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Microwave-assisted synthesis of thieno[2,3-*c*]pyridine derivatives as a new series of allosteric enhancers at the adenosine A_1 receptor

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Abstract—The microwave-assisted aromatization method has been used for the synthesis of a series of novel thieno[2,3-*c*]pyridines. This rapid method produces compounds in good yield within minutes in comparison with conventional heating method. The synthesized molecules have been evaluated as a potential new series of allosteric enhancers acting at the adenosine A_1 receptor. In a functional assay, one compound (**3h**) showed activity comparable with that of reference compound PD 81,723.

Allosteric effects are observed when there are interactions between two binding processes that occur simultaneously or sequentially: the binding of one ligand affects the binding of another ligand. The flexible nature of interactions between receptors and various allosteric modulators, together with the potential for subtype selectivity, makes allosteric sites attractive for therapeutic intervention.[1] 2-Amino-3-benzoyl thiophenes are allosteric enhancers acting at the adenosine A_1 receptor.[2,3] Bruns and coworkers reported that this class of derivatives are capable of enhancing both the binding and activity of reference A_1 receptor agonists, such as N^6-cyclopentyladenosine (CPA), at the adenosine A_1 receptor. This effect is manifested as a slowing of the rate of dissociation of the agonist from the receptor.[4] Bruns also reported that these compounds were capable of acting as competitive antagonists at the same receptor, usually at higher concentrations.[5] Therefore, the concentration range where these compounds can enhance the effects of agonists is limited. Among the compounds tested by Bruns, PD 81,723 (2-amino-4,5-dimethylthien-3-yl)-[3-(trifluoromethyl)phenyl]-methanone (**1**) represents a specific and selective allosteric enhancer of agonist binding to the A_1 receptor, with the best ratio of enhancement to antagonistic action at this receptor.[6]

PD 81,723 is selective for adenosine A_1 receptor, having no effects on other adenosine receptor subtypes or on other classes of receptors. While the exact molecular mechanism(s) through which PD 81,723 exerts its allosteric actions remains unknown, the available data indicate that PD 81,723 functions to stabilize a high affinity or agonist-preferring state of the A_1 receptor.[7]

To study the role of various substitutions on the phenyl ring and the importance of the 4,5-dimethyl group on the thienyl ring, several research groups[5,8] have described the synthesis and biological evaluation of compounds useful as potent, yet selective allosteric enhancers of the adenosine A_1 receptor. The 2-amino and 3-benzoyl groups were found to be crucial for the activity. It was evident from previous SAR studies that substitution on the benzoyl moiety at the 3-position of the thiophene ring with electron-withdrawing substituents, such as chlorine

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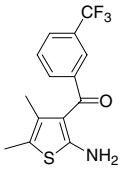
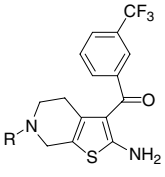
and trifluoromethyl, resulted in higher enhancement activity. Substitution at the 4-position of the thiophene ring with simple alkyl or aryl groups increased activity,[2–4] while bulky 5-alkyl substitution had little or no beneficial effect on activity, with the exception of a small series of thiophene 5-bromo derivatives.[8e] In addition, several studies indicated that the addition of a fused ring on the thiophene provided improved allosteric enhancer activity. Among these compounds, 2-amino-3-aroyl-4,5,6,7-tetrahydro[2,3-*c*]pyridine derivatives with general formula **2** were considered worthy of further attention. In the series of molecules which bear the 3-(trifluoromethyl)benzoyl substituent of PD 81,723, N^6-benzyl substitution (derivative **2a)** was beneficial, while its removal (**2b**) or substitution with a methyl (**2c**) was detrimental for the enhancer activity.[5,8a]

In this article, we report the synthesis and evaluation as allosteric enhancers at the adenosine A_1 receptor of a novel class of ligands with general formula **3**, based on the 2-amino-thieno[2,3-*c*]pyridines' molecular skeleton, that contains different aroyl groups at the 3-position. For compounds **3b**–**h**, the substituents on the benzoyl group were identified which further improved activity (e.g., trifluoromethyl, bromo, chloro, methyl, and methoxy).[3,4,8] Replacing the aroyl for a naphthoyl moiety also yielded potent enhancers.[2,3] By the synthesis of 3-(1-naphthoyl) derivative **3i**, we seek to examine if the receptor environment neighboring the benzoyl binding site is lipophilic. Finally, we prepared **3j** to further explore the effect of the replacement of the phenyl at the 3-position with an isosteric/isoelectronic 2-thienyl group.

