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Synthesis and biological activity of 2-aminothiazoles as novel inhibitors of PGE_2 production in cells

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ABSTRACT

This Letter presents the synthesis and biological evaluation of a collection of 2-aminothiazoles as a novel class of compounds with the capability to reduce the production of PGE_2 in HCA-7 human adenocarcinoma cells. A total of 36 analogs were synthesized and assayed for PGE_2 reduction, and those with potent cellular activity were counter screened for inhibitory activity against COX-2 in a cell free assay. In general, analogs bearing a 4-phenoxyphenyl substituent in the R^2 position were highly active in cells while maintaining negligible COX-2 inhibition. Specifically, compound **5l** (R^1 = Me, R^2 = 4-OPh-Ph, R^3 = CH(OH)Me) exhibited the most potent cellular PGE_2 reducing activity of the entire series (EC_{50} = 90 nM) with an IC_{50} value for COX-2 inhibition of >5 μM in vitro. Furthermore, the anti-tumor activity of analog **1a** was analyzed in xenograft mouse models exhibiting promising anti-cancer activity.

Inflammatory processes are implicated in 25% of all cancers and chronic inflammation has been shown to promote the growth of malignant tissues. Indeed, prostaglandin E_2 (PGE_2) has been identified as a key mediator of pain and inflammation and is overexpressed in various cancers. Definitive evidence suggests that PGE_2 is the dominant prostaglandin involved in the growth of tumors associated with colon, lung, breast, head, and neck cancers.[1–4] Interestingly recent studies support a role for PGE_2 in most if not all of the six hallmarks of cancer named by Hanahan and Weinberg; the mechanisms by which PGE_2 induces these cellular capabilities can vary amongst cancer types.[4–6]

Traditionally, prostaglandin synthesis has been modulated primarily through the use of non-steroidal anti-inflammatory drugs (NSAIDs), including non-specific cyclooxygenase (COX) -1 and 2 inhibitors (Aspirin, Tylenol, and Ibuprofen) and specific COX-2 inhibitors (Bextra®, Celebrex®, and Vioxx®). Incidentally, patients taking NSAIDs for chronic inflammatory disorders such as rheumatoid arthritis are associated with a decreased incidence of colon cancer, and high doses of NSAIDs have been shown to cause colorectal tumor regression.[1–4] Furthermore, exogenous treatment with PGE_2 blocks NSAID-induced tumor regression.[1] Therefore, it is believed that the anti-cancer effects of COX inhibitors are ultimately due to a reduction of PGE_2 levels, although there is a downside of NSAID treatment in that the high doses and chronic usage required for anti-cancer activity lead to adverse target-based side effects including gastrointestinal intolerance, heart attacks, and blood clotting.[1–4] Thus, these safety implications have motivated research efforts focused on the reduction of PGE_2 levels through alternate mechanisms.[7–11]

Herein we report the biological evaluation of a series of 2-aminothiazole analogs as a novel class of small molecules with PGE_2 reducing character in HCA-7 colon cancer cells. The general structure for analogs **1–5** is shown in Figure 1.

2-Aminothiazole analogs depicted in Table 1 (**1a–h, 2a–g, 3a–d, 4a–e**) as well as structures **5c–i** from Table 2 were assembled by employing a microwave assisted Hantzsch reaction (Scheme 1).[12,16] Compounds **5j** and **5k–l** were obtained by reduction of the ketone functionality of **5i** and **5f**, respectively (Scheme 1). Alternately, compounds **5a** and **5b** were synthesized in two-steps proceeding through an amidino-thiourea intermediate (**6**) (Scheme 2).[13] Purities of all analogs were assessed by HPLC/MS analysis. All compounds showed purity level greater than 95%, as judged by UV absorbance at 254 and 214 nm as well as evaporative light scattering (ELS).

