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2-Aminothiazoles: A New Class of Agonist Allosteric Enhancers of A_1 Adenosine Receptors

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Abstract—This report describes the synthesis and structure–activity relationships of a new class of A_1 adenosine receptor agonist allosteric enhancers, 2-aminothiazolium salts. The EC_{50} of compounds **6a**, **6b**, **7**, and **8** were 0.3, 4.5, 3.8, and 1.2 μM, substantially lower than that of the 'Gold Standard' 2-amino-3-benzoyl thiophene (PD 81,723), which has an EC_{50} of 38 μM.

The four kinds of G protein-coupled adenosine receptors [ARs] (A_1, A_{2A}, A_{2B}, and A_3) are important physiological regulators in many tissues.[1–3] The A_1AR couples to several effectors, inhibiting adenylate cyclase, opening K^+ channels and stimulating phospholipases. A_1AR density is high in the CNS but lower in kidney and heart.[4] A_1AR activation decreases excitatory neurotransmitter release in brain, suppresses pacemaker activity and AV node conduction in the heart, mediates preconditioning in heart, brain and other organs, modulates tubulo-glomerular feedback in the kidney and increases glucose uptake in adipocytes.

An agonist allosteric enhancer [AE] binds to a site on the receptor distinct from the orthosteric site that binds the natural ligand and amplifies the action of the agonist by stabilizing the agonist-receptor–G protein ternary complex.[5] Because the activity of an AE depends on the presence of the natural ligand, it is tissue- and event-specific. Bruns[6] found that 2-amino-3-aroylthiophenes, particularly PD 81,273 (Fig. 1), allosterically enhance binding of agonists to the A_1AR. These compounds were selective for the A_1AR, with little or no effect on agonist binding to A_{2A}-adenosine, M_2-muscarinic or α_2-adrenergic receptors. Since hypoxic or ischemic tissues produce adenosine, a protective metabolite, an AE might be useful clinically when it is desirable to enhance the action of endogenous adenosine.

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The present study shows that 2-aminothiazole derivatives are a new class of AE at the human A_1AR.

A one-step synthesis[7] (Scheme 1) yielded the 2-aminothiazolium salts from their respective ketones, thiourea, and iodine. Workup consisted of concentrated, washed with ether and treated with water to yield a yellow to red-brown solid for characterization by ^1H and ^{13}C NMR. The notes[8] report general procedure for their synthesis and spectral characterization data for some of the new compounds.

Routine synthetic procedures (Scheme 2) afforded some indanone and tetralone derivatives not commercially available. Those ketones were characterized by ^1H and ^{13}C NMR and their purity was analyzed by TLC and GCMS.

DMSO was the solvent for stock solutions of the thiazolium salts. Binding assays employed membranes from CHO-K1 cells stably expressing recombinant human A_1ARs. Figure 2 summarizes the assay method.[9] The first phase consisted of incubating the radiolabeled agonist, ^{125}I-ABA, with membranes for 120 min. The second phase consisted of the addition of the candidate AE and incubation for 5 min, a time sufficient to produce allosteric enhancement without disturbing equilibrium ^{125}I-ABA binding. Dissociation of ^{125}I-ABA was initiated by addition of 50 μM 8-CPT (antagonist) and 50 μM GTPγS. After 10 min, the assay was stopped by rapid filtration of membranes over glass fiber filters.

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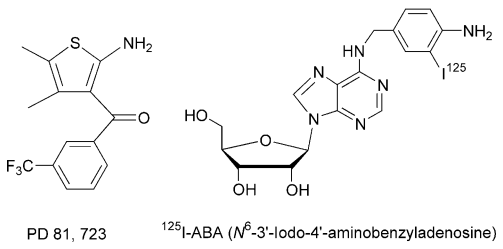
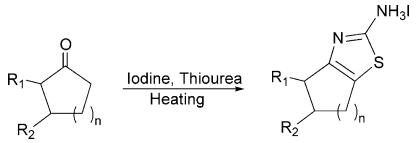
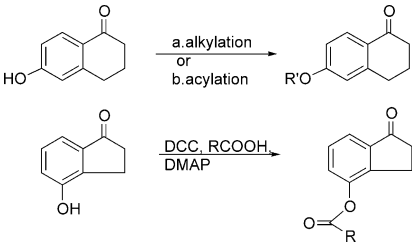


Figure 1. Structures of PD 81,723 and ^{125}I-ABA (agonist).



