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C-Nucleoside Analogues of Furanfurin as Ligands to A_1 Adenosine Receptors

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Abstract—Furanfurin (2-β-D-ribofuranosylfuran-4-carboxamide) derivatives and analogues were synthesized and their affinity for adenosine receptors was determined. The agonistic behavior of furanfurin against A_1 receptors is preserved only when the furan ring is substituted with isosteric pentatomic ring systems such as oxazole, thiazole or thiophene, and the carboxamide group is unsubstituted. Replacement of the hydrogen atoms of the carboxamide group with alkyl, cycloalkyl or arylalkyl groups generates compounds endowed with moderate antagonistic activity.

Introduction

The *C*-nucleoside tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, **1**) and its selenium analogue selenazofurin (**2**) are potent antitumor agents.[1] Phase I and phase II clinical studies of these nucleosides are under way in order to evaluate their therapeutic potential. It was demonstrated that the antitumor activity of these compounds derives from a combination of cytotoxicity and maturation-inducing activities.[2] Both effects result from inhibition of target enzyme inosine monophosphate dehydrogenase (IMPDH), which induces the shutdown of guanine nucleotide synthesis. In sensitive cells, tiazo- and selenazofurin are metabolized to nicotinamide adenine dinucleotide (NAD) analogues, called TAD and SAD, respectively, which are potent inhibitors of the enzyme. The clinical studies pointed out that human recipients of intravenous tiazofurin frequently complain of headaches as well as displaying personality changes and obtundation.[3] It has been hypothesized that the thiazole ring of tiazofurin, which confers on the molecule a kind of purine-like property, enables the drug to interact with the adenosine receptors of the central nervous system. Actually, on the basis of binding studies, we ascertained that tiazofurin is able to bind selectively to A_1 adenosine receptors (bovine cortical membranes), albeit with moderate affinity ($K_i = 1.6\times10^{-3}$ M).[4] This affinity was unrelated to IMPDH inhibition because the oxazole analogue of tiazofurin (oxazofurin, **3**),[5a,b] a *C*-nucleoside inactive as antitumor agent and unable to inhibit this enzyme, proved to be more potent than tiazofurin as agonist at A_1 adenosine receptors ($K_i = 2.4\times10^{-4}$ M). We also ascertained that the nitrogen atom at 3-position in the thiazole and oxazole ring of tiazofurin and oxazofurin does not play an important role in the binding to A_1 adenosine receptors. In fact, thiophenfurin (2-β-D-ribofuranosylthiophene-4-carboxamide, **4**) and furanfurin (2-β-D-ribofuranosylfuran-4-carboxamide, **5**)[6] were found to have an affinity for A_1 receptors similar or superior to that of the parent compound.[4]

From the binding studies, furanfurin emerged as the most potent compound among these *C*-nucleosides with a K_i of 59 μM.

To further investigate the structure–activity relationships of this type of A_1 adenosine receptor ligands, we synthesized a series of furanfurin derivatives obtained through modification of the carboxamido group, and a series of furanfurin analogues which were tested in binding assays to evaluate their affinity for A_1 and A_{2A} receptors.

Chemistry

Furanfurin and compounds **6**–**8**, reported in Figure 1, and **9** were synthesized as reported by Franchetti and co-workers.[6]1

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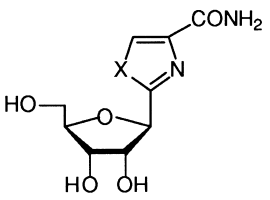
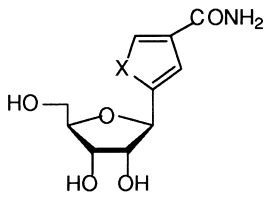
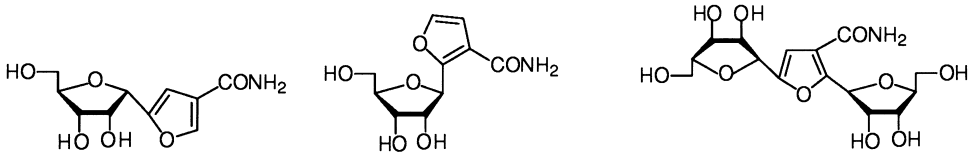


X = S Tiazofurin (**1**)
X = Se Selenazofurin (**2**)
X = O Oxazofurin (**3**)



