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Design and synthesis of novel iminothiazinylbutadienols and divinylpyrimidinethiones as ARE inducers

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Abstract

Novel iminothiazinylbutadienols and divinylpyrimidinethiones were designed and synthesized as analogues of curcumin with its diketone moiety masked as a heterocyclic adduct with thiourea. The chemical stability of these novel heterocyclic compounds was improved as compared to curcumin. They exhibit longer half-lives and do not react with nucleophilic thiols under physiological conditions. In an ARE-luciferase reporter assay, some of these new curcumin analogues are more effective ARE activators than curcumin and isothiocyanates.

Keywords

Curcumin; Iminothiazinylbutadienols; Divinylpyrimidinethiones; Anti-inflammatory Agents; ARE induction

Curcumin, the yellow principal polyphenol curcuminoid, was isolated from the popular Indian spice turmeric (*Curcuma longa*).¹ It has been shown to activate antioxidant response element (ARE) genes. The activation of ARE genes lead to the enhanced expression of oxidative stress response enzymes that function as a cytoprotective shield against carcinogens, reactive intermediates, and inflammation.^{2–6} Curcumin exhibits a variety of biological and cellular activities including antioxidant, anti-inflammatory, anti-carcinogenic and hypocholesterolemic properties.^{6–11} It has been evaluated in clinical trials as therapeutic and chemopreventive agents for conditions involving inflammation such as multiple myeloma, pancreatic cancer, colon cancer, psoriasis, and Alzheimer's disease.^{12–18}

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Unfortunately, the potential clinical utility of curcumin is limited by its low potency, fast metabolism, poor bioavailability and selectivity.^{18–22}

During the last decade, many synthetic modifications of curcumin have been carried out to enhance its biological activities and to improve its pharmacokinetic properties. The potential utility of curcumin is limited partly due to its chemical and metabolic instability. Curcumin decomposes rapidly under neutral and basic conditions.²² The presence of the active methylene group and diketone moiety contributes to the instability of curcumin under physiological conditions and its fast metabolism.^{22–24} *In vivo*, recent studies indicate that the diketone moiety appears to be a specific substrate of a series of aldo-keto reductases and can decompose rapidly.^{24–26} The stability, metabolic profiles and biological activities of curcumin could be enhanced by modifying its diketone moiety.^{15,27–29} Heterocycles are commonly found in synthetic bioactive small molecules that exert specific biological effects.³⁰ Curcumin analogues bearing certain heterocycles also showed improved anticancer and anti-inflammatory activities.^{29,31,32} Thus, our design is to mask the chemically reactive diketone moiety of curcumin through the conjugation with different thioureas and the introduction of one heterocyclic ring structure to curcumin while retaining most of the other curcumin structure features in efforts to reduce its cytotoxicity and systemic side effects and to increase chemical stability without adversely affecting its anti-inflammatory activity. The introduction of basic heterocyclic nitrogen may provide an opportunity to convert the target compounds into their salt forms and potentially benefit the aqueous solubility. The rigidity and hydrogen bonding potential of the heterocyclic ring introduced might also provide additional conformational restriction and achieve certain target selectivity for these curcumin analogues.^{30,33}

Curcumin has two Michael acceptors when it exists in the keto form. The diketone moiety can accept a variety of nucleophiles such as thiols and amines via Michael addition reactions.^{32,34,35} However, the tautomeric enol form is energetically more stable which means one of the two Michael acceptors in curcumin is more reactive towards nucleophilic attack. Thus, we masked as shown in Fig. 1 one of the reactive Michael acceptors via conjugation with thioureas derived from the chemopreventive isothiocyanates and formed a novel class of heterocyclic curcumin analogues iminothiazinylbutadienols (**Ia–g**), which could rearrange to form the second novel class of heterocyclic analogues, divinylpyrimidinethiones (**IIa–g**). The second series of compounds effectively masks both of the Michael acceptors in curcumin. We further evaluated the effect of introducing different substituents to the imino group on the heterocyclic thiazine ring in series **I** analogues and to the pyrimidine nitrogen in series **II** analogues to derive valuable structure-activity relationships (SAR).

The synthesis of the novel heterocyclic curcumin analogues is shown in Scheme 1. Thioureas are either commercially available or readily prepared from the reaction between isothiocyanates and ammonia. The acid-catalyzed addition of thioureas to curcumin was carried out in the presence of 4 N HCl in dioxane. Nucleophilic attack of the sulfur in thioureas to the β -carbon of the Michael acceptor in the enol ketone form of curcumin under the acidic conditions give the Michael adduct intermediates, which then cyclize with the elimination of water to afford the desired iminothiazinylbutadienol products (**Ia–g**). The thiazine ring can open up in the presence of K_2CO_3 in DMF and then cyclize through the addition of the thiourea nitrogen to the carbonyl carbon of the second Michael acceptor with the elimination of water to give the second series of desired curcumin analogues, divinylpyrimidinethiones (**IIa–g**). Generally, the reactions proceed in good yields ranging from 44% to 79%.

The chemical reactivity of curcumin is well known as evidenced by the adduct formation when it is incubated with glutathione (GSH).³⁶ Indeed, when curcumin was incubated with 1 mM GSH in pH 7.4 buffer at 37 °C, it forms a 1:2 adduct with GSH as indicated by LC-MS analysis (data not shown). However, when our novel heterocyclic curcumin analogues were incubated with GSH under the same conditions, no GSH adducts were detected using LC-MS. Their lack of reactivity towards GSH suggests that we have successfully masked the reactive Michael acceptors of curcumin in the iminothiazinylbutadienol and divinylpyrimidinethione analogues.