Scheme 1. General synthesis of 2-aminothiazoliums.



Scheme 2. Derivatization of ketones. (a) NaH/RBr, THF, reflux; (b) RCOCl, NEt_3, RT: R′ = R or COR.

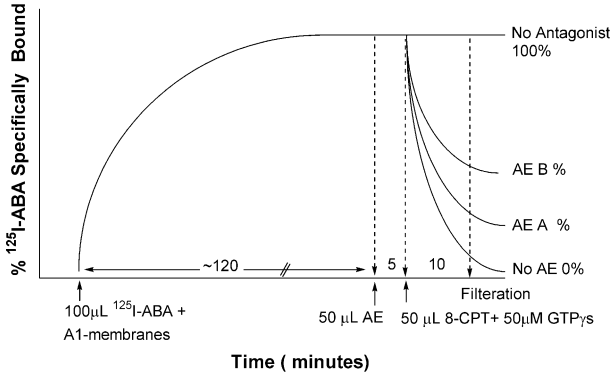
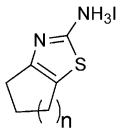
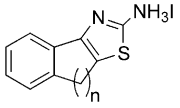
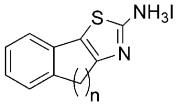
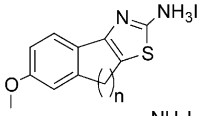
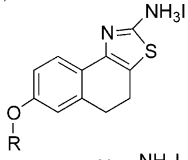
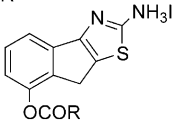
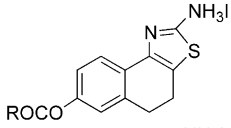
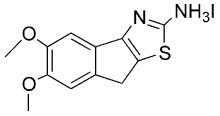
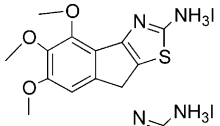
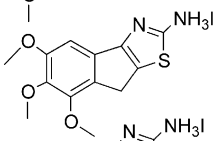
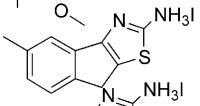
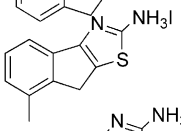
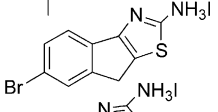
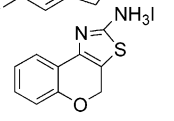


Figure 2. Determination of % score AE activities. Score for AE(X) = %(AE(X)−No AE)/(No Antagonist−No AE) measured 10 min after the initiation of dissociation, and ranges from 0 to 100%. In the examples shown B slows dissociation more than A and has a higher enhancer score.

Specific ^{125}I-ABA bound to the A_1AR (Fig. 2) is the difference obtained by subtracting unspecific equilibrium binding in membranes treated with 8-CPT and GTPγS but not AE, from residual radioactivity on the filter. AE activity is the ratio of specific binding at the end of dissociation to specific binding at the end of 120 min of equilibration, expressed as per centum. % AE activity values vary from 0%, reflecting no enhancement, to 100%, corresponding to complete prevention of agonist dissociation.

Table 1 summarizes the chemical and biological data. Figure 3 compares dose–response curves for two of the thiazolium salts with that of PD 81,273.