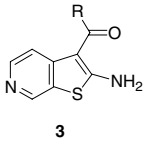
Compounds **3a**–**j** were obtained by an oxidative aromatization procedure under microwave irradiation, starting from the corresponding 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives.



1, **PD 81,723**



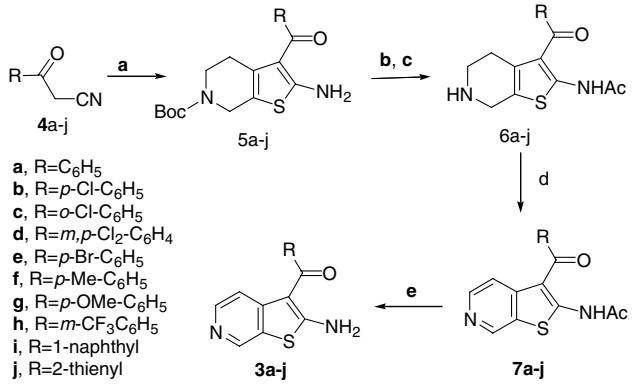
2a, R=Bn
2b, R=H
2c, R=Me



3

R=substitued and unsubstituted phenyl, 1-naphthyl, 2-thienyl.

The thieno[2,3-*c*]pyridine system was previously prepared in low yield by reaction of 3-bromo-4-cyanomethylpyridine with phenylisothiocyanates and sodium hydride.[9] Thieno[2,3-*c*]pyridine analogs with general structure **3** were synthesized by a five-step synthesis described in Scheme 1. Bicyclic N^6-*tert*-butoxycarbonyl (Boc) protected 2-amino-3-aroyl-4,5,6,7-tetrahydrothieno[*b*]pyridines **5a**–**j** were obtained by the Gewald reaction[10] applied to β-ketonitriles **4a**–**j**[11] and *N*-Boc-4-piperidone. Acetylation of the amino group using acetyl chloride followed by removal of the N^6-Boc protecting group with trifluoroacetic acid (TFA) afforded the 4,5,6,7-tetrahydro[2,3-*c*]pyridine derivatives **6a**–**j**. The subsequent aromatization by treatment with manganese dioxide (MnO_2) in toluene under microwave irradiation



Scheme 1. Reagents and conditions: (a) *N*-Boc-4-piperidone, S_8, morpholine, EtOH, 70 °C for 1 h then 1 h at rt; (b) AcCl, pyridine, DCM, rt, 2 h; (c) TFA, DCM. 2 h, rt; (d) MnO_2 toluene, 120 °C, 5 min, microwave (CEM, 300 W, ramp time 2 min); (e) NaOH, EtOH, reflux, 1 h.

(MW) furnished the thieno[2,3-*c*]pyridines **7a**–**j**. In comparison with conventional (thermal) heating, microwave heating reduced the reaction time (3 h vs. 5 min, respectively), although we did not notice any yield improvement (53–65%). These latter derivatives were transformed by saponification into the final products **3a**–**j**.

The effects of compounds **3a**–**j** were determined on forskolin-stimulated cAMP accumulation in the presence of an A_1-adenosine agonist (CPA) in Chinese hamster ovary (CHO) cells expressing the cloned human adenosine A_1 receptor (hA_1AR). Activation of these receptors causes an inhibition of the activity of adenylyl cyclase and a reduction of cAMP content of CHO cells.[12] The results are shown in Table 1.

Allosteric enhancement was measured as the ability of compounds **3a**–**j** at four different concentrations (0.01, 0.1, 1, and 10 μM) to further reduce the cAMP content of CHO:hA_1 cells. The reference compound for comparison was PD 81,723. As shown in Table 1, PD 81,723

Table 1. Effect of compounds **3a**–**j** on cAMP cell content in CHO cells expressing human adenosine A_1 receptor

