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Synthetic studies on selective adenosine A_{2A} receptor antagonists: Synthesis and structure–activity relationships of novel benzofuran derivatives

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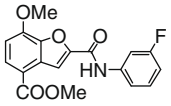
ABSTRACT

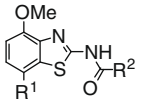
A series of benzofuran derivatives were prepared to study their antagonistic activities to the A_{2A} receptor. Replacement of the ester group of the lead compound **1** with phenyl ring improved the PK profile, while modifications of the amide moiety showed enhanced antagonistic activity. From these studies, compounds **13c**, **13f,** and **24a** showed good potency in vitro and were identified as novel A_{2A} receptor antagonists suitable for oral activity evaluation in animal models of catalepsy.

Adenosine receptors have been extensively characterized and divided into four different subtypes (A_1, A_{2A}, $A_{2B,}$ and A_3).[1] These four receptors play central roles in a variety of biological responses that could be beneficial in a number of clinical applications. The A_{2A} receptor is abundant in the medium-sized neurons of the caudate, putamen, and accumbens nuclei, and stimulates adenylyl cyclase.[2] Epidemiological studies have shown that consumption of caffeine, an A_{2A} receptor antagonist, is associated with a decreased risk of developing Parkinson's disease (PD).[3] PD is a very serious neurological disorder, a chronic and progressive degenerative disease of the brain that impairs motor control, speech, and other functions, and current methods of treatment fail to achieve long-term control. Selective antagonism of the A_{2A} receptor in a primate model of PD ameliorated motor depression.[4] Phase III clinical studies of KW-6002 (Istradefylline) in PD patients with L-dopa-related motor complications yielded promising results with regard to motor symptom relief without motor side effects.[5] Selective A_{2A} antagonists should provide a novel non-dopaminergic approach to PD therapy.

Some xanthine and non-xanthine compounds have been found to have high A_{2A} affinity with varying degrees of A_{2A} versus A_1 selectivity.[6] The search for a new A_{2A} antagonist that is capable of crossing the blood–brain barrier in order to achieve efficacy in rodent model of PD started with benzofuran derivative **1,** identified by high-throughput screening of our compound library (Fig. 1).



1



2

Figure 1.

Previous non-xanthine A_{2A} adenosine receptor antagonists from the literature include compound **2**.[7] These compounds share amide bonds and 5,6-fused heterocyclic skeletons substituted by a methoxy group as a common structural motif. In an effort to better understand this class of compounds as A_{2A} antagonists, we have extensively investigated the effect of modifying the 2- and 4-position of the molecule on the potency and pharmacokinetic parameters. Here, we report the structure–activity relationship of a series of benzofuran derivatives as potent and selective A_{2A} antagonists.

Scheme 1 details the synthesis of benzofuran derivatives with modifications at the 2- and 4-position. The synthesis started with 2-hydroxy-3-methoxybenzaldehyde (**3**). Treatment of compound **3** with ethyl bromoacetate afforded benzofuran **4**. Subsequent reaction with dichloromethyl methyl ether in the presence of $TiCl_4$ provided 4-formyl benzofuran derivative **5**. Hydrolysis of the ester followed by amidation of the resulting carboxylic acid gave the desired benzofurans **7**. Methyl ester **8** was synthesized from 4-formyl benzofurans **7**.

The synthesis of 4-aryl benzofurans is outlined in Scheme 2. Biphenyls **10** were synthesized via Suzuki coupling reaction using

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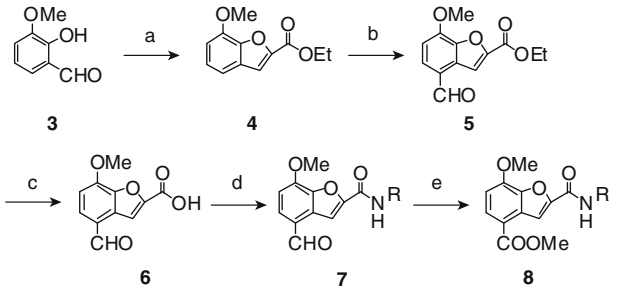
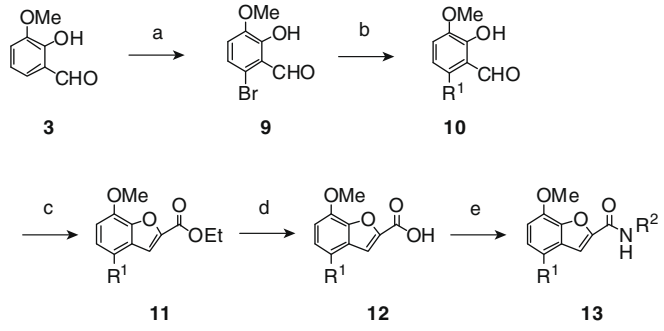