Table 1. Structure, yields, and biological data for 2-aminothiazolium salts

	Compd	Yield (%)	% AE activity[a]	EC_{50}[b]
1		a. n = 2, 70 b. n = 3, 72 c. n = 8, 72	19 ± 3 NS 19 ± 4	ND ND ND
2		a. n = 1, 76 b. n = 2, 82 c. n = 3, 64	91 ± 3 80 ± 6 NS	38 17 ND
3		a. n = 1, 93 b. n = 2, 80	NS NS	ND ND
4		a. n = 1, 72 b. n = 2, 71	66 ± 4 78 ± 3	50 8.7
5		a. R = C_5H_{11}, 76 b. R = C_8H_{17}, 79 c. R = C_5H_5, 70	41 ± 20* 45 ± 19* NS*	ND ND ND
6		a. R = CH_3, 52 b. R = C_5H_9, 82 c. R = C_6H_{11}, 89	74 ± 1* 85 ± 1 NS	0.3 4.5 ND
7		a. R = CH_3, 67 b. R = C_6H_5, 62	95 ± 10 54 ± 2*	3.8 ND
8		78	78 ± 1	1.2
9		76	24 ± 3	ND
10		80	60 ± 5*	17
11		77	72 ± 2	4.2
12		69	81 ± 3	ND
13		60	NS	ND
14		77	62 ± 15	ND

[a]% Score values are means of 2–3 experiments, ±SEM, concentration of AE 50 μM and in some cases where * is mentioned concentration is 5 μM (NS = not significantly active).
[b]EC_{50} values are in μM concentrations; ND, not done.

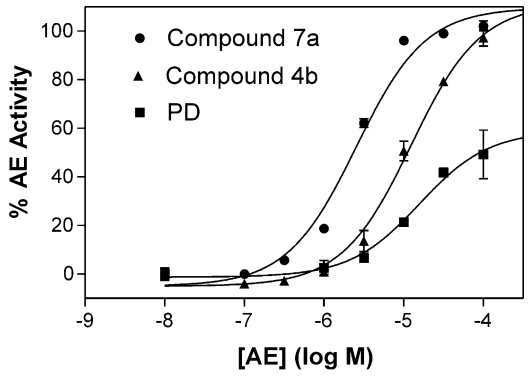


Figure 3. Typical dose–response curves.

An established synthesis gave new thiazolium salts **4a**, **5a**–**b**, **6a**–**c**, **9**, **10**, **11**, and **12** for this investigation. The new compounds have simple structures, contain a variety of functional groups, and can be easily transformed into a variety of analogues. The only structural feature that these 2-amino thiazolium salts share with PD 81,723 is a five-membered aromatic ring containing sulfur. The 2-aminothiazolium salts lack the 3-aroyl moiety thought to be important for the AE activity of 2-amino-3-aroyl thiophenes[6] and yet were potent allosteric enhancers at the A_1AR. Indeed, some were substantially more potent than PD 81,723. Examples are: **4b**, 8.7 μM; **6a**, 0.3μM; **6b**, 4.5μM; **7a**, 3.8 μM; **8**, 1.2 μM, and **12**, 4.2 μM.

The structure–activity profile of the 2-aminothiazolium salts indicates that bicyclic 2-aminothiazolium derivatives have less AE activity than tricyclic salts (compare **1a**–**c** with **2a**–**c**). Among the tricyclic derivatives the potency order of allosteric enhancement is 6:5:5 (**2a**) ~6:6:5 (**2b**) > 6:7:5 (**2c**).

Thus, planarity of the overall skeleton and the dihedral angle between the thiazole ring and the aromatic ring might be important. Exchanging the positions of nitrogen and sulfur as in case of derivatives **2a**–**b** and **3a**–**b** has a marked effect on enhancer activity, suggesting that the disposition of the nitrogen is important for molecular recognition. Steric hindrance exerted by the phenyl groups of **3a**–**b** is an alternative explanation for the difference in AE activity. An electron donor group on the aromatic ring improves allosteric enhancer activity (compare **2b** with **4b**).

Compounds **6a**, **7a**, and **8** are interesting as lead compounds for additional AE's. Although more information is needed to define the structure–activity rules more precisely, the discovery of AE activity in 2-aminothiazoles advances our understanding of these rules.

A few of these compounds were tested for their subtype selectivities within adenosine receptors subfamily and were found to be more potent and highly efficacious on A_1 compared to their A_{2A} and A_3 AR counterparts. For example compounds **4b**, **6a**, **7a**, and **8** displayed AE maximal scores (pEC_{50} value) at A_1 94 (8.7), 80 (0.3), 92 (3.8), 99 (1.2)% as against at A_{2A} 18 (30), 19 (1.8), 24 (23), 36 (12)% and at A_3 8 (not determined), 22 (1.8), 30 (15), 29 (3.1)%, respectively. These compounds are not yet tested for AE activities against other receptor types such as adrenergic and muscarinic. Further studies are progressing in that direction will be reported in due course.

