,4-pentanedione (Scheme 5).

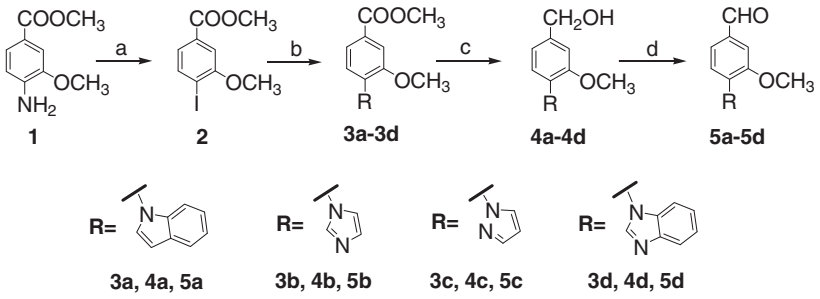
As shown in Scheme 1, aryl iodide **2** was yielded by the diazotization-iodination of aryl amine **1**.[37] Copper-diamine-catalyzed cross-coupling of *N*-H containing heterocycles with aryl iodide **2** (Ullmann Coupling Reaction) led to the respective *N*-arylated heterocycles **3a–3d**,[38,39] which were easily transformed into the desired 4-heterocyclic substituted benzaldehyde precursors **5a–5d** in two efficient steps: reduction and oxidation. The reduction step of benzoate moiety was usually performed with $LiAlH_4$, whereas the following oxidation reaction was achieved by using different but appropriate oxidant for particular intermediate benzyl alcohol. As described in Scheme 2, in addition to being a building block for *N*-arylated heterocycles, aryl iodide **2** itself was also transformed into the corresponding 4-iodo-substituted



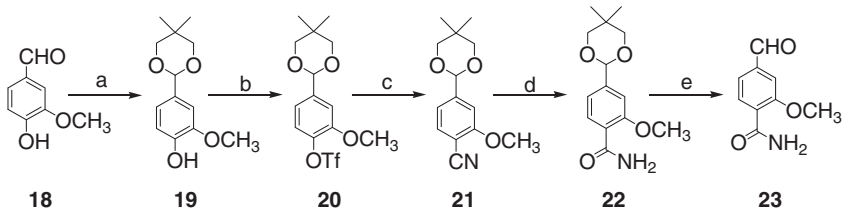
Scheme 2. Synthesis of benzaldehyde precursors Part II. Reagents and conditions: (a) $LiAlH_4$, dry THF, rt, 6 h, 84%; (b) PCC, CH_2Cl_2, rt, 4 h, 88%; (c) $NaBrO_3$, $NaHSO_3$, CH_3CN/H_2O, rt, 4 h, 71%; (d) PCC, CH_2Cl_2, rt, 4 h, 75%.



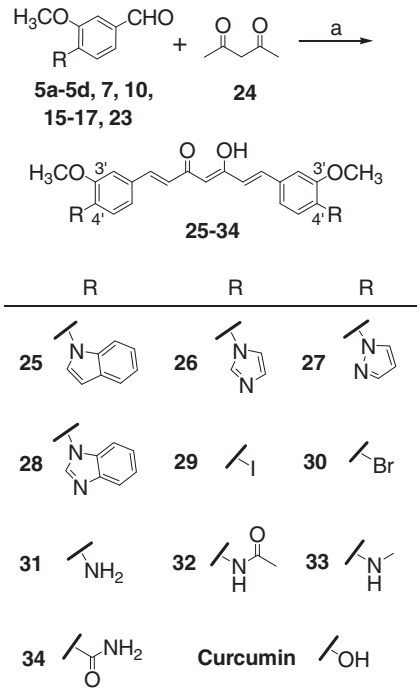
Scheme 3. Synthesis of benzaldehyde precursors Part III. Reagents and conditions: (a) CH_3COCl, Et_3N, dry CH_2Cl_2, rt, 0.5 h, 98%; (b) NsCl, Py, dry CH_2Cl_2, rt, 5 h, 95%; (c) CH_3I, NaH, dry DMF, rt, 18 h, 99%; (d) $HSCH_2COOH$, DBU, CH_3CN, rt, 3 h, 82%; (e) (i) $LiAlH_4$, dry THF, rt, 0.5–4 h, 68–87%; (ii) for **15**: MnO_2, THF, reflux, 3 h, 65%; for **16**: $(COCl)_2$, DMSO, dry CH_2Cl_2, −60 °C, N_2, 12 h, 86%; for **17**: MnO_2, CH_2Cl_2, reflux, 24 h, 51%.



