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

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ABSTRACT

Based on the previously reported lead compound, a series of benzofuran derivatives were prepared to study their antagonistic activities to A_{2A} receptor. The replacement of the phenyl group at the 4-position with a heterocyclic ring improved the PK profile and aqueous solubility. From these studies, we discovered a potent new A_{2A} antagonist, **12a**, which has both a good oral bioavailability and in vivo efficacy on motor disability in MPTP-treated common marmosets.

Adenosine receptors have been extensively characterized and are 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 exploited for a number of clinical applications. Of the four adenosine receptor subtypes, the A_{2A} receptor is the focus of intense research within the neurological field, particularly in relation to the hypoactive and hyperactive movement disorders.[2] Our own interest in this area stemmed from the potential benefit of adenosine A_{2A} antagonists in the central nervous system (CNS) area and, specifically, for the treatment of basal ganglia disorders such as Parkinson's disease (PD).[3] PD is a very serious neurological disorder which involves the chronic and progressive degeneration of the brain and impairs motor control, speech, and other functions. L-Dopa has long been the gold standard of therapy for Parkinson's disease, but its long-term shortcomings are motor response complications such as dyskinesias, end-of-dose deterioration or wearing-off, and the fluctuating regulation of motor symptoms.[4] The selective antagonism of the A_{2A} receptor in a primate model of PD can ameliorate motor depression.[5] 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.[6] Selective A_{2A} antagonists should provide a novel nondopaminergic 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.[7] We have previously reported the identification of new A_{2A} antagonists with a benzofuran skeleton.[8] In an effort to understand this novel class of A_{2A} antagonists, we have investigated the effect of chemically modifying the 2- and 4-positions of the molecule on compound potency and pharmacokinetic parameters. It became apparent that reducing the intrinsic lipophilicity of the compounds was desirable for more potent compounds. In the current study, a variety of six-membered heterocyclic moieties with one or two nitrogen atoms in the ring were incorporated to investigate their contribution to various pharmacokinetic parameters. In most cases, the introduction of heterocyclic moieties improved the metabolic stability and aqueous solubility, thus resulting in the discovery of a potent new A_{2A} antagonist, **12a**, which demonstrated good oral bioavailability and in vivo efficacy on motor disability in MPTP-treated common marmosets.

Scheme 1 describes the preparation of 4-substituted benzofurans by reactions involving the bromo-functionalized analogue **3**. Stille coupling of **3** with various tributylstannyl pyrazines and pyridines gave the arylated analogues **4a–g**. Hydrolysis of the esters followed by amidation of the resulting carboxylic acids yielded the desired benzofurans **6a–g**.

4-Morpholinobenzofurans were prepared as shown in Scheme 2. The nitration of 2-ethoxycarbonyl-7-methoxybenzofuran **7** with nitric acid in acetic anhydride gave the isomeric mixture of **8** and **13**, which was separated by recrystallization from methanol and

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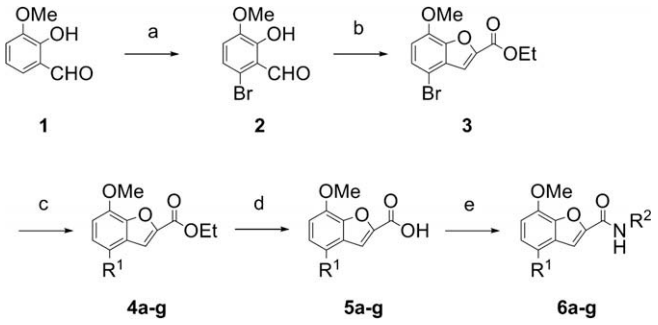
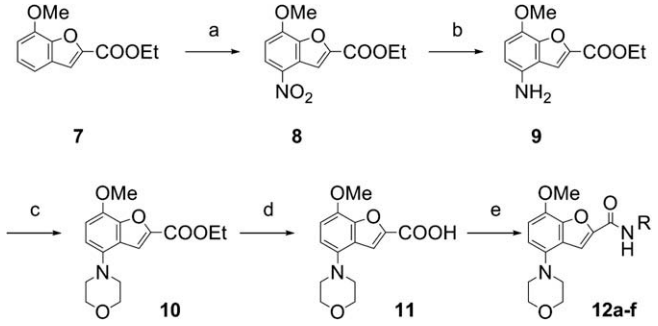


Scheme 1. Reagents and conditions: (a) Br_2, AcOH; (b) ethyl chloroacetate, K_2CO_3, KI, DMF; (c) R^1SnBu_3, $Pd(PPh_3)_4$, toluene; (d) LiOH·H_2O, THF, H_2O; (e) R^2NH_2, WSC·HCl, HOBt·H_2O, Et_3N, DMF.