Initially, all compounds (Figs. 2 and 3) were screened for their ability to reduce PGE_2 production in HCA-7 colon cancer cells at 1 μM concentration; activities are summarized in Tables 1 and 2 as percentage reduction of PGE_2 levels.[17] As a means for tuning out COX-2 activity, compounds that exhibited reduction of PGE_2

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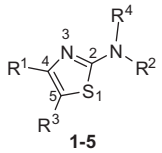
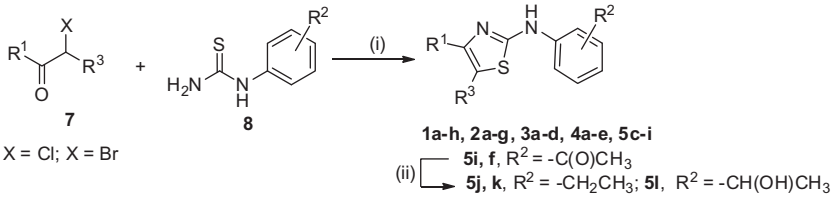
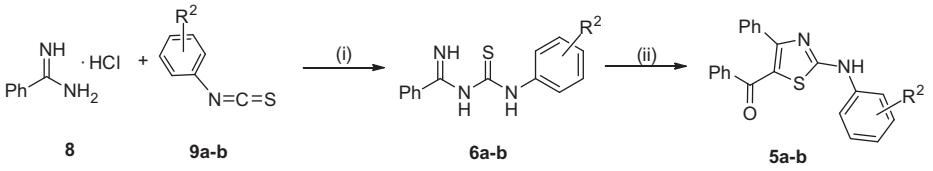


1-5

Figure 1. General structure of aminothiazoles **1**–**5**.



Scheme 1. Synthesis of aminothiazoles **1a**–**h**, **2a**–**g**, **3a**–**d**, **4a**–**e**, **5c**–**l**. Reagents and conditions: (i) Ethanol, microwave irradiation, 110 °C, 10 min; (ii) $LiAlH_4$, dry THF, 0 °C to rt (**5j**, R^1 = Me, R^2 = 4-OCH_3, R^3 = Et, 40%; **5k**, R^1 = Me, R^2 = 4-OPh, R^3 = Et, 20%; **5l**, R^1 = Me, R^2 = 4-OPh, R^3 = –$CH(OH)CH_3$, 7%).



Scheme 2. Synthesis of aminothiazoles **5a**–**b**. Reagents and conditions: (i) DIEA, 2-propanol, 0 °C to rt; (**6a**, R^2 = 3,4-methylenedioxy, 50%; **6b**, R^2 = 4-CN, 81%); (ii) 2-bromo-1-phenylethanone, DBU, DMF, 0 °C to rt (**5a**, R^2 = 3,4-methylenedioxy, 88%; **5b**, R^2 = 4-CN, 83%).

levels higher than 70% were tested for COX-2 inhibition at 5 μM in an in vitro cell free assay,[18] with Celecoxib incorporated as a positive control in both PGE_2 and COX-2 assays.[14] IC_{50} values for COX-2 inhibition were determined only for compounds that exhibited inhibitory activity against COX-2 greater than 50%. Only compounds that exhibited more than 70% reduction of PGE_2 levels, but did not show more than 50% COX-2 inhibition were tested further for EC_{50} determination.

Aminothiazole analogs **1a–h** consist of a *para*-chlorophenyl group at the C-4 position (R^1) of the thiazole ring and varying substituents at other positions. Presence of 4-phenoxyphenyl (**1a**), 3,4-methylenedioxyphenyl (**1b**), or 3-methoxyphenyl (**1c**) at the R^2 position leads to good activity (reduction in PGE_2 levels = 76–94%). Compounds **1d–e** were characterized by lower activity (reduction in PGE_2 levels = 51%) when compared to compounds **1a–c**. Substitution with a polar cyano group (**1f**) also resulted in significant loss of activity (reduction in PGE_2 levels = 23%) possibly due to a decreased ability to permeate the cellular membrane. Surprisingly, analog **1g** bearing a methyl group on the amino functionality (R^4), resulted in good cellular activity despite the polar 4-hydroxyphenyl substituent at R^2 (reduction in PGE_2 levels = 86%). Similarly, substitution of a methyl group at the C-5 position (R^3) of the aminothiazole ring, as in compound **1h**, is also well tolerated (reduction in PGE_2 levels 89%). Compounds from this group exhibited relatively strong inhibition of COX-2 in vitro (IC_{50} = 0.84–1.39 μM), with exceptions represented by **1a** and **1h** (IC_{50} >5 μM). These two compounds were then tested for EC_{50} determination and show similar EC_{50} values for cellular PGE_2 reduction (0.28 and 0.29 μM, respectively).