Scheme 1. Synthesis of benzaldehyde precursors Part I. Reagents and conditions: (a) 20% H_2SO_4, $NaNO_2$, KI, aq., −5 °C, 1.5 h, 86%; (b) indole or imidazole or pyrazole or benzoimidazole, (1*S*,2*S*)-cyclohexane-1,2-diamine or *N*,*N*-dimethylglycine hydrochloride, CuI, anhydrous K_3PO_4 or K_2CO_3, dry Tol or DMF or DMSO, 100–110 °C, N_2, 13–24 h, 25–58%; (c) $LiAlH_4$, dry THF, rt, 0.5–18 h, 64–88%; (d) for **5a**: PCC, dry CH_2Cl_2, rt, 12 h, 88%; for **5b**: $(COCl)_2$, DMSO, dry CH_2Cl_2, −60 °C, N_2, 5 h, 72%; for **5c**: PCC, dry CH_2Cl_2, rt, 4 h, 75%; for **5d**: DMP, dry CH_2Cl_2, rt, 4 h, 85%.



Scheme 4. Synthesis of benzaldehyde precursors Part IV. Reagents and conditions: (a) 2,2-dimethylpropane-1,3-diol, cat. TsOH, benzene, reflux, 24 h, 94%; (b) $(CF_3SO_2)_2O$, Py, dry CH_2Cl_2, 0 °C, 2 h, 88%; (c) $NiCl_2(PPh_3)_2$, PPh_3, Zn, KCN, dry CH_3CN, 80 °C, N_2, 4 h, 70%; (d) KOH, *t*-BuOH, reflux, 3 h, 85%; (e) CF_3COOH/H_2O, rt, 16 h, 54%.



Scheme 5. Synthesis of curcumin analogues. Reagents and conditions: (a) (i) B_2O_3, 2,2-dimethoxypropane or tributyl borate, *n*-$BuNH_2$, dry DMF, N_2, 70 °C, 2-4 h; (ii) 5% HOAc (aq), 70 °C, 0.5 h; yields range for **25**–**34**: 2–34%.

benzaldehyde precursor (**7**) by successive reduction and oxidation, and the 4-bromo-substituted precursor (**10**) was synthesized from **8** via regioselective bromination[40] followed by PCC oxidation. As outlined in Scheme 3, the amino group of **1** was functionalized with electron-withdrawing acetyl group and electron-donating methyl group, respectively, to give **11** and **14**.[20] The benzoates **1**, **11** and **14** were further transformed into the corresponding benzaldehydes **15**, **16** and **17**. Preparation of **23**, the reverse amide analogue of **16** shorter by one atom,[41,42] was summarized in Scheme 4. Starting from vanillin (**18**), protection of the aldehyde function as a cyclic acetal, followed by sulfonylation of the phenolic hydroxyl, resulted in phenyl triflate (trifluoromethanesulfonate) **20**. Since the triflate anion is an excellent leaving group, Ni(0)-catalyzed cyanation of **20** was easily carried out to provide benzonitrile **21**,[43] which was hydrolyzed to benzamide **22** under strong basic condition. Deprotection of **22** with TFA finally afforded benzaldehyde precursor **23**.

General synthesis of curcumin analogues from corresponding benzaldehyde precursors was carried out by modified literature methods (Scheme 5).[12,44,45] Under anhydrous condition, using aliphatic amine as catalyst and 2,2-dimethoxypropane or tributyl borate as dehydrating agent, aldol condensation of acetylacetone–boron complex with different benzaldehyde precursors (**5a–5d**, **7**, **10**, **15–17** and **23**), followed by subsequent elimination, eventually gave the desired curcumin analogues (**25–34**).

The cytotoxicity of these curcumin analogues was assessed in a panel of human tumor cell lines using MTT assay. The IC_{50} values for 48 h exposure were summarized in Table 1. Although **33** was reported previously,[20] it was included here to evaluate its cytotoxicity in other cell lines and to provide a comparison with the new analogues.