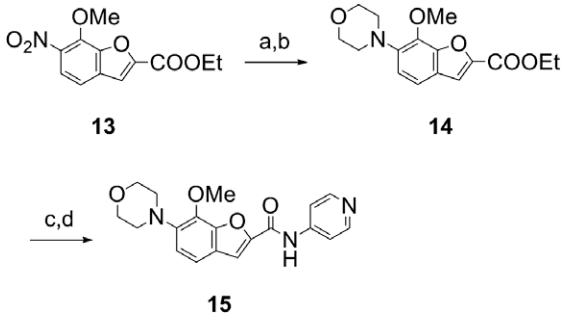
Scheme 2. Reagents and conditions: (a) concd HNO_3, Ac_2O; (b) H_2, Pd/C, EtOH; (c) bis(bromoethyl)ether, $^iPr_2NEt$, toluene; (d) LiOH·H_2O, THF, H_2O; (e) RNH_2, WSC·HCl, HOBt·H_2O, Et_3N, DMF.

chloroform to yield 4-nitrobenzofuran **8**. The subsequent hydrogenation of the nitro group and cyclization of the amino group of **9** with bis(bromoethyl)ether gave the desired 4-morpholinobenzofuran **10**. Compounds **12a–f** were obtained by the same procedure described in Scheme 1.

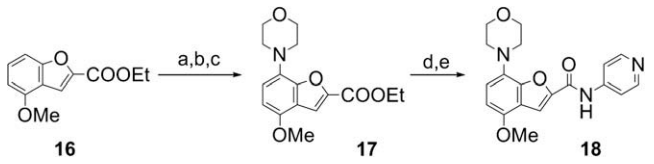
Similarly, 6-morpholinobenzofuran **15** was obtained from the 6-nitrobenzofuran **13**, which was formed as a byproduct in the nitration of **7** (Scheme 3).

7-Morpholinobenzofuran **18** was prepared from 2-ethoxycarbonyl-4-methoxybenzofuran **16** according to the same method described for the synthesis of compounds **12a–f** (Scheme 4).

The compounds were evaluated for affinity to the three subtypes of adenosine receptors in binding assays. The assays were performed with recombinant human receptors using the same radioligand protocol as described previously.[8] Our previous findings on the SAR of benzofuran derivatives indicated that the incorporation of a 4-phenyl group improved the PK profile, while



Scheme 3. Reagents and conditions: (a) H_2, Pd/C, EtOH; (b) bis(bromoethyl)ether, $^iPr_2NEt$, toluene; (c) LiOH·H_2O, THF, H_2O; (d) 4-aminopyridine, WSC·HCl, HOBt·H_2O, Et_3N, DMF.

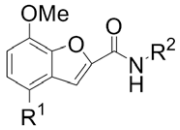


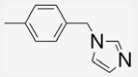
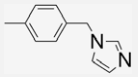
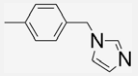
Scheme 4. Reagents and conditions: (a) concd HNO_3, Ac_2O; (b) H_2, Pd/C, EtOH; (c) bis(bromoethyl)ether, $^iPr_2NEt$, toluene; (d) LiOH·H_2O, THF, H_2O; (e) 4-aminopyridine, WSC·HCl, HOBt·H_2O, Et_3N, DMF.

modifications of the amide moiety showed enhanced A_{2A} antagonistic activity. The reduction of the intrinsic lipophilicity of the compounds was performed by an alternative strategy to further improve the metabolic stability by human liver microsomes. A study for the initial set of compounds was carried out to determine the effect of replacing the phenyl group at 4-position with either pyridine or pyrazine. Changes to the heteroaryl substituents at the 4-position influenced potency, as demonstrated by the assay data summarized in Table 1. The 2-pyridine analogue **6a** and pyrazine analogues **6b** and **6c** were significantly less potent than the corresponding previously reported phenyl analogue **19**,[8] thus suggesting an unfavorable interaction with the nitrogen in the ring. However, the introduction of 3-pyridyl (**6d**, **6e**) and 4-pyridyl (**6f**, **6g**) groups to the same position provided analogues that were roughly equipotent to **19**. In addition, substitutions of the phenyl group at the amide moiety with (1*H*-imidazol-1-yl)methyl group were beneficial for the antagonistic activity, regardless of the substituent at 4-position (**6d** vs **6e** and **6f** vs **6g**). The 4-phenylbenzofuran derivative **19** showed a poor pharmacokinetic profile and exhibited a high clearance rate. This resulted in low bioavailability, which was possibly due to a high first-pass metabolism. On the other hand, changing the phenyl group at 4-positon to pyridyl and pyrazyl groups significantly improved the pharmacokinetic profile. On the basis of potent in vitro activities at A_{2A} and good pharmacokinetic profiles, compounds **6d**–**g** were evaluated in the mouse CGS 21680-induced catalepsy model,[9] which is commonly used to assess the activity of compounds for anti-parkinsonian effects. Despite their improvement in clearance rates over the previous compound **19**, these new compounds still had a moderate efficiency when dosed at 10 mg/kg in this model, and therefore that there remains significant room for improvement. Next, we compared some of the physicochemical properties for the potent compounds in the series. The data indicated that the aqueous solubility of these compounds was not sufficient (<6 μg/ml) in a solution such as JP2 fluid ('2nd fluid' as described in the disintegration test of Japanese Pharmacopoeia).