Compounds **1a** and **2a–g** consist of a *para*-phenoxyphenyl group on the amino functionality (R^2). These compounds show a moderate to high reduction of PGE_2 levels (42–89%). In this series, compounds characterized by a *para*-fluorophenyl (**2b**) or a methoxyphenyl (**2c–e**) substituent at C-4 of the 2-aminothiazole core (R^1) exhibited the highest level of PGE_2 reduction (80–89%). Movement of the methoxy group amongst *ortho*, *meta*, and *para* positions on the phenyl ring at R^1 did not significantly influence the observed biological activity (**2c–e**). However, the activity of compound **2a** (reduction of PGE_2 levels 80%) suggested that the presence of a substituent on the phenyl ring of R^1 was not essential. Presence of bulky lipophilic substituents (naphthyl, **2f**; 4-cyclohexylphenyl, **2g**) at the R^1 position results in lower activity (reduction in PGE_2 levels = 54% and 42%, respectively) when compared to compound **2a**. None of these compounds **2a–g** showed significant COX-2 inhibitory activity (IC_{50} values >5 μM). Notably, analogs **2b** and **2c** exhibited the lowest EC_{50} values for PGE_2 reduction of this group (0.12 and 0.18 μM, respectively). Other analogs showed EC_{50} values similar to analog **1a** (0.24–0.33 μM). In general, analogs bearing a *para*-phenoxyphenyl substituent at R^2 (**1a** and **2a–e**) showed negligible inhibitory activity on COX-2 while still strongly reducing PGE_2 levels in cells.

Replacement of the previous *para*-phenoxyphenyl group at R^2 (**2a–g**) with a *para*-methoxphenyl group (**3a–c**) resulted in an analogous activity trend. Compounds **2e** and **3c** bearing a *para*-methoxyphenyl at R^1 both show similar reduction of PGE_2 levels (80% and 74%, respectively). Additionally, compounds **3a** and **3b** bearing 4-methylphenyl and 3,4-dimethylphenyl substituents, respectively, showed cellular activities comparable to analogs **2a, 2f**, and **2g** also containing hydrophobic moieties at C-4 (reduction in PGE_2 levels = 37–74%, **3a–b** and 42–80%, **2a, f–g**). Interestingly, when the 4-methoxyphenyl group on R^2 of analog **3c** was exchanged with a 4-methylphenyl to give analog **3d**, an increase in cellular PGE_2 reducing activity was observed (reduction in PGE_2 levels = 74% and 92%, respectively). Although transitioning from a *para*-phenoxyphenyl (**2a–g**) to a *para*-methoxyphenyl (**3a–c**) at R^2 does not significantly affect PGE_2 reducing activity, the COX-2 inhibition of **3b–c** (IC_{50} = 1.20 and 1.37 μM, respectively) is considerably stronger than for **2a–e** (IC_{50} values >5 μM).

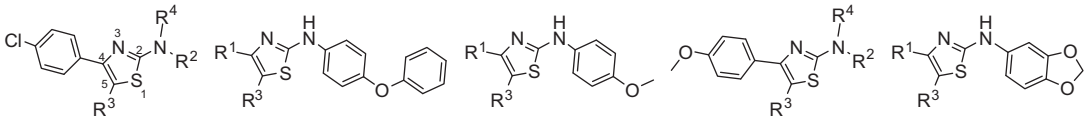


Figure 2. Structure of 2-aminothiazoles analogs **1**–**4**.