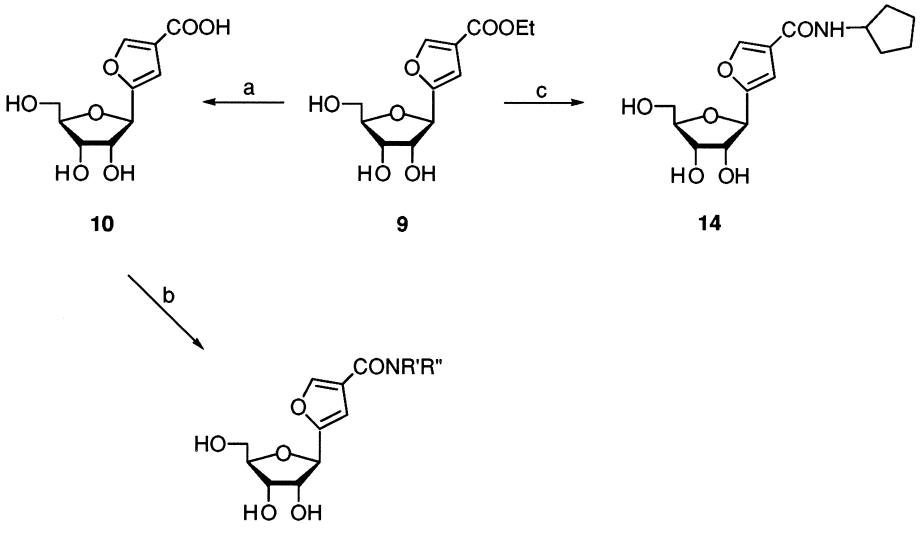
X = S Thiophenfurin (**4**)
X = O Furanfurin (**5**)

Furanfurin derivatives **10**–**14** were synthesized as reported in Scheme 1. Hydrolysis of ester **9** with sodium hydroxide gave acid **10** (78% yield). Treatment of **10** with L-amphetamine, D-amphetamine or diethylamine in tetrahydrofuran, in the presence of 1-hydroxybenzotriazole and *N,N′*-dicyclohexylcarbodiimide, gave the amides **11**, **12** and **13** (40, 70 and 41% yield, respectively). Amide **14** was obtained by reaction of **9** with cyclopentylamine (45% yield). The 5-carboxamide analogue of furanfurin (**21**) was synthesized as reported in Scheme 2. The reaction of ethyl 2-furoate (**15**) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**16**) in 1,2-dichloroethane in the presence of stannic chloride afforded the 5-glycosylated regioisomer as a mixture of α- and β-anomers (**17** and **18**, 4.8:1 ratio) in 85% yield. The anomers were separated by flash chromatography and converted to deblocked ethyl esters **19** and **20** by treatment with a catalytic amount of sodium ethoxide (76 and 65% yield, respectively). The glycosylation position was determined by ^{1}H NMR and proton–proton nuclear Overhauser effect (NOE) difference spectroscopy. The ^{1}H NMR spectra of compounds **17** and **18** showed that the signal of H-5 proton of furan had disappeared, indicating that the glycosylation position was at C-5. The structures of compounds **19** and **20** were further supported by NOE experiments. When the H-1′ signal of these compounds



6 **7** **8**

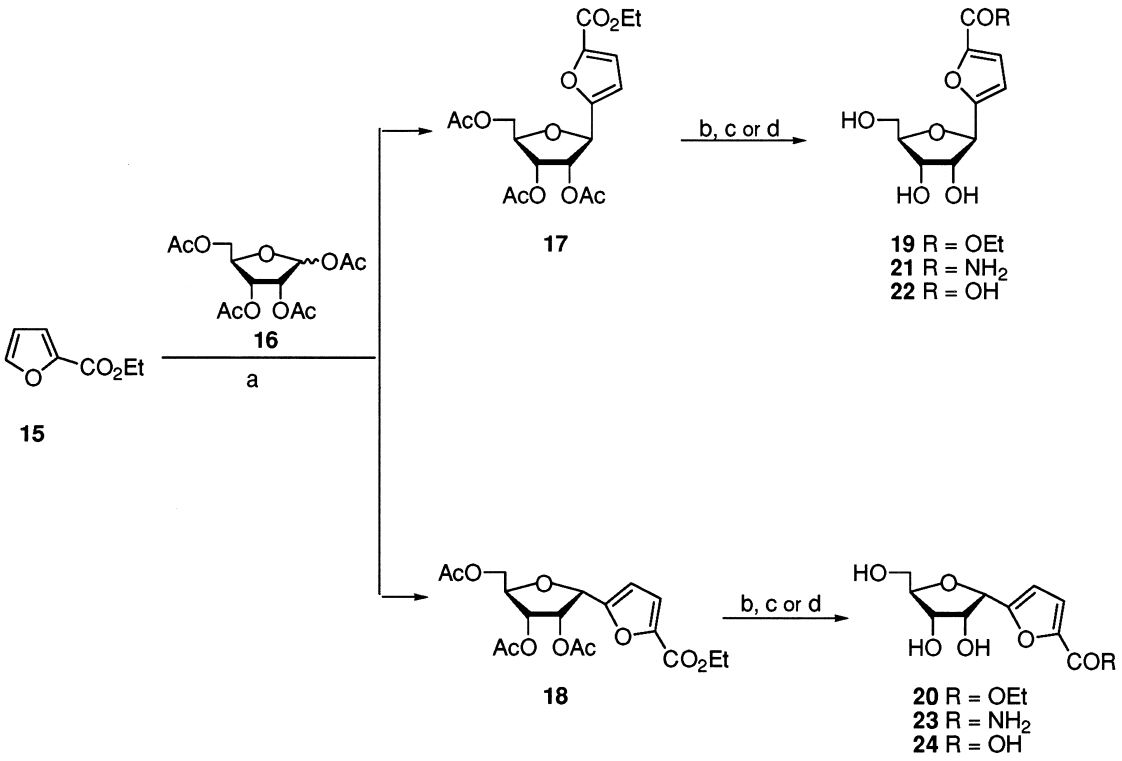
Figure 1.