With this information in hand, we prepared a series of 4-morpholinobenzofuran derivatives designed to improve the aqueous solubility (Table 2). This modification resulted in a dramatic improvement in both the aqueous solubility and metabolic stability while retaining the desired antagonistic activity on A_{2A} receptor. The incorporation of (1*H*-imidazol-1-yl)methyl group (**12b**), which proved to be highly beneficial in previous SAR studies, produced a slight improvement in potency, albeit the solubility of this compound in water was very low. Compound **12c**, containing an additional water-solubilizing group at the amide moiety, also demonstrated a slight improvement in potency while retaining the desirable aqueous solubility. Aliphatic substituents appended to the amide nitrogen were tolerated and yielded good activities, with potency increasing as the size of the substituent increased (**12e** (R = Et) >**12d** (R = Me)). This trend did not extend to the larger heteroaliphatic ring substituted analogue **12f**, however, which exhibited a very low activity. The position of attachment of the morpholine ring to the benzofuran nucleus was investigated as shown by the comparison of the 6-subtituted (**15**) and 7-substituted (**18**) analogues. Compound **15** displayed much lower potency

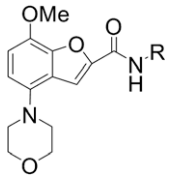
Table 1
Structure–activity relationships and in vitro clearances for 4-heteroarylbenzofurans

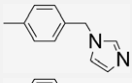
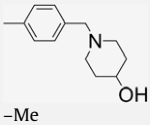
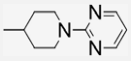


Compd	R^1	R^2	A_{2A}[a] lnhibn. (%) $10^{-8}/10^{-7}$ (mol/L)	Human CL_{int}/f_m[b] (L/h/kg)	CGS catalepsy lnhibn. (%) 10 mg/kg, po
19			29/87	34.8	40
6a			4/27	2.23	NT
6b			24/22	9.21	NT
6c			20/44	<0.47	NT
6d			27/67	3.42	38
6e			46/83	3.18	60
6f			18/72	1.97	48
6g			56/90	2.44	60

[a] Average of triplicate measurements.
[b] Compounds were incubated with human liver microsomes for 60 min in the presence of NADPH. CL_{int} was determined by in vitro $T_{1/2}$ method.[10] NT = not tested.

Table 2
Structure–activity relationships, in vitro clearances and aqueous solubilities for 4-morpholinobenzofurans



Compd	R	A_{2A}[a] lnhibn. (%) $10^{-8}/10^{-7}$ (mol/L)	Marmoset CL_{int}[b] (L/h/kg)	Solubility (μg/mL) JP2	CGS catalepsy lnhibn. (%) 10 mg/kg, po
12a		30/73	<0.43	87	76
12b		50/89	8.0	2	NT
12c		39/74	NT	47	50
12d	–Me	23/58	1.3	97	NT
12e	–Et	37/87	NT	NT	44
12f		−2/26	NT	NT	NT
15		−16/−5	NT	NT	NT
18		26/77	NT	NT	NT

[a] Average of triplicate measurements.
[b] Compounds were incubated with marmoset liver microsomes for 60 min in the presence of NADPH. CL_{int} was determined by in vitro $T_{1/2}$ method.[10] NT = not tested.

as compared to the corresponding compound **12a**, while the 7-morpholino analogue **18**, which has the regiochemically opposite substitution pattern to **12a**, exhibited an equipotent antagonistic activity. Some of the more potent compounds were evaluated for their anti-parkinsonian effects in vivo. The excellent activity of compound **12a** against the catalepsy induced by the intracerebroventricular administration of CGS 21680 can be explained by the improvement in aqueous solubility and metabolic stability.