Table 1
Biological activity of 2-aminothiazoles analogs **1**–**4**[a,b,c,d,e,f]

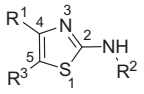
Compd	R^1	R^2	R^3	R^4	PGE_2[c] (%)	COX-2[d] (%)	COX-2[e] (IC_{50}, μM)	PGE_2[f] (EC_{50}, μM)
1a	4-Cl-Ph	4-OPh-Ph	H	H	75.5 ± 7	32.1 ± 5	>10	0.28 ± 0.11
1b	4-Cl-Ph	3-OCH_2O-4-Ph	H	H	94.2 ± 3	68.8 ± 8	0.84 ± 0.20	—
1c	4-Cl-Ph	3-OMe-Ph	H	H	78.3 ± 3	55.1 ± 3	1.39 ± 0.34	—
1d	4-Cl-Ph	Ph	H	H	51.0 ± 8	—	—	—
1e	4-Cl-Ph	1-naphthyl	H	H	51.4 ± 8	—	—	—
1f	4-Cl-Ph	4-CN-Ph	H	H	23.1 ± 10	—	—	—
1g	4-Cl-Ph	4-OH-Ph	H	Me	85.8 ± 8	84.2 ± 13	1.08 ± 0.37	—
1h	4-Cl-Ph	4-OMe-Ph	Me	H	89.3 ± 2	12.0 ± 1	>5	0.29 ± 0.10
2a	Ph	4-OPh-Ph	H	H	79.6 ± 10	31.0 ± 1	>5	0.33 ± 0.15
2b	4-F-Ph	4-OPh-Ph	H	H	87.9 ± 1	43.4 ± 8	>5	0.12 ± 0.04
2c	2-OMe-Ph	4-OPh-Ph	H	H	82.0 ± 3	38.9 ± 5	>5	0.18 ± 0.02
2d	3-OMe-Ph	4-OPh-Ph	H	H	88.9 ± 1	40.3 ± 4	>5	0.29 ± 0.13
2e	4-OMe-Ph	4-OPh-Ph	H	H	80.4 ± 2	40.8 ± 5	>5	0.24 ± 0.05
2f	2-Napthyl	4-OPh-Ph	H	H	53.5 ± 1	—	—	—
2g	4-Cy-Ph	4-OPh-Ph	H	H	41.6 ± 16	—	—	—
3a	4-Me-Ph	4-OMe-Ph	H	H	37.3 ± 6	—	—	—
3b	3,4-diMe-Ph	4-OMe-Ph	H	H	73.9 ± 2	69.3 ± 5	1.20 ± 0.19	—
3c	4-OMe-Ph	4-OMe-Ph	H	H	73.6 ± 3	66.8 ± 3	1.37 ± 0.08	—
3d	4-OMe-Ph	4-Me-Ph	H	H	91.5 ± 1	73.1 ± 6	0.81 ± 0.16	—
4a	Ph	3-OCH_2O-4-Ph	H	H	98.2 ± 1	114.2 ± 1	0.41 ± 0.11	—
4b	2-Cl-Ph	3-OCH_2O-4-Ph	H	H	85.9 ± 3	84.8 ± 3	0.91 ± 0.10	—
4c	2-OMe-Ph	3-OCH_2O-4-Ph	H	H	89.0 ± 4	91.3 ± 4	0.93 ± 0.02	—
4d	3-OMe-Ph	3-OCH_2O-4-Ph	H	H	94.4 ± 2	86.0 ± 8	0.92 ± 0.38	—
4e	4-OMe-Ph	3-OCH_2O-4-Ph	H	H	96.2 ± 1	94.4 ± 2	0.91 ± 0.06	—

[a] Celecoxib was used as a positive control (PGE_2, 5.70 ± 3.28% and EC_{50} = 1.15 ± 0.06 nM; COX-2, 85.6 ± 4.4% inhibition and IC_{50} = 0.03 ± 0.01).[12]
[b] Not determined.
[c] % of inhibition of PGE_2 levels in HCA-7 cells at 1 μM concentration ± SD (n = 3).
[d] % of inhibition of COX-2 levels in vitro at 5 μM concentration ± SD (n = 3).
[e] In vitro IC_{50} for COX-2 inhibition ± SD (n = 3).
[f] EC_{50} for PGE_2 level reduction in HCA-7 cells ± SD (n = 3).