Scheme 1. Reagents and conditions: (a) ethyl bromoacetate, K_2CO_3, DMF; (b) Cl_2CHOCH_3, $TiCl_4$, CH_2Cl_2; (c) LiOH·H_2O, THF, H_2O; (d) RNH_2, WSC·HCl, HOBt·H_2O, Et_3N, DMF; (e) I_2, KOH, MeOH.



Scheme 2. Reagents: (a) Br_2, AcOH; (b) $R^1B(OH)^2$, $PdCl_2(P(o\text{-tol})_3)_2$, K_2CO_3, H_2O, EtOH, toluene; (c) ethyl bromoacetate, K_2CO_3, DMF; (d) LiOH·H_2O, THF, H_2O; (e) R^2NH_2, WSC·HCl, HOBt·H_2O, Et_3N, DMF.

palladium catalyst from 6-bromo-2-hydroxy-3-methoxybenzaldehyde (**9**). A screening of palladium catalysts and ligands revealed that $PdCl_2(P(o\text{-tol})_3)_2$ was a good catalyst for the reaction. The 4-aryl benzofurans **13** were obtained by the same procedure described in Scheme 1.

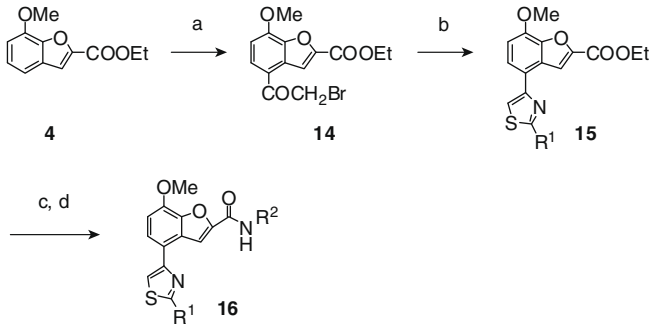
For the preparation of 4-thiazolyl benzofurans, compound **4** was bromoacetylated by Friedel–Crafts reaction, followed by the cyclization using thioamide to give compound **15**. Subsequent hydrolysis and amidation gave the desired compound **16** (Scheme 3).

The reverse-amide compound was prepared through the route described in Scheme 4. To avoid the use of 2-aminobenzofuran, which is difficult to treat because of the instability of the compound, an indirect synthetic method was developed. First, 2-cyanobenzofuran **17** was prepared by cyclization of compound **9** with bromoacetonitrile, followed by Suzuki coupling reaction to give 4-phenylbenzofuran **18**. Treatment of compound **18** with aniline afforded amidine **19**. The reaction of compound **19** with iodobenzene diacetate gave the rearranged product **20**, which was further treated with aniline to afford *N*-(7-methoxy-4-phenylbenzofuran-2-yl)acetamide **21**.

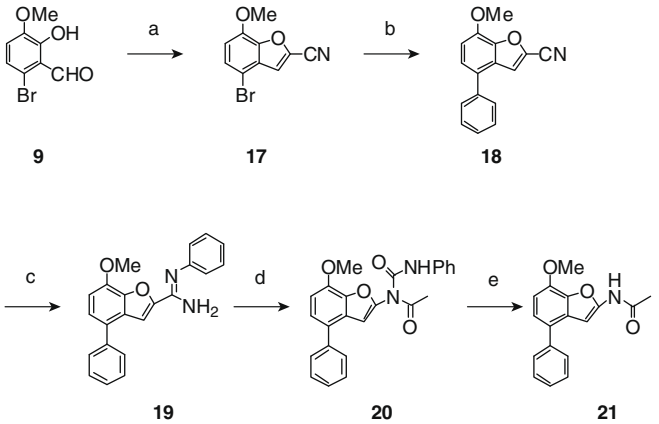
Carbamates **24** and urea **25** were prepared starting with the hydrazination of ester **11f**. The reaction of hydrazine with sodium nitrite provided azide **23**, followed by Curtius rearrangement with alcohols or anilines, providing the desired compounds (**24** and **25**) (Scheme 5).