10 **9** **14**

11 R' = (*R*)-phenylisopropyl, R" = H
12 R' = (*S*)-phenylisopropyl, R" = H
13 R' = R" = C_2H_5

Scheme 1. (a) 1N NaOH, Dowex 50/H^+; (b) L-amphetamine or D-amphetamine or diethylamine, 1-hydroxybenzotriazole, DCC, THF; (c) cyclopentylamine.



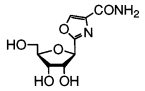
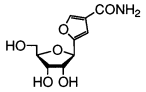
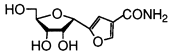
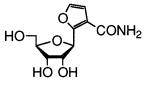
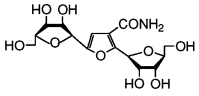
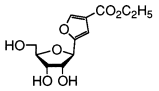
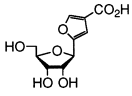
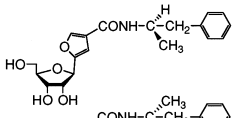
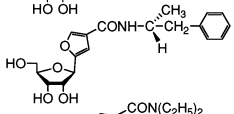
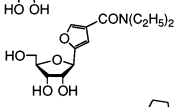
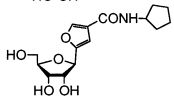
Scheme 2. (a) $SnCl_4/ClCH_2CH_2Cl$; (b) EtONa, EtOH; (c) 30% NH_4OH; (d) 1N NaOH.

was irradiated, an NOE effect was observed at H-4, confirming that the ribosyl moiety resides at C-5. The anomeric configuration was also assigned on the basis of NOE experiments. In fact, selective irradiation of the anomeric proton signal of **19** increased the intensity of the H-4′ signal, while the intensity enhancement of the H-3′ signal was zero; this indicates that H-1′ and H-4′ are located on the same face of the ribosyl ring.[7] Finally, amide **21** and its α-anomer **23** were obtained by ammonolysis of **19** and **20** with 30% ammonium hydroxide (60 and 72% yield, respectively). Hydrolysis of **19** and **20** with 1N sodium hydroxide gave the acids **22** and **24** (58 and 75% yield, respectively).

Results and Discussion

Furanfurin derivatives and analogues were tested in radioligand binding assays to determine their affinity toward A_1 and A_{2A} adenosine receptors. Affinity (K_i) was determined in competition assays in bovine cerebral cortex membranes (A_1) and bovine striatal membranes (A_{2A}) using, respectively, [^{3}H]CHA and [^{3}H]CGS21680 as radioligands. Oxazofurin (**3**), furanfurin (**5**), CPA and CPX were used as reference compounds. Agonist and antagonist behavior of tested compounds was established by Borea's method,[8] through evaluation of the ratios of the inhibitory binding constants K_i at 0 and 25 °C. According to this method, compounds for which the K_i value ratios at the two temperatures are greater than unity should be considered agonists, while compounds for which the ratios are smaller than unity should be considered antagonists. All compounds were found inactive at A_{2A} receptors at the highest tested concentration (5 mM). The affinity of furanfurin derivatives and analogues for bovine brain A_1 receptors are reported (Table 1). Oxazofurin and furanfurin were confirmed to be selective agonists for A_1 adenosine receptors with K_i values of 240 and 59 μM, respectively. The structure of furanfurin appears to be essential for the affinity to adenosine receptor because neither the α-anomer **6** nor the C2-ribosylated isomer **7** was active. The introduction of a second ribosyl moiety at 2-position of furanfurin (compound **8**) brought about the conversion of a moderately potent A_1 agonist to a moderately potent A_1 antagonist. Also, the modification in the carboxamido group of furanfurin caused the appearance of antagonistic properties. The most active antagonist proved to be the carboxylic acid **10** with a K_i value of 34 μM, while its ethyl ester **9** was found to be 3.8 times less potent. Replacement of one hydrogen atom in the carboxamido group on furanfurin with an (*R*)-phenylisopropyl (**11**) or cyclopentyl (**14**) group gave compounds endowed with similar moderate affinity for A_1 receptors. Interestingly, the *S*-enantiomer of **11** (compound **12**) was found to be inactive. So, the stereochemical requirement for receptor affinity shown by **11** and **12** is similar to that observed in the case of *R*- and *S*-enantiomers of PIA and of *R*- and *S*-enantiomers of the selective A_1 antagonist 1,3-dipropyl-8-(phenyl-isopropyl)xanthine.[9] This finding supports the hypothesis that the carboxamido substituent of **11** and **12** binds in the same region as does the N^6 substituent of an adenosine derivative agonist, and the C^8 substituent of a xanthine antagonist. Finally, we found that the substitution of both hydrogen atoms in the furanfurin carboxamido group with alkyl substituents (compound **13**) eliminated the activity at adenosine receptors. The 5-carboxamide analogues **19**–**24** were found inactive.