Introduction of a 3,4-methylenedioxyphenyl substituent on the amine nitrogen (R^2) resulted in a dramatic reduction of cellular PGE_2 levels (**4a–e**, 98–86%). Compound **4a**, characterized by an un-substituted phenyl ring at the C-4 position of the 2-aminothiazole (R^1) showed the highest activity (reduction in PGE_2 levels = 98%). Unfortunately, the high levels of cellular PGE_2 reducing activity observed with this group of compounds is always associated with relatively strong inhibition of COX-2 activity (**4a–e**; IC_{50} = 0.41–0.93 μM). Thus, the strong cellular activity of **4a–e** as well as analogs **3b–c** may arise, at least in part, from inhibitory effects on COX-2. However the overall cellular PGE_2 reducing activity of these analogs cannot be fully explained by COX-2 inhibition and rather is likely the result of inhibitory activity on more than one target. Furthermore, although tuning out COX-2 inhibition has proven to be clinically important in terms of safety, PGE_2 reducing therapeutics functioning primarily through a mechanism not involving COX, but maintaining weak COX-2 inhibition, may still be therapeutically useful if PGE_2 biosynthesis can be blocked while COX activity remains at least at a basal level.[1–4]

Compounds **5a–i** containing a keto moiety (acetyl or benzyl group) at the R^3 position (Table 2) were moderately active to inactive (0–59%; except **5f**, reduction of PGE_2 levels = 88%). Overall, analogs characterized by the presence of a methyl at C-4 (R^1) and an acetyl group at C-5 (R^3) of the aminothiazole (**5e–i**) exhibited higher PGE_2 reducing character than analogs **5a–d**. Of interest, when the acetyl group of **5i** was reduced to the corresponding ethyl chain (R^3, **5j**) a marked reduction of PGE_2 levels was observed (94%). In contrast, reduction of the ketone functionality of **5f** to the corresponding ethyl group (**5k**) did not lead to a significant change in activity (reduction in PGE_2 levels = 88% and 87%, respectively). Interestingly, compound **5l** with –CH(OH)Me at R^3 was the most potent compound identified in this report (EC_{50} = 0.09 μM). Compound **5l** was slightly more active than its parent analog **5f** (EC_{50} = 0.39 μM) and its fully reduced derivative **5k** (EC_{50} = 0.37 μM). Active compounds **5f** and **5j–l** were analyzed for COX-2 activity, showing little to no inhibition at 5 μM.

In conclusion, we have synthesized and tested a series of 2-aminothiazole congeners with the ability to reduce the production of PGE_2 in HCA-7 cells. A total of 36 aminothiazoles were evaluated, and active compounds with limited COX-2 inhibition were identified. Compound **1a** was also evaluated for its effect on tumor growth in mouse xenograft models under three different cell lines, and was confirmed to have promising anti-cancer activity. Tumor versus control values (T/C) were 61% and 40% for HCA-7 human colonic adenocarcinoma and SW837 rectum adenocarcinoma cell lines, respectively, under a dosing schedule of 200 mg/kg over 5 days (P <0.05). Additionally, a T/C value of 38% was observed in A549 human lung adenocarcinoma cells after treatment with 100 mg/kg over a period of 10 days (P <0.05).[15] Work is on-going to identify alternate modes of action that may feasibly lead to



5a-l

Figure 3. Structure of 2-aminothiazoles analogs **5**.