2-Aminothiazolium salts are a new class of allosteric enhancers of A_1ARs distinct from the 2-amino-3-aroyl thiophenes. Some of these compounds are more potent than PD 81,723.

Acknowledgement

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References and Notes

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6. Bruns, R. F.; Fergus, J. H. *Mol. Pharmocol.* **1990**, *38*, 939.
7. King, L. C.; Hlavacek, R. J. *J. Am. Chem. Soc.* **1950**, *72*, 3722.
8. General procedure for synthesis of 2-aminothiazolium salts: Thiourea (3–4 mmol) and iodine (1.1 mmol) were added to a solution of ketone (1.0 mmol) in absolute ethanol (2 mL). The mixture was heated for 2–3 h in an open vessel on oil bath at 100 °C. Heating evaporated the ethanol. The crude residue was washed with ether (3×5 mL) and recrystallized from hot water. Drying under reduced pressure yielded yellow to red-brown solids. A few of these compounds in free amine form are reported in literature and their structural characterization was not carried out thoroughly for example compounds **1a**–**b**, **2a**–**c**, **3a**–**b**, **4a**, **8** and **14**. ^{1}H and ^{13}C-spectral data for selected compounds: Compound **4a**, 5-methoxy-8*H*-indeno[1,2-*d*]thiazol-2-ylamine hydroiodide: ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 6.82 (dd, J = 2.4, 7.8 Hz, 1H), 7.37 (d, J = 2.4 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 9.5 (br s). Compound **8**, 5,6-dimethoxy-8*H*-indeno[1,2,-*d*]thiazole-2-ylamine hydroiodide: ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 7.20 (1H, CH, ArH), 7.25 (s, 1H, ArH), 9.00 (br s); ^{13}C NMR (DMSO-d_6) δ 33.6, 55.6, 55.8, 102.4, 109.8, 119.1, 137.8, 147.6, 148.0, 173.7. Compound **9**, 5,6,7-trimethoxy-8*H*-indeno[1,2-*d*]thiazol-2-ylamine hydroiodide: ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 7.11 (s, 1H, ArH), 8.72 (br s); ^{13}C NMR (DMSO-d_6) δ 34.0, 56.3, 60.6, 61.5, 106.3, 118.5, 119.2, 140.2, 141.2, 144.7, 152.7, 174.4. Compound **10**, 4,5,6-trimethoxy-8*H*-indeno[1,2-*d*]thiazol-2-ylamine hydroiodide: ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 7.11 (s, 1H, ArH), 8.72 (br s); ^{13}C NMR (DMSO-d_6) δ 31.4, 56.1, 60.2, 60.6, 98.6, 121.2, 128.2, 128.8, 139.6, 143.1, 149.5, 153.2, 173.8. Compound **11**, 6-methyl-8*H*-indeno[1,2-*d*]thiazol-2-ylamine hydroiodide: ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H), 3.73 (s, 2H, CH_2), 7.07 (dd, 1H, J = 1.5, 7.8 Hz, ArH), 7.38 (d, 1H, J = 1.5 Hz, 7.42 (d, 1H, J = 7.8 Hz, ArH), 9.03 (br s);

^{13}C NMR (DMSO-d_6) δ 21.1, 33.6, 118.8, 122.1, 124.8, 126.4, 132.9, 136.1, 142.3, 142.6, 173.9. Compond **12**, 4-methyl-8*H*-indeno[1,2-*d*]thiazol-2-ylamine hydroiodide: (DMSO-d_6) δ 2.33 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 7.09 (d, $J=7.8$ Hz, 1H, ArH), 7.27 (t, 1H, $J=7.5$, 7.8 Hz, ArH), 7.40 (d, 1H, $J=7.5$ Hz, ArH), 9.02 (br s); ^{13}C NMR (DMSO-d_6) δ 18.0, 32.8, 116.0, 121.6, 127.0, 127.2, 132.4, 133.9, 143.2, 143.8, 173.8.

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