Compound	cAMP content as percent of control (mean ± SEM)[a]			
	Concentration of compounds			
	0.01 μM	0.1 μM	1 μM	10 μM
3a	91 ± 8	89 ± 10	90 ± 11	100 ± 8
3b	104 ± 6	102 ± 4	96 ± 10	114 ± 10
3c	103 ± 11	98 ± 5	97 ± 5	111 ± 8
3d	113 ± 5	108 ± 8	103 ± 5	105 ± 7
3e	97 ± 6	89 ± 5	84 ± 6	104 ± 4
3f	102 ± 9	107 ± 9	107 ± 6	121 ± 5
3g	95 ± 10	97 ± 9	90 ± 6	94 ± 9
3h	101 ± 5	95 ± 6	82 ± 6	63 ± 4
3i	113 ± 4	112 ± 7	107 ± 5	66 ± 8
3j	111 ± 6	104 ± 5	107 ± 8	98 ± 9
PD 81,723	97 ± 3	93 ± 3	81 ± 3	52 ± 2

[a] The results are the average of six experiments at each of four concentrations of tested compound.

inhibited forskolin-stimulated cAMP accumulation in a concentration-dependent manner from 1 to 10 μM with a maximum inhibition of 48%. The pyridine ring fused to the 2-amino-3-aroyl thiophene ring system led to a significant loss of activity. Thus, these compounds generally have no potency or efficacy as allosteric enhancers through the concentration range tested. Only one compound of this series (**3h**) exhibited a concentration-dependent activity comparable with that of the reference compound, PD 81,723, at all tested concentrations, with 37% inhibition at 10 μM. Replacing the benzoyl with a bulky 1-naphthoyl yielded compound (**3i**), active only at 10 μM.

Since many known allosteric enhancers are also adenosine A_1 receptor antagonists at some (usually higher) concentration, the fact that most of the synthesized compounds did not show a greater efficacy than did 10 μM PD 81,723 could be explained by possible antagonist properties. Compounds **3a**–**j** were tested at a concentration of 10 μM for their ability to displace the binding of [^{3}H]DPCPX, [^{3}H]ZM 241385, and [^{3}H]MRE-3008F20 to the ligand binding site of CHO:-hA_1, CHO:hA_{2A}, and CHO:hA_3 adenosine receptors (AR), respectively (Table 2).[13]

The prototype enhancer, PD 81,723, did not inhibit the binding of a radiolabeled antagonist to A_1 and A_{2A} receptors, but it reduced by 21% the binding of [^{3}H]MRE-3008F20 to the A_3 receptor. None of the selected compounds inhibited binding at the hA_{2A}AR and hA_3AR, but some derivatives (**3b**, **3f**, and **3j**) inhibited binding to the hA_1 AR to some extent (35–50%). For the only compound of this series active as an allosteric enhancer (**3h**), it was possible to achieve a good separation between enhancing activity and binding to the orthosteric site, being devoid of antagonist activity on A_1, A_{2A}, and A_3 receptors.

In conclusion, we have reported a new and general methodology for the construction of a series of 2-amino-3-aroyl-thieno[2,3-*c*]pyridines as potential allosteric enhancer at the A_1-adenosine receptor. This route is convenient for its simplicity, availability of starting materials, and good yields obtained. One compound from this series (**3h**) was shown to have activity as an allosteric enhancer and thus the thieno[2,3-*c*]pyridine nucleus could be useful as a precursor for the synthesis of further compounds.

Table 2. Percent Inhibition activity of enhancer compounds **3a**–**j**

Compound	hA_1[a]	hA_{2A}[b]	hA_3[c]
3a	0 ± 0	0 ± 0	0 ± 0
3b	50 ± 5	0 ± 0	0 ± 0
3c	0 ± 0	0 ± 0	0 ± 0
3d	0 ± 0	0 ± 0	0 ± 0
3e	0 ± 0	0 ± 0	0 ± 0
3f	45 ± 5	0 ± 0	0 ± 0
3g	0 ± 0	0 ± 0	0 ± 0
3h	0 ± 0	0 ± 0	0 ± 0
3i	0 ± 0	0 ± 0	0 ± 0
3j	35 ± 4	0 ± 0	0 ± 0
PD 81,723	0 ± 0	0 ± 0	21 ± 2

Inhibition activity was expressed as percent displacement value (±STD, $n = 3$) of 1 nM of [^{3}H]DPCPX[a], [^{3}H]ZM 241385[b] or [^{3}H]MRE 3008F20[c] by 10 μM of tested compound.