Table 2
Biological activity of 2-aminothiazoles analogs **5a–l**[a,b,c,d,e,f,g,h]

Compd	R^1	R^2	R^3	PGE_2[d] (%)	COX-2[e] (%)	COX-2[f] (IC_{50}, μM)	PGE_2[g] (EC_{50}, μM)
5a	Ph	3-OCH_2O-4-Ph	C(O)Ph	NR	—	—	—
5b	Ph	4-CN-Ph	C(O)Ph	NR	—	—	—
5c	Ph	4-OMe-Ph	C(O)Ph	NR	—	—	—
5d	Ph	4-OPh-Ph	C(O)Ph	42.0 ± 6	—	—	—
5e	Me	Ph	C(O)Me	29.9 ± 1	—	—	—
5f	Me	4-OPh-Ph	C(O)Me	87.9 ± 1	28.9 ± 15	>5	0.39 ± 0.07
5g	Me	3-OCH_2O-4-Ph	C(O)Me	25.3 ± 3	—	—	—
5h	Me	4-CN-Ph	C(O)Me	22.4 ± 4	—	—	—
5i	Me	4-OMe-Ph	C(O)Me	58.5 ± 4	—	—	—
5j	Me	4-OMe-Ph	Et	94.4 ± 1	49.3 ± 4	>5	0.32 ± 0.14
5k	Me	4-OPh-Ph	Et	87.1 ± 3	41.3 ± 5	>5	0.37 ± 0.07
5l	Me	4-OPh-Ph	CH(OH)Me	80.4 ± 3	−16.9 ± 10 [h]	>5	0.09 ± 0.04

[a] Celecoxib was used as a positive control (PGE_2, 5.70 ± 3.28% and EC_{50} = 1.15 ± 0.06 nM; COX-2, 85.6 ± 4.4% inhibition and IC_{50} = 0.03 ± 0.01).[12]
[b] NR, no observed reduction.
[c] Not determined.
[d] % of inhibition of PGE_2 levels in HCA-7 cells at 1 μM concentration ± SD (n = 3).
[e] % of inhibition of COX-2 levels in vitro at 5 μM concentration ± SD (n = 3).
[f] In vitro IC_{50} for COX-2 inhibition ± SD (n = 3).
[g] EC_{50} for PGE_2 level reduction in HCA-7 cells ± SD (n = 3).
[h] To be considered as no inhibition of activity rather than induction of activity.

reduced PGE_2 levels. Future synthetic efforts will be focused on further development of aminothiazoles with diverse substitution patterns that will continue to reveal important structure activity relationship features and aid in the identification of more potent compounds with anticancer activity in vivo.

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16. *General procedure for the preparation of* ***2e***: 2-Bromo-1-(4-methoxyphenyl) ethanone **7** (0.437 mmol, 100 mg) and *N*-(4-phenoxyphenyl)thiourea **8** (0.437 mmol, 107 mg) were suspended in ethanol (2 mL) and subjected to microwave irradiation at 110 °C for 10 min. The resulting solution was diluted with CH_2Cl_2 (10 mL) and washed with a saturated aqueous solution of Na_2CO_3 (20 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The resulting crude residue was purified by silica gel column chromatography (EtOAc–hexanes, gradient) using a ISCO™ system to give aminothiazole **2e** as a light orange solid (0.246 mmol, 92 mg, 56%). mp: 125.5–127 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.25 (br s, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.77 (d, J = 8.9 Hz, 2H), 7.37 (t, J = 7.8 Hz, 2H), 7.14 (s, 1H), 7.13–7.02 (m, 3H), 7.02–6.94 (m, 4H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.9, 159.7, 158.6, 150.9, 150.8, 138.4, 130.8, 128.3, 127.9, 123.5, 120.9, 119.2, 118.4, 114.8, 101.4, 56.0. LC–MS $[MH]^+$ 375.1.
17. *PGE_2 production assay*: Cells were seeded in 6-well plates and incubated overnight in DMEM/10% FBS. They were serum starved for the next 18 h. Cells were then treated with 10 ng/ml IL-1β and increasing concentration of compounds (dissolved in DMSO) in 1 mL serum-free medium. After 72 h of incubation, the supernatants were collected for PGE_2 level detection using the PGE_2 EIA kit (R&D Systems).
18. *COX-2 cell-free assay*: COX-2 activity was measured by a COX Fluorescent inhibitor screen assay kit following the manufacturer's instructions (Cayman Chemical, http://www.caymanchem.com).