Table 1. Affinity of furanfurin derivatives and analogues in radioligand binding assays at bovine brain A_1 receptors

Compound	Structure	K_i (μM)[a] (0 °C)	K_i (μM)[a] (25 °C)	K_i Ratio
3		> 1000	240±19	> 1
5		> 1000	59±3.2	> 1
6		[b]	[b]	—
7		[b]	[b]	—
8		269±24	> 1000	< 1
9		128±11	> 1000	< 1
10		34±2.1	> 1000	< 1
11		85±7.0	150±10	< 1
12		[b]	[b]	—
13		[b]	[b]	—
14		147±11	262±21	< 1
CPA[c]		1.5 nM±0.1	0.81 nM±0.06	> 1
CPX[d]		0.064 nM±0.005	0.114 nM±0.01	< 1

[a]Bovine cerebral cortex membranes were incubated with [^{3}H]CHA and increasing drug concentrations as described in Experimental.
[b]Inactive at the highest tested concentration (5 mM).
[c]N^6-Cyclopentyladenosine.
[d]8-Cyclopentyl-1,3-dipropyl-xanthine.

We also tested some selected agonist compounds (tiazofurin, oxazofurin and furanfurin) in functional assays measuring the inhibition of adenylyl cyclase in isolated rat adipocytes (A_1) and in human platelets (A_{2A}). None of these compounds proved to be active at IC_{50} values lower than 100 μM (data not shown). In the functional assay, antagonist **10** proved unable to block the effects on adenylyl cyclase of A_1 agonist (CHA) and A_{2A} agonist (NECA). The inactivity of these compounds in functional assays might be due to their low affinity for the adenosine receptors.

Conclusion

In conclusion it was found that, among the studied *C*-nucleosides, only furanfurin behaved as a moderate agonist at A_1 adenosine receptors. The inversion of the configuration of the ribofuranosyl moiety or its shifting from the 5- to the 2-position of the furan ring abolished the activity. The substitution of the carboxamido group in the 3-position with the ethoxycarbonyl or carbonyl one, or the *N*-monosubstitution with (*R*)-phenylisopropyl or cyclopentyl, converted a moderate A_1 selective agonist into an A_1 selective antagonist.

Experimental

Chemistry

Melting points were determined on a Buchi apparatus and are uncorrected. Elemental analyses were determined on an EA 1108 CHNS-O (Fisons Instruments) analyzer. Thin layer chromatography (TLC) was run on silica gel 60 F_{254} plates and RP-18 F_{254} S (Merck); silica

gel 60 (Merck) (70–230 and 230–400 mesh) for column chromatography was used. Nuclear magnetic resonance ^{1}H spectra were determined at 300 MHz with a Varian VXR spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O.

5-β-D-Ribofuranosylfuran-3-carboxylic acid (10). The title compound was obtained from treatment of **9**[6] (1 g, 3.67 mmol) with 1N NaOH (10 mL) for 30 min at room temperature. The mixture was neutralized with the ion exchange resin Dowex 50wx8 (H^+ form), filtered, and the filtrate was evaporated to dryness. The solid residue was crystallized by treatment with acetone as a white solid (78% yield); mp 125–128 °C. TLC (CH_3CN:H_2O, 80:20): R_f = 0.37. ^{1}H NMR (Me_2SO-d_6) δ 3.45 (m, 2H, H5, H5′), 3.75 (dd, J = 4.6, 8.7 Hz, 1H, H4′), 3.90 (t, J = 4.7 Hz, 1H, H3′), 4.07 (t, J = 5.8 Hz, 1H, H2′), 4.59 (d, J = 6.8 Hz, 1H, H1′), 4.80 (t, J = 5.6 Hz, 1H, OH), 5.02, 5.15 (2br s, 2H, OH), 6.72 (s, 1H, H4), 8.25 (s, 1H, H2), 12.70 (br s, 1H, COOH). Anal. calcd for $C_{10}H_{12}O_7$: C 49.19, H 4.95. Found: C 49.28, H 5.02.

General procedure for the synthesis of the carboxamide derivatives 11–13. To a solution of **10** (100 mg, 0.41 mmol) in dry THF (10 mL), the corresponding amines (0.37 mmol), 1-hydroxybenzotriazole (0.40 mmol) and then *N,N*-dicyclohexylcarbodiimide (0.40 mmol) were added. The mixture was stirred at room temperature for 4 h. The white precipitate was filtered off and the filtrate was washed with saturated $NaHCO_3$ (3×10 mL). The organic layers were dried (Na_2SO_4) and evaporated in vacuo to give a crude oily residue which was chromatographed on a silica gel column eluting with 8% of MeOH in $CHCl_3$ or 5% for **13**.