Acknowledgment

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12. A stock solution (10 mM) of each compound was prepared in DMSO at the initial time of use and stored at −20 °C. Chinese hamster ovary (CHO) cells expressing recombinant human A_1-adenosine receptors were used to test A_1-adenosine receptor allosteric enhancers. These cells expressed the A_1 receptor at a density of approximately 5 pmol/mg cellular protein. Cells were grown in 48-well culture plates with Ham's F12 culture medium, fetal bovine serum, and antibiotic G-418 for 48–72 h (1 day preconfluent). To begin an experiment, the culture medium was removed from each well, and warm Hanks' balanced salt solution (HBSS) was added. This wash solution was removed after 6 min at 37 °C and replaced with fresh HBSS solution that included adenosine deaminase

(0.5 U/ml) and 20 μM rolipram with or without 1 μM forskolin, 0.03 nM CPA, and varying concentrations of T-compounds or PD 81,723. After a 6-min incubation at 37 °C, the incubation solution was aspirated, and HCl (0.5 ml, 50 mM) was added to each well to terminate drug action. The cAMP content in each well was determined by a radioimmunoassay. Briefly, a standard curve was prepared in duplicate with tubes containing 100 μl cAMP (0.001–10 pmol) in 50 mM HCl. The 48-well plates containing the samples were gently agitated to mix the contents of the wells. A 5 μl aliquot was then transferred from each well to test tubes containing 100 μl of 50 mM HCl. Each sample was then acetylated by the addition of 4.5 μl triethylamine and acetic anhydride solution (3.5:1), and immediately vortexed. A 10 μl aliquot of [^{125}I]-ScAMP-TME containing 20,000 cpms was added, followed by 100 μl cAMP antibody in a solution of 50 mM Na-acetate buffer (pH 4.75) containing 0.125% BSA. The tubes were then incubated either at room temperature for 2 h or at 4 °C overnight. Following the incubation, 50 μl hydroxyapatite in a 1:1 suspension with water was added to each tube and incubated for 10 min at room temperature. Using a Brandel cell harvester, samples were filtered and rinsed 3 times with ice-cold 10 mM Tris buffer, pH 7.0. The filters were placed in vials and [^{125}I]-ScAMP-TME bound was measured using a Beckman Gamma Counter. In each experiment (3 plates, 48 wells per plate), the response to the tested compounds and PD 81,723 was determined in quadruplicate at concentrations of 0.01, 0.1, 1.0, and 10 μM. Additionally, each plate included controls: basal, 1 μM forskolin, 1 μM forskolin with 100 nM CPA, and 1 μM forskolin with 0.03 nM CPA. Each experiment was repeated at least 3 times. The data were analyzed using Microsoft Excel and graphed using GraphPad Prism. The results are expressed as the means ± SEM.

13. Competition experiments of 1 nM [^{3}H]DPCPX to CHO:-hA_1 membranes were performed incubating membranes (100 μg of protein/assay) at 25 °C for 150 min. Competition experiments were performed in duplicate in a final volume of 250 μl in test tubes containing 50 μM Tris–HCl buffer, pH 7.4, and 100 μl of membranes and at least six to eight different concentrations of the tested compounds. Non-specific binding was defined as the binding in the presence of 1 μM DPCPX and was about 25% of total binding. Competition experiments of 2 nM [^{3}H]ZM241385 to CHO:hA_{2A} membranes were performed incubating membranes (100 μg of protein/assay) at 4 °C for 60 min. Competition experiments were performed in duplicate in a final volume of 250 μl in test tubes containing 50 μM Tris–HCl buffer, 10 μM $MgCl_2$, pH 7.4, and 100 μl of membranes, and at least six to eight different concentrations of the tested compounds. Non-specific binding was defined as the binding in the presence of 1 μM ZM241385 and was about 30% of total binding. Competition experiments of 2 nM [^{3}H]MRE-3008F20 to CHO:hA_3 membranes were performed incubating membranes (100 μg of protein/assay) at 4 °C for 150 min. Competition experiments were performed in duplicate in a final volume of 250 μl in test tubes containing 50 μM Tris–HCl buffer, 10 μM $MgCl_2$, 1 mM EDTA, pH 7.4, and 100 μl of membranes, and at least six to eight different concentrations of the tested compounds. Non-specific binding was defined as the binding in the presence of 1 μM MRE-3008F20 and was about 30% of total binding.