To investigate the stability of curcumin analogs under cell culture conditions, we determined the changes in HPLC peak area of curcumin and its analogues upon incubation in Dulbecco's Modified Eagle's Medium (pH 7.4) containing 5% methanol in the absence or presence of 1% FBS. Methanol was used to make sure that curcumin and its analogues were fully soluble under the incubation conditions. As shown in Fig. 2, it was found curcumin degraded very rapidly with a half-life of about 46 min in the absence of FBS. In the presence of 1% FBS, the stability of curcumin was also poor, about 50% of the curcumin was degraded after 2 h of incubation. In contrast, the novel heterocyclic curcumin analogues we prepared are much more stable. For example, compound **Ib** and **IIb** had only about 25% decomposition after 72 h of incubation under the same conditions in the presence or absence of FBS (Fig. 2).

The new curcumin analogs were submitted to NCI Developmental Therapeutics Program (DTP) for cell growth inhibition evaluation in the NCI-60 Human Tumor Cell Line Screen.³⁷ The NCI60 screening at NCI is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 μ M. Compounds that exhibit significant growth inhibition are evaluated further against the 60 cell panel at five concentrations. As shown in Table 1, four out of the 14 compounds submitted did not show significant cytotoxicity and were not selected for further evaluation and the ten that were evaluated in the five-point dose-response assays demonstrated cytotoxicity similar to that of curcumin and isothiocyanates.

Representative heterocyclic curcumin analogues were then evaluated for their ability to induce ARE gene expression in an ARE-Luciferase reporter assay in SW480 cell line. As shown in Fig. 3 and Table 1, curcumin (Cur) and sulforaphane (SFN) all showed strong ARE-induction (5–18 fold) at 10 μ M. Curcumin exhibited nearly 20-fold ARE-induction at 10 μ M. Among the novel heterocyclic curcumin derivatives tested, **Ia** and **IIc** exhibited the strongest ARE-induction with 28–35-fold increase in luciferase activity at 10 μ M. These results are encouraging and the new compounds are being further evaluated in additional ARE-induction and antioxidative assays.

In summary, we have designed and synthesized two series of novel heterocyclic curcumin analogues combining with the structure features of isothiocyanates. These novel analogues were designed to mitigate the chemical reactivity of both curcumin and isothiocyanates, both of which are known to react with biological nucleophiles and be cytotoxic. The new compounds are chemically more stable with much longer half-lives under physiological conditions and do not react with nucleophilic thiols such as GSH while some of them demonstrated stronger ARE induction in an ARE-luciferase reporter assay. Further evaluation of these promising heterocyclic curcumin derivatives are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Design of novel iminothiazinylbutadienols (**Ia-g**) and divinyldiprimidinethiones (**IIa-g**) as conjugates of curcumin and thiourea.

Fig. 2. Stability study of curcumin and representative analogues **Ib** and **IIb** as monitored by changes in peak area at 220 nm using an HPLC assay.

Fig. 3. ARE-inducing activity of heterocyclic curcumin derivatives synthesized in ARE-luciferase reporter assay at 1 and 10 μ M in SW480 cells in comparison to curcumin and sulforaphane (SFN).

Scheme 1.

Synthesis of novel iminothiazinylbutadienols (**Ia-g**) and divinylpyrimidinethiones (**IIa-g**) from curcumin and isothiocyanates.

Table 1

The cytotoxicity and ARE induction of novel iminothiazinybutadienols and divinylpyrimidinethiones in the NCI60 Screen.

Compd	R	NSC# (Lab ID)	% Mean Cell Growth (% at 10 μ M) ^a	Average GI ₅₀ ^b (μ M)	ARE induction ^c
Ia	-H	762482 (LH502)	37.84	2.17	507, 3317
Ib		762483 (LH503)	3.61	2.07	131, 1083
Ic		762485 (LH505)	70.93	16.5	94, 420
Id		762487 (LH507)	0.50	2.32	f
Ie		762489 (LH510)	60.56	54.9	f
If		762484 (LH504)	101.57	d	f
Ig		762520 (LH509)	10.85	2.41	f
IIa	-H	762502 (LH525)	93.53	d	93, 77

Compd	R	NSC# (Lab ID)	% Mean Cell Growth (% at 10 μ M) ^a	Average GI ₅₀ ^b (μ M)	ARE induction ^c
IIb		762503 (LH526)	36.61	6.87	125, 1635
IIc		762505 (LH528)	85.22	^d	124, 4217
IId		762507 (LH530)	-18.35	1.67	^f
IIe		762509 (LH532)	36.95	9.51	^f
IIf		762504 (LH527)	66.43	^d	^f
IIg		762508 (LH531)	-5.50	2.88	^f
CUR		32982	37.2	8.04	198, 2093
PEITC		87868	52.18	1.09	^f
SFN		749790	87.25	^e	413, 582

^a Mean Cell Growth (% relative to control) in the NCI60 single dose assay at 10 μ M.

^b Average GI₅₀ (μ M) in the NCI60 5-dose testing.

^c ARE-Luciferase reporter assay in SW480 cells at 1 μ M and 10 μ M (for comparison, the blank control has a luminescence count of 119 $\pm$ 11).

^d Not selected for 5-dose testing.

^e 5-dose testing data not available in the NCI60 database.

^f Not tested.