Curcuminoids have been reported to interact with multiple molecular targets, such as NF-κB, STATs, AP-1, PPAR-g, etc.[3,4] Therefore, the observed cytotoxic effects may be derived from the interactions of curcumin analogues with either a single or multiple targets. The situation is so complicated that it is extremely difficult to reveal the SAR of curcumin analogues. To our delight, in this study, several 4′-substituted analogues (**30**, **31**, **32** and **34**) exhibited significant selective cytotoxicity against human epidermoid carcinoma cell line A-431 and human glioblastoma cell line U-251, which could facilitate our understanding of the structural requirements of curcumin analogues for selectivity for certain tumor cell lines.

In the series of 4′-heterocyclic substituted analogues (**25**–**28**), only **26** bearing an imidazole moiety retained partial cytotoxicity of curcumin, suggesting that rigid and bulky aromatic systems are not well tolerated at 4′-position. Since the nitrogen atoms in the heterocyclic rings would serve as hydrogen bond acceptors, the difference in potency between 4′-imidazolyl analogue (**26**) and 4′-pyrazolyl analogue (**27**) could be connected with the location of the hydrogen bond acceptors. Compared with N^2-nitrogen of 4′-pyrazole group, N^3-nitrogen of 4′-imidazole group was evidently preferred in the H-bonding interaction with active sites. This finding implicated that in the structural requirements of active analogues, besides the need for a hydrogen bond acceptor, a two-atom spacer between the hydrogen bond acceptor and the phenyl ring was preferred, which was confirmed later by the observation in the case of 4′-acetylamino analogue (**32**).

Bromine-substituted derivative (**30**) showed weak but selective inhibition on the proliferation of A-431 and U-251 cells, suggesting that the bromine atom played a critical role in the selective recognition. This could be attributed to the halogen bond donating capacity of the bromine atom. Halogen bonding plays an important role in conferring specificity and affinity for halogenated ligands, and it is the electrostatic interaction between a classical hydrogen bond acceptor and a polarizable, partially electropositive halogen atom (X = Cl, Br, I) that acts like the hydrogen bond donor in the hydrogen bonding.[32–34] The iodine atom in **29** was also a halogen bond donor. However, unlike bromine, it did not provide a positive impact on the cytotoxicity, which indicated that the size of the halogen (I > Br) could also affect the selective recognition and a small substituent was preferred. Considering the similar electrostatic nature of halogen bonding and hydrogen bonding, electropositive halogen bond donor (X = Cl, Br, I) could be treated as a form of hydrogen bond donor.[34] Thus, we speculated that small hydrogen bond donors at 4′-positions were needed for the selective cytotoxicity of curcumin analogues to A-431 and U-251 cell lines, which was verified in the case of 4′-NH_2 analogue (**31**).

As expected, replacing 4′-OH groups with amino groups resulted in 4′-NH_2 analogue (**31**) with selective and significant

Table 1
In vitro cytotoxicity of curcumin analogues against a panel of tumor cells

Compound	IC_{50} (μM)							
	HeLa	HepG2	SkoV3	A549	A-431	U-251	MCF-7	HEp-2
Curcumin	27.4	31.6	37.7	42.6	13.8	13.8	48.0	56.9
25	>100	>100	>100	>100	>100	NT[a]	>100	>100
26	45.4	82.5	66.9	>100	16.8	22.0	>100	>100
27	>100	>100	>100	>100	>100	NT	>100	>100
28	>100	>100	>100	>100	>100	NT	>100	>100
29	>100	>100	>100	>100	>100	>100	>100	>100
30	>100	>100	>100	>100	55.9	84.8	>100	>100
31	>100	>100	>100	>100	15.8	24.5	>100	>100
32	37.1	71.6	55.5	>100	7.2	7.1	>100	>100
33	>100[b]	>100[b]	>100[b]	>100[b]	>100	>100	>100	>100
34	23.8	43.6	38.3	>100	9.4	7.7	49.2	>100

Cell lines: human cervix adenocarcinoma HeLa, human hepatocellular carcinoma HepG2, human ovary adenocarcinoma SkoV3, human lung carcinoma A549, human epidermoid carcinoma A-431, human glioblastoma U-251, human breast adenocarcinoma MCF-7, human laryngeal carcinoma HEp-2.

[a] NT, not tested.