The corresponding benzothiophene analogues were synthesized as shown in Scheme 6. The starting 3-fluoro-2-formyl-4-methoxybiphenyl (**26**) was cyclized in the presence of methyl 2-mercaptoacetate and then hydrolyzed to give 2-carboxy substituted benzothiophene **27**. The reaction of the carboxylic acid **27** with amines afforded the amides **28**.

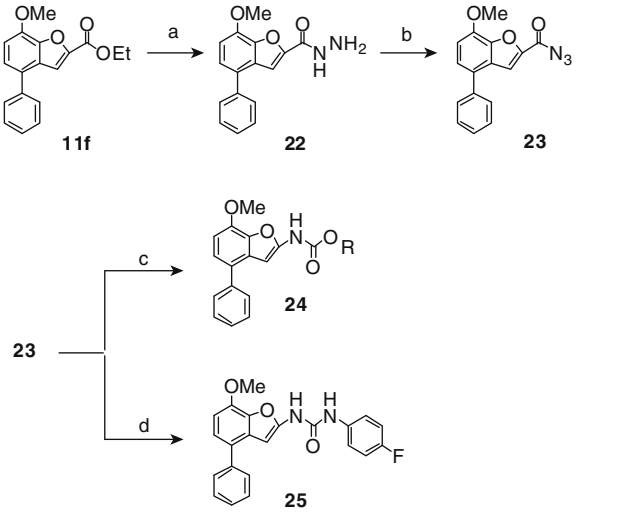
The compounds were evaluated in binding assays to the three subtypes of adenosine receptors. The assays were performed with



Scheme 3. Reagents: (a) $BrCOCH_2Br$, $AlCl_3$, CH_2Cl_2; (b) R^1CSNH_2, dioxane; (c) LiOH·H_2O, THF, H_2O; (d) R^2NH_2, WSC·HCl, HOBt·H_2O, Et_3N, DMF.

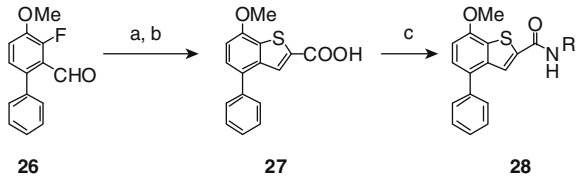


Scheme 4. Reagents: (a) bromoacetonitrile, K_2CO_3, DMF; (b) $PhB(OH)_2$, $PdCl_2(P(o\text{-tol})_3)_2$, K_2CO_3, H_2O, EtOH, toluene; (c) aniline, $AlCl_3$, CH_2Cl_2; (d) iodobenzene diacetate, toluene; (e) aniline, toluene.



Scheme 5. Reagents: (a) N_2H_4, EtOH; (b) $NaNO_2$, AcOH, H_2O; (c) ROH, toluene; (d) 4-fluoroaniline, toluene.

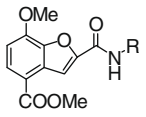
recombinant human receptors using the same radioligand protocol as described previously.[8] A study for the initial set of compounds aimed at determining the effect of substituents on the 2-position of the benzofuran ring. The effects of alterations made to the aniline ring of compound **1** on the inhibitory activity are summarized in Table 1. The non-substituted (**8a**), 4-chloro (**8b**), and 4-methoxy (**8c**) substituted analogues showed equivalent potency to lead

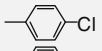
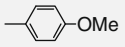
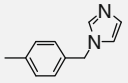


26 27 28

Scheme 6. Reagents: (a) methyl 2-mercaptoacetate, NaH, DMSO; (b) LiOH·H_2O, THF, H_2O; (c) RNH_2, WSC·HCl, HOBt·H_2O, Et_3N, DMF.