Compound **11** was separated as a foam which was crystallized by methanol/ethyl ether to obtain a white solid (40% yield); mp 134–136 °C. TLC ($CHCl_3$:MeOH, 90:10): R_f = 0.27. ^{1}H NMR (Me_2SO-d_6) δ 1.15 (d, J = 6.5 Hz, 3H, CHCH_3), 2.72 (dd, J = 6.3, 12.9 Hz, 1H, CH_2–C_6H_5), 2.83 (dd, J = 7.2, 13.0 Hz, 1H, CH_2–C_6H_5), 3.48 (m, 2H, H5, H5′), 3.78 (m, 1H, H4′), 3.90 (m, 1H, H3′), 4.05 (q, J = 5.9 Hz, 1H, CHCH$_3$), 4.15 (m, 1H, H2′), 4.58 (d, J = 6.6 Hz, 1H, H1′), 4.76 (t, J = 5.5 Hz, 1H, OH), 5.0 (d, J = 4.7 Hz, 1H, OH), 5.15 (d, J = 6.2 Hz, 1H, OH), 6.80 (s, 1H, H4), 7.25 (m, 5H, arom.), 7.98 (d, J = 8.1 Hz, 1H, NH), 8.10 (s, 1H, H2). Anal. calcd for $C_{19}H_{23}NO_6$: C 63.15, H 6.41, N 3.88. Found: C 63.31, H 6.27, N 3.92.

Compound **12** was separated as a white solid (70% yield); mp 134–136 °C. TLC ($CHCl_3$:MeOH, 92:8): R_f = 0.21. ^{1}H NMR (Me_2SO-d_6) δ 1.18 (d, J = 6.5 Hz, 3H, CHCH_3), 2.70 (dd, J = 6.3, 12.9 Hz, 1H, CH_2–C_6H_5), 2.83 (dd, J = 7.2, 13.0 Hz, 1H, CH_2–C_6H_5), 3.45 (m, 2H, H5, H5′), 3.78 (m, 1H, H4′), 3.88 (m, 1H, H3′), 4.05 (q, J = 5.9 Hz, 1H, CHCH$_3$), 4.17 (m, 1H, H2′), 4.58 (d, J = 6.6 Hz, 1H, H1′), 4.78 (t, J = 5.5 Hz, 1H, OH), 5.0 (d, J = 4.7 Hz, 1H, OH), 5.15 (d, J = 6.2 Hz, 1H, OH), 6.80 (s, 1H, H4), 7.25 (m, 5H, arom.), 7.98 (d, J = 8.1 Hz, 1H, NH), 8.10 (s, 1H, H2). Anal. calcd for $C_{19}H_{23}NO_6$: C 63.15, H 6.41, N 3.88. Found: C 63.09, H 6.45, N 3.79.

Compound **13** was obtained as a foam (41% yield). TLC ($CHCl_3$:MeOH, 95:5): R_f = 0.3. ^{1}H NMR (Me_2SO-d_6) δ 1.15 (t, J = 7.1 Hz, 3H, CH_2CH_3), 3.45 (q, m, 6H, H5, H5′, CH_2CH_3), 3.78 (q, J = 4.4 Hz, 1H, H4′), 3.92 (q, J = 5.0 Hz, 1H, H3′), 4.05 (q, J = 6.4 Hz, 1H, H2′), 4.60 (d, J = 6.6 Hz, 1H, H1′), 4.80 (t, J = 5.7 Hz, 1H, OH), 4.98 (d, J = 5.1 Hz, 1H, OH), 5.12 (d, J = 6.5 Hz, 1H, OH), 6.63 (s, 1H, H4), 7.95 (s, 1H, H2). Anal. calcd for $C_{14}H_{21}NO_6$: C 56.18, H 7.07, N 4.68. Found: C 56.22, H 6.97, N 4.70.

***N*-Cyclopentyl-(5-β-D-ribofuranosyl)furan-3-carboxamide (14).** The title compound was obtained by treatment of **9** (1.25 mmol) with (30%) aqueous solution of cyclopentylamine (125 mmol) at room temperature for 60 h. The reaction mixture was evaporated to dryness and the brown residue was chromatographed on a silica gel column eluting with 10% of MeOH in $CHCl_3$ to give **14** as a white foam (45% yield). TLC ($CHCl_3$:MeOH, 90:10): R_f = 0.23. ^{1}H NMR (Me_2SO-d_6) δ 1.40–1.95 (m, 8H, cyclop.), 3.45 (m, 2H, H5, H5′), 3.76 (q, J = 4.2 Hz, 1H, H4′), 3.90 (q, J = 4.5 Hz, 1H, H3′), 4.04 (q, J = 5.9 Hz, 1H, H2′), 4.16 (q, J = 6.5 Hz, 1H, CH), 4.55 (d, J = 6.4 Hz, 1H, H1′), 4.75 (t, J = 5.5 Hz, 1H, OH), 4.98 (d, J = 5.1 Hz, 1H, OH), 5.10 (d, J = 6.5 Hz, 1H, OH), 6.84 (s, 1H, H4), 7.97 (d, J = 7.5 Hz, 1H, NH), 8.12 (s, 1H, H2). Anal. calcd for $C_{15}H_{21}NO_6$: C 57.87, H 6.8, N 4.5. Found: C 57.93, H 6.69, N 4.58.