Common marmosets exhibiting parkinsonian symptoms following MPTP treatment appear to be the most suitable pharmacological models of Parkinson's disease because their response to anti-parkinsonian agents mimics that of human patients with similar side effects, such as vomiting, stereotyped behavior, and L-DOPA-induced dyskinesias.[11] Compound **12a** was selected for further in vivo testing based on its superior potency in the mouse CGS 21680-induced catalepsy model as well as the excellent

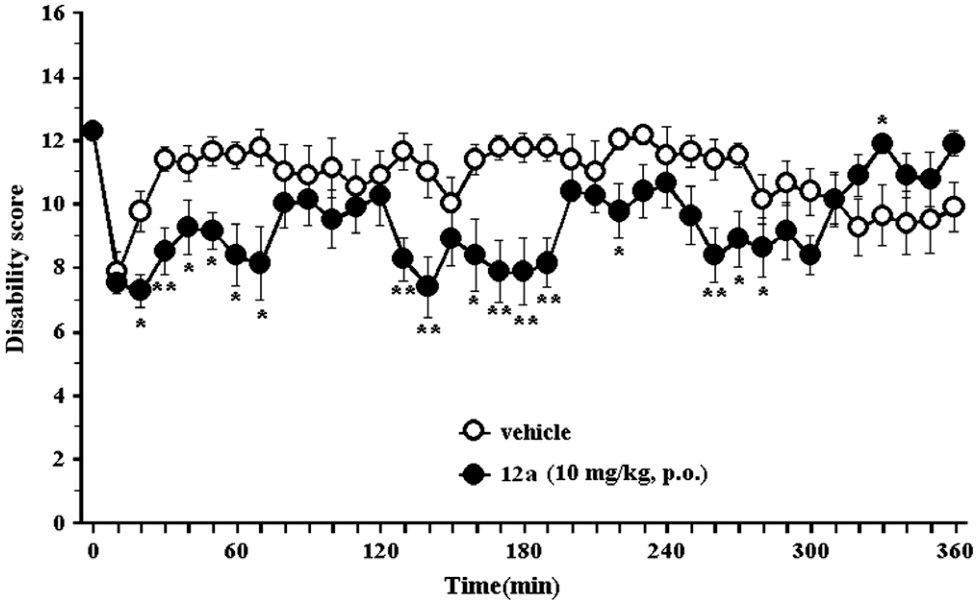


Figure 1. Time course of the effect of compound **12a** (10 mg/kg, po) on motor disability. Each point represents the mean disability score/10 min (±SEM, $n = 8$). Open circles show the vehicle treatment group. Closed circles show the compound **12a** treatment group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with vehicle control: Sign–Wilcoxon test.

selectivity profile (4% inhibition for A_1 and 21% inhibition for A_{2B} at 1 μmol/L, respectively) and marmoset pharmacokinetics (CL <0.43 L/h/kg). As shown in Figure 1, compound **12a** (10 mg/kg, po) caused a long-lasting (up to 5 h) reduction in motor disability,[12] and increased locomotor activity (data not shown). In addition, neither nausea nor vomiting was observed. This result indicates that compound **12a** is a promising candidate for future pre-clinical evaluations.

In summary, a novel class of adenosine A_{2A} antagonists was identified by the replacement of the phenyl group at 4-position of the previously reported benzofuran derivative **19**. The improved aqueous solubility and pharmacokinetic property with a water-solubilizing group at the 4-position suggested that this strategy of reducing the intrinsic lipophilicity of the compounds is a promising approach. Moreover, the SAR studies uncovered some important factors for the design of potent A_{2A} antagonists in the benzofuran series, and identified some potent analogues that exhibited an acceptable in vivo efficacy in mice. This investigation culminated in the discovery of compound **12a**,[13] which demonstrated an excellent in vivo efficacy in MPTP-treated common marmosets. Additional SAR analyses, as well as further evaluations of the in vivo efficacy of related compounds, will be reported in the future.

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12. The motor disability score was evaluated using the rating scale as described in detail in Ref. 10. Trained observers continuously monitored the animals for scoring periods and scored them every 10 min.
13. *Spectroscopic data of* **12a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ: 3.12–3.15 (m, 4H), 3.91–3.94 (m, 4H), 4.01 (s, 3H), 6.68 (d, $J = 8.4$ Hz, 1H), 6.88 (d, $J = 8.4$ Hz, 1H), 7.68 (dd, $J = 1.6$, 4.9 Hz, 2H), 7.69 (s, 1H), 8.51 (br, 1H), 8.58 (dd, $J = 1.6$, 4.9 Hz, 2H). Anal. Calcd for $C_{19}H_{19}N_3O_4{\cdot}0.5H_2O$: C, 62.97; H, 5.56; N, 11.60. Found: C, 62.77; H, 5.42; N, 11.34. MS (APCI): 354 $[M+H]^+$.