Table 1
Structure–activity relationships for 4-methoxycarbonylbenzofurans



Compound	R	A_{2A}[a] lnhibn. (%) 10^{-8}/ 10^{-7}/10^{-6} (mol/L)	CGS catalepsy lnhibn. (%) 10 mg/kg, po
1		14/58/86	48
8a		6/49/86	NT
8b		1/43/77	NT
8c		–/53/91	NT
8d		40/80/98	0

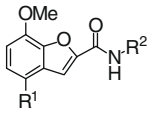
[a] Average of triplicate measurements; NT = not tested.

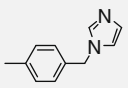
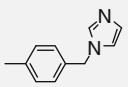
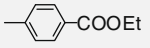
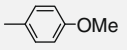
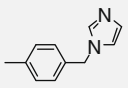
compound **1**. From the structural similarity to Roche's compounds **2**,[7] the introduction of (1*H*-imidazol-1-yl)methyl group was evaluated, and this analogue **8d** exhibited increased potency. However, compound **8d** showed no activity in the mouse CGS21680-induced catalepsy model, which is commonly used to assess the activity of compounds for antiparkinsonian effects. This result may be due to the pharmacokinetic instability of the ester group at the 4-position.

Therefore, we turned our attention to the introduction of a variety of substituents at the 4-position that are capable of replacing the methoxycarbonyl group. While the introduction of the formyl, carboxyl, and acyl groups was detrimental to the antagonistic activity (data not shown), the analogues with phenyl group at the 4-position exhibited equipotent activities to those of the 4-methoxycarbonyl analogues (Table 2). Substitutions of the amide with methyl (**13a**), phenyl (**13b**), morpholino (**13c**), 4-pyridyl (**13d**), or (1*H*-imidazol-1-yl)methyl (**13e**) group were also tolerated in 4-phenyl benzofuran derivatives. In particular, 2,6-dichloro-4-pyridyl analogue **13f** showed the most potent activity. Oral administration of the compounds at 10 mg/kg was found to be active in the mouse model CGS-induced catalepsy. The introduction of the chloro (**13g, h**) or ethoxycarbonyl (**13i**) group at the 4-position of the phenyl group showed significantly less potency, while the 4-methoxy analogue **13j** was approximately equipotent. The analogue **16a–d**, with 2-thiazolyl substituent at the 4-position, significantly diminished the antagonistic activity (see Table 3).

The next set of compounds was prepared to study the influence of the replacement of the oxygen atom by a sulfur atom in the benzofuran ring. The benzothiophene derivatives **28a, b** were found to be less potent than the corresponding benzofuran derivatives. This result suggested the superiority of benzofuran skeleton with respect to its affinity for the A_{2A} receptor.

Table 2
Structure–activity relationships for 4-phenylbenzofurans



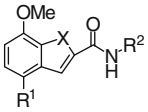
Compound	R^1	R^2	A_{2A}[a] lnhibn. (%) 10^{-8}/ 10^{-7}/10^{-6} (mol/L)	CGS catalepsy lnhibn. (%) 10 mg/kg, po
1	–COOMe		14/58/86	48
13a		–Me	57/88/—	18
13b			13/49/67	NT
13c			39/81/103	78
13d			29/87/100	40
13e			24/64/95	12
13f			83/101/—	74
13g			18/28/—	NT
13h			8/33/—	NT
13i			–/–2/–8	NT
13j			24/63/90	54

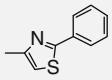
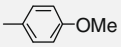
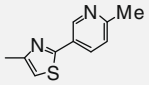
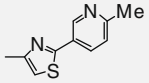
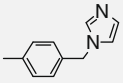
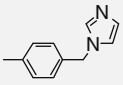
[a] Average of triplicate measurements; NT = not tested.

The amide template was further modified by replacing it with a reversed amide, carbamate, or urea template, as shown in Table 4. Reversed amide bond was tolerated, and the analogue containing a methylcarbamate exhibited good in vivo efficacy. This result indicated that the pharmacological characteristics of compound **24a** are advantageous for penetration of the blood–brain barrier. Encouraged by this result, we next examined aryl carbamates and ureas to adjust the physicochemical properties of the compounds in this series. However, these analogues displayed significantly lower potencies as compared to the corresponding amide derivatives.