Ethyl 5-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)furan-2-carboxylate (17) and ethyl 5-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)furan-2-carboxylate (18). A solution of **15** (10 g, 71.35 mmol) in dry 1,2-dichloroethane (100 mL) was reacted with **16** (22.7 g, 71.35 mmol). After cooling at 0 °C, $SnCl_4$ (4.6 mL) was added and the reaction mixture was stirred at room temperature for 5 h. The black mixture was washed with H_2O (2×80 mL), neutralized with saturated $NaHCO_3$ and extracted with $CHCl_3$ (3×100 mL). The combined organic layers were dried (Na_2SO_4), filtered and evaporated in vacuo. The yellow oily residue was purified by flash chromatography on silica gel eluting with 20% of ethyl ether in hexane. Compound **17** was obtained from the first eluate as an oil (15% yield). TLC (hexane:ethyl ether, 70:30): R_f = 0.55. ^{1}H NMR (Me_2SO-d_6) δ 1.30 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 2.08 (3s, 9H, OCOCH_3), 4.12 (m, 3H, H4′, H5, H5′), 4.28–4.40 (m, 4H, H3′, H2′, OCH_2CH_3), 5.05 (d, J = 5.5 Hz, 1H, H1′), 6.80 (d, J = 3.4 Hz, 1H, H4), 7.12 (d, J = 3.4 Hz, 1H, H3).

Evaporation of the following fraction gave **18** as an oil (72% yield). TLC (ethyl ether:hexane, 70:30): R_f = 0.49. ^{1}H NMR (Me_2SO-d_6) δ 1.30 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 2.08 (3s, 9H, OCOCH_3), 4.10–4.45 (m, 5H, H4′, H5, H5′, OCH_2CH_3), 5.18–5.28 (m, 2H, H2′, H3′), 5.47 (d, J = 3.8 Hz, 1H, H1′), 6.32 (d, J = 3.5 Hz, 1H, H4), 7.30 (d, J = 3.5 Hz, 1H, H3).

Ethyl 5-β-D-ribofuranosylfuran-2-carboxylate (19). A mixture of compound **17** (1.8 g, 4.5 mmol) and sodium

ethoxide (18 mmol) was stirred for 1 h at room temperature. Dowex 50wx8 (H^+ form) resin (1.0 g) was added (pH 6) and the mixture was stirred for 1 h. The resin was filtered off and washed with EtOH. Evaporation of the solvent gave a yellow oily residue which was chromatographed on a silica gel column. Evaporation of the fractions eluted with 5% of MeOH in $CHCl_3$ afforded the compound **19** as a colorless oil (76% yield). TLC ($CHCl_3$:MeOH, 90:10): $R_f = 0.47$. ^1H NMR (Me_2SO-d_6) δ 1.28 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 3.44 (m, 2H, H5, H5′), 3.80 (q, $J = 4.6$ Hz, 1H, H4′), 3.94 (q, $J = 4.8$ Hz, 1H, H3′), 4.08 (q, $J = 5.8$ Hz, 1H, H2′), 4.30 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3), 4.65 (d, $J = 6.2$ Hz, 1H, H1′), 4.80 (t, $J = 5.6$ Hz, 1H, OH), 5.05 (d, $J = 5.2$ Hz, 1H, OH), 5.20 (d, $J = 6.2$ Hz, 1H, OH), 6.68 (d, $J = 3.4$ Hz, 1H, H4), 7.28 (d, $J = 3.6$ Hz, 1H, H3). Anal. calcd for $C_{12}H_{16}O_7$: C 52.94, H 5.92. Found: C 52.78, H 6.05.

Ethyl 5-α-D-ribofuranosylfuran-2-carboxylate (20). The title compound was obtained from **18**, as reported for **19**, as an oil (65% yield). TLC ($CHCl_3$:MeOH, 90:10): $R_f = 0.46$. ^1H NMR (Me_2SO-d_6) δ 1.30 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 3.45 (m, 1H, H5), 3.62 (2dd, $J = 2.6$, 5.1 Hz, 1H, H5′), 3.82 (m, 1H, H4′), 4.10 (2m, 2H, H2′, H3′), 4.28 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 4.73 (t, $J = 5.6$ Hz 1H, OH), 4.93 (m, 3H, H1′ and 2 OH; changes to a d with D_2O, $J = 2.9$ Hz), 6.55 (d, $J = 3.6$ Hz, 1H, H4), 7.25 (d, $J = 3.6$ Hz, 1H, H3). Anal. calcd for $C_{12}H_{16}O_7$: C 52.94, H 5.92. Found: C 52.85, H 5.97.