[b] Previously reported.[20]

cytotoxicity against A-431 and U-251 cell lines. In addition, compared to 4′-bromine analogue (**30**), an approximately 3.5-fold improvement in potency was observed for 4′-NH_2 analogue (**31**), which could be ascribable to the better hydrogen bond donor capacity of amino group. To our best knowledge, this is the first report of highly selective cytotoxic activities against human epidermoid carcinoma cell line and human glioblastoma cell line with respect to curcuminoids, and these findings have important practical significance. First, **30** and **31** have the potential to be developed as either skin cancer-specific or glioma-specific chemotherapeutic agents. Second, they can be used as probes to identify the specific molecular target(s) responsible for this apparent cancer cell line selectivity and thereby provide significant implications for the treatment of skin cancer and glioma.

Interchange of amino and hydroxyl groups has been successfully employed in the development of various pharmacological agents. In this study, distinct differences were observed between curcumin and 4′-NH_2 analogue (**31**) in biological activities. The differences could be related to the size and hydrogen-bond donating capacity of the hydroxyl and amino groups. In view of the facts that (a) the size of the substituents, described as van der Waals radius, increased in the sequence: OH < NH_2 < Br < I,[35,46] and (b) the hydrogen-bond donating capacity of the substituents, represented as the local positive charge or electrostatic potential on the molecular surface, increased in the order: Br < I < NH_2 < OH,[32–34] it was possible to draw the following conclusions: (1) bulky substituents without good hydrogen bonding capacity, such as iodine atoms, could not be tolerated at 4′-positions, which was further supported by the observation of complete loss of potency for 4′-methylamino analogue (**33**); (2) the presence of small but strong hydrogen bond donors such as OH groups at 4′-positions could lead to increase in potency but decrease in selectivity; (3) moderate hydrogen bond donors of appropriate size, such as NH_2 groups, at 4′-positions were required for the special selectivity for A-431 and U-251 cell lines. According to these findings, we supposed that the thiol group might be an attractive alternative to the 4′-amino group to achieve the special selective cytotoxicity against A-431 and U-251 cell lines because of their similar size and hydrogen bond donor strength.[34,35]

Since the biological activities of curcumin analogues would be adversely affected by the increased size of 4′-substituents, the acetylamino groups ($NHCOCH_3$) were not originally expected to be well tolerated at 4′-positions. However, to our delight, introduction of acetylamino groups (generating **32**) led to a positive impact on the potency and selectivity. An approximately 2-fold increase in cytotoxicity toward A-431 and U-251 cell lines was achieved for 4′-acetylamino analogue (**32**) over curcumin. Evidently, the 4′-acetylamino groups having both strong hydrogen bond donor and acceptor functionalities were essential for this potent and highly selective cytotoxicity. It is well known that the carbon–nitrogen bond in amide group has a partial but significant double-bond character. For the $NHCOCH_3$ group, the electron delocalization makes the amide NH moiety a strong donor and the amide oxygen atom a strong hydrogen-bond acceptor. Therefore, we speculated that the amide (NHCO) bioisosteres which possess both strong hydrogen bond donor and acceptor functionalities, such as reversed amide (CONH), urea (NHCONH), thioamide (NHCS), carbamate ($NHCO_2$), sulfonamide ($NHSO_2$), thiourea (NHCSNH) and thiocarbamate (NHCOS), might be suitable replacements for 4′-amide moieties in **32** to provide new curcumin analogues with potent and highly selective cytotoxicity against A-431 and U-251 cell lines.[34,35] This deduction was further supported by the subsequent observation. The 4′-retro-amide ($CONH_2$) curcumin analogue (**34**), resulting from reversal of the amide functionalities in **32**, was the most potent analogue in this study and likewise displayed significant selectivity for A-431 and U-251 cell lines.

In summary, several novel curcumin analogues, especially **32** and **34**, exhibited selective and potent cytotoxic activity against human epidermoid carcinoma cell line A-431 and human glioblastoma cell line U-251, implying their specific potential in the chemoprevention and chemotherapy of skin cancer and glioma. The preliminary SAR information extracted from the results suggested that introduction of appropriate substituents to the 4′-positions could be a promising approach for the further development of new cytotoxic curcumin analogues with special selectivity for A-431 and U-251 cell lines. Thus, besides the assessment of the metabolic stability of these analogues, further structural modifications at the 4′-positions of curcumin are in progress in our laboratory to develop new selective cytotoxic agents and to disclose more SAR information, and the results will be reported in due course.

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Supplementary data

Supplementary data (experimental procedures and ^1H NMR, ^{13}C NMR, HRMS data of compounds **25**–**34**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.020.

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