Most of the benzofuran derivatives showed remarkable selectivity of A_{2A} over A_1 and A_{2B}. Specifically, the compounds **13e** and **13j,** having an imidazolyl group, showed no activity against A_1 or A_{2B} receptors at 10^{-5} mol/L, which implies the nature and size of the substituents at the amide moiety are critical for good selectivity. The compounds with good inhibitory activity were selected and their rat liver microsomal stability was evaluated. The results are shown in Table 5. For a direct comparison of the in vitro metabolic stability, CL_{int}/f_m can be calculated from the experimental apparent intrinsic clearance, CL_{int}, by correcting for free fraction of test compounds in the incubations. As discussed above, the stability of the benzofuran derivative **8d** having an ester group at 4-position was relatively low. On the other hand, 4-phenyl benzofurans were demonstrably better in terms of their

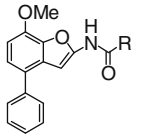
Table 3
Structure–activity relationships for 4-(2-thiazolyl)benzofurans and 4-phenylbenzothiophenes

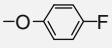
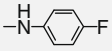


Compound	R^1	R^2	X	A_{2A}[a] lnhibn. (%) 10^{-8}/ 10^{-7}/10^{-6} (mol/L)
16a			O	1/15/60
16b			O	6/11/−19
16c			O	17/6/4
16d			O	12/23/20
28a			S	11/31/—
28b			S	25/20/—

[a] Average of triplicate measurements.

Table 4
Structure–activity relationships for reversed amide, carbamate, and urea derivatives



Compound	R	A_{2A}[a] lnhibn. (%) 10^{-8}/ $1C^{-7}$ (mol/L)	CGS catalepsy lnhibn. (%) 10 mg/kg, po
21	–Me	62/91	28
24a	–OMe	28/85	86
24b	–OPh	23/65	34
24c		11/32	NT
25		22/37	NT

[a] Average of triplicate measurements; NT = not tested.

metabolic stability. Above all, compound **13c** showed the best stability. The lower lipophilicity of **13c** may be partially responsible for the improvement in the CL_{int}/f_m value. It was apparent that an alternative strategy of reducing the intrinsic lipophilicity of the compounds was desirable to design more potent compounds.

Table 5
Selectivities and in vitro clearances for selected compounds

Compound	A_1[a] lnhibn. (%) 10^{-6}/ 10^{-5} (mol/L)	A_{2B}[a] lnhibn. (%) 10^{-6}/ 10^{-5} (mol/L)	*c*Log *P*	CLint/f_m (L/h/kg)
8a	NT	NT	3.52	45.5
13c	37/82	81/98	3.57	1.22
13d	20/77	21/58	4.50	34.8
13e	9/10	1/1	4.84	156
13j	−4/−83	−19/4	4.77	NT

In summary, we have prepared a series of A_{2A} antagonists based on the lead compound **1**. The SAR studies uncovered some important factors for the design of potent A_{2A} antagonists in the benzofuran series, and identified **13c**, **13f,** and **24a** that exhibited excellent in vivo efficacy. Further exploration of the SAR and biology of the benzofuran series will be reported in due course.

References and notes

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8. Adenosine A_{2A} binding assay: The human A_{2A} receptor transfectant membrane (Receptor Biology Inc., Beltsville, MD) was diluted 20-fold with incubation buffer (50 mmol/L Tris (pH 7.4), 120 mmol/L NaCl, 5 mmol/L KCl, 10 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$, and 2 U/mL adenosine deaminase) to prepare the membrane fraction. The membrane fraction (20 μL), [^{3}H]CGS21680 (75 μL, final concentration: 75 nmol/L) and the test drug (20 μL) were placed in a tube for assay and added with the incubation buffer to make the total volume of 200 μL. After incubation at 25 °C for 90 min, the reaction mixture in the tube was filtrated with a 0.5% polyethyleneimine-treated 934 A/H filter (Whatman, Maidstone, UK) using a cell harvester M-24R (Brandel, Graithersburg, MD). The filtrated filter was transferred to a scintillation vial and dried. The filter was then added with 0.5 mL of Ultima gold (Packard, Downers Grove, IL), and the radioactivity was determined by means of a scintillation counter (LS 6500, Beckman CO., Ltd, Fullerton, CA). The nonspecific binding was determined as the radioactivity measured in the presence of 50 μmol/L NECA.