5-β-D-Ribofuranosylfuran-2-carboxamide (21). Compound **19** (300 mg, 1.1 mmol) was stirred with 30% ammonium hydroxide (20 mL) for 7 h at room temperature. After evaporation of the mixture, the residue was coevaporated with absolute ethanol and then was purified by chromatography on a silica gel column using 10% of MeOH in $CHCl_3$ to give **21** as a white foam (60% yield). TLC ($CHCl_3$:MeOH, 80:20): $R_f = 0.44$. ^1H NMR (Me_2SO-d_6) δ 3.42–3.55 (m, 2H, H5, H5′), 3.78 (q, $J = 4.6$ Hz, 1H, H4′), 3.92 (q, $J = 4.5$ Hz, 1H, H3′), 4.12 (q, $J = 6.1$ Hz, 1H, H2′), 4.62 (d, $J = 6.4$ Hz, 1H, H1′), 4.75 (t, $J = 5.7$ Hz, 1H, OH), 5.05 (d, $J = 5.1$ Hz, 1H, OH), 5.15 (d, $J = 6.3$ Hz, 1H, OH), 6.58 (d, $J = 3.3$ Hz, 1H, H4), 7.07 (d, $J = 3.3$ Hz, 1H, H3), 7.38, 7.72 (2br s, 2H, NH_2). Anal. calcd for $C_{10}H_{13}NO_6$: C 49.38, H 5.39, N 5.76. Found: C 49.44, H 5.23, N 5.71.

5-α-D-Ribofuranosylfuran-2-carboxamide (23). The title compound was prepared from **20**, as reported for **21**, as a white solid (72% yield); mp 129–131 °C. TLC ($CHCl_3$:MeOH, 80:20): $R_f = 0.43$. ^1H NMR (Me_2SO-d_6) δ 3.48 (m, 1H, H5), 3.62 (2dd, $J = 2.5$, 5.2 Hz, 1H, H5′), 3.85 (m, 1H, H4′), 4.12 (m, 2H, H3′, H2′), 4.77 (t, $J = 5.7$ Hz, 1H, OH), 4.98 (3d, $J = 3.5$ Hz, 3H, H1′, 2 OH, changes to a d with D_2O, $J = 2.9$ Hz), 6.48 (d, $J = 3.4$ Hz, 1H, H4); 7.05 (d, $J = 3.4$ Hz, 1H, H3), 7.30, 7.68 (2br s, 2H, NH_2). Anal. calcd for $C_{10}H_{13}NO_6$: C 49.38, H 5.39, N 5.76. Found: C 49.24, H 5.41, N 5.66.

5-β-D-Ribofuranosylfuran-2-carboxylic acid (22). The title compound was obtained from **19**, as reported for **10**, as a white solid (58% yield); mp 175–177 °C. TLC (CH_3CN:H_2O, 80:20): $R_f = 0.71$. ^1H NMR (Me_2SO-d_6) δ 3.35, 3.50 (2m, 2H, H5, H5′), 3.80 (q, J = 4.6 Hz, 1H, H4′), 3.90 (q, $J = 4.6$ Hz, 1H, H3′), 4.08 (t, $J = 4.7$ Hz, 1H, H2′), 4.48 (br s, 1H, OH), 4.65 (d, $J = 6.2$ Hz, 1H, H1′), 4.80, 5.10 (2br s, 2H, OH), 6.63 (d, $J = 3.4$ Hz, 1H, H4), 7.18 (d, $J = 3.4$ Hz, 1H, H3), 12.50 (br s, 1H, COOH). Anal. calcd for $C_{10}H_{12}O_7$: C 49.19, H 4.95. Found: C 49.08, H 4.82.

5-α-D-Ribofuranosylfuran-2-carboxylic acid (24). Compound **24** was prepared from **20**, as reported for **10**, as a white solid (yield 75%); mp 180–182 °C. TLC (CH_3CN:H_2O, 80:20): $R_f = 0.69$. ^1H NMR (Me_2SO-d_6) δ 3.40, 3.65 (2dd, $J = 5.1$, 10.1 Hz, 2H, H5, H5′), 3.80 (q, $J = 4.6$ Hz, 1H, H4′), 4.10 (m, 2H, H2′, H3′), 4.70, 4.82, 4.95 (3br s, 3H, OH), 4.95 (d, $J = 2.3$ Hz, 1H, H1′), 6.40 (d, $J = 3.1$ Hz, 1H, H4), 6.85 (d, $J = 3.1$ Hz, 1H, H3), 12.52 (br s, 1H, COOH). Anal. calcd for $C_{10}H_{12}O_7$: C 49.19, H 4.95. Found: C 49.23, H 4.76.

Biological methods

[^{3}H]CHA (sp.act. 32.5 Ci/mmol) and [^{3}H]CGS 21680 (37.5 Ci/mmol) were purchased from NEN Life Science Products). Adenosine deaminase was obtained from Boehringer-Mannheim (Mannheim, Germany). (*R*)-PIA, NECA and other compounds were purchased from Sigma Chemical Co. (St. Louis, MO).

Receptor binding assay

Bovine brains were obtained from the local slaughterhouse. Cortex and striatal tissue were isolated and membranes prepared as previously described.[10a,b] In brief, cerebral cortex was homogenized in 10 volumes of ice-cold buffer containing 0.25 M sucrose, 5 mM EDTA, 0.1 mM PMSF, 200 μg/mL bacitracine, 160 μg/mL benzamidine, and 10 mM Tris:HCl, pH 7.7, and centrifuged at 1000×*g* for 10 min at 4 °C. The resulting supernatant was centrifuged at 48,000×*g* for 20 min at the same temperature. The pellet was resuspended in 10 volumes of ice-cold buffer A (1 mM EDTA, 4 mM $MgCl_2$, and 50 mM Tris:HCl, pH 7.7) containing protease inhibitors (as above) and centrifuged at 48,000×*g* for 20 min at 4 °C. The pellet was resuspended in 5 volumes of buffer A containing protease inhibitors and adenosine deaminase (2 UI/mL). After incubation for 30 min at 37 °C, the suspension was centrifuged at 48,000×*g* for 20 min at 4 °C. The final pellet was stored in aliquots at −80 °C until the time of assay. Cortical membranes were suspended in buffer A, and [^{3}H]CHA binding to A_1 receptors was measured in triplicate, as previously described.[11] Striatal tissue was homogenized in 20 volumes of ice-cold, 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$ (buffer B), containing protease inhibitors (20 μg/mL soybean trypsin inhibitor, 200 μg/mL bacitracine, and 160 μg/mL benzamidine) and centrifuged at 48,000×*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer B containing protease inhibitors and 2 UI/mL of adenosine deaminase (ADA) to 50 mg/mL of original tissue weight, incubated at 37 °C for 30 min to remove endogenous adenosine, then recentrifuged, and the final pellet was frozen at −20 °C until the time of assay. Striatal membranes were suspended in buffer B, and the [^{3}H]

CGS21680 binding to A_{2A} receptors was performed as previously described.[10b] Compounds were dissolved in assay buffer, and at least six different concentrations of each compound were used. IC_{50} values were derived from semilog plots of data from agonist/antagonist displacement experiments. The Cheng–Prusoff equation was used to calculate K_i values from IC_{50} values.[12] Values represent the means±SE derived from (n) experiments conducted in triplicate. To investigate agonistic and antagonistic behavior of A_1 adenosine receptor ligands, we performed two-temperature measurements of in vitro inhibitory binding constants as previously described.[8]

Determination of cyclic AMP levels in rat fat cells and in human platelets. Isolated rat fat cells were prepared essentially according to the method of Rodbell.[13] Determination of cyclic AMP levels in rat fat cells was performed essentially according to Borea et al.[14] Isolated adipocytes were suspended in 400 mL of Krebs–Ringer buffer, pH 7.4, containing 1.0 IU/mL of ADA and 0.5 mM 4-(3-butoxy-4-methoxy-benzyl)-2-imidazolidinone (Ro 20-1724) as phosphodiesterase inhibitor, and incubated for 10 min at 37 °C in a shaking bath. Then the compounds plus 1 μM forskolin were added and after 5 min incubation the reaction was stopped by adding ice-cold 6% trichloroacetic acid (TCA). TCA suspensions were centrifuged at 200×g for 10 min at 4 °C, and supernatant acidity was extracted four times with water-saturated ethyl ether. The final aqueous solution was frozen at −80 °C and used for the determination of cAMP levels. Washed human platelets (8×10^4 cells/mL) obtained from the peripheral blood of healthy volunteers were prepared as described by Korth et al.[15] Measurements of cAMP levels in human platelets were carried out according to Varani et al.[16] Human platelets were incubated for 10 min at 37 °C in 0.5 mL of Tyrode buffer, pH 7.4, containing 1.0 IU/mL of ADA and 0.5 mM Ro 20-1724. Then the compounds examined plus 1 μM forskolin were added to the mixture and the incubation was continued for a further 5 min. The reaction was stopped by adding ice-cold 6% TCA and the final aqueous solution was tested for cAMP by a competitive protein binding assay. In particular, [^{3}H]cAMP was added to each tube at a total assay volume of 500 mL containing the binding protein, previously prepared from bovine adrenals essentially according to Brown et al.[17] These were incubated at 4 °C for 150 min and centrifuged at 2000×g for 10 min. Clear supernatant (200 mL) was mixed with 4 mL scintillation liquid and counted in a spectrometer (Beckmann LS 1800 Irvine, CA, USA) at a counting efficiency of about 55%.

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