Synthesis and Biological Evaluation of Novel 1-Deoxy-1-[6-[((hetero)arylcarbonyl)hydrazino]-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide Derivatives as Useful Templates for the Development of A_{2B} Adenosine Receptor Agonists

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The lack of molecules endowed with selective and potent agonistic activity toward the hA_{2B} adenosine receptors has limited the studies on this pharmacological target and consequently the evaluation of its therapeutic potential. We report the design and the synthesis of the first potent (EC_{50} in the nanomolar range) and selective hA_{2B} adenosine receptor agonists consisting of 1-deoxy-1-[6-[((hetero)arylcarbonyl)-hydrazino]-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide derivatives. The concurrent effect of 6-substitution of the purine nucleus with a ((hetero)arylcarbonyl)hydrazino function and a 2-chloro substitution has been investigated in such NECA derivatives.

Introduction

Adenosine, a naturally occurring nucleoside, is well-known to be involved in a large variety of physiological and pathophysiological processes that are modulated through the interaction of the endogenous ligand with specific cell membrane G-protein-coupled receptors classified into four subtypes, A_1, A_{2A}, A_{2B}, and A_3.[1] Of these, the A_{2B} subtype has been identified on the basis of functional assays on rat brain slices by Daly et al.,[2] and subsequently the existence of this pharmacological target has been confirmed by receptor cloning experiments conducted in various species such as rat and human.[3] The A_{2B} receptor, described as a low-affinity subtype, shows a well-preserved interspecies sequence with 85% identity between human and mouse and 95% identity between rat and mouse.[4] Quantitative tissue distribution of the A_{2B} adenosine receptors is so far unknown because of the lack of potent radioligands endowed with sufficient receptor selectivity. Determination of receptor-coding mRNA levels furnished important information about A_{2B} tissue distribution,[4] assuming that high mRNA levels should correspond to high receptor protein expression. On this basis, high concentrations of adenosine A_{2B} receptors have been suggested in caecum, large intestine, and urinary bladder, while a lower expression level has been suggested in lung, blood vessels, eye, and mast cells. Adipose tissue, adrenal gland, brain, kidney, liver, ovary, and pituitary gland are thought to have a very low concentration of A_{2B} adenosine receptor.[5] Recently it has been demonstrated that activation of A_{2B} receptors in primary cultures of mouse cortical astrocytes leads to an increase of glycogen synthesis through the modulation of gene expression,[6] suggesting that adenosine probably exerts a fundamental role in brain energy metabolism. A study by Zeng and co-workers highlighted the positive effect of adenosine on the release of angiogenic factors (IL-8) from the glioblastoma cell line U87MG, which seems to be correlated to an overexpression of A_{2B} receptors on tumor cell surfaces.[7] Because of the widespread distribution of A_{2B} adenosine receptors and the involvement of this receptor subtype in important (patho)-physiological processes both in peripheral tissues and in the central nervous system, many efforts have been carried out in order to identify potent and selective A_{2B} ligands endowed with noteworthy therapeutic potential. Treatment of asthma with selective A_{2B} adenosine receptor antagonists has been, up to now, the most interesting therapeutic goal.[8] However, several remarkable therapeutic applications have been proposed for the employment of A_{2B} receptor agonists. It has been shown that activation of A_{2B} is related to an inhibition of fibroblasts[9] and smooth muscle proliferation. Therefore, A_{2B} agonists have been suggested for the treatment of cardiac diseases such as hyperplasia consequent to hypertension, heart attacks, and arteriosclerosis.[10,11] Since interaction of adenosine with A_{2B} receptors inhibits production of the proinflammatory cytokine TNFα in monocytes,[12] A_{2B} agonists have been proposed for treatment of septic shock. Moreover, A_{2B} agonists may be useful for the treatment of cystic fibrosis[13] and impotence,[14] as antidiarrhoeal drugs,[15] and as coronary dilatatory agents.[16]

Identification of potent and selective A_{2B} adenosine receptor agonists has been an ambitious goal for years in order to characterize the potential physiological role of A_{2B} receptors, especially in tissues where all four adenosine receptor subtypes are coexpressed. From a pharmacological point of view, the lack of highly selective agents has so far hampered efforts to better characterize the adenosine A_{2B} receptor subtype and consequently to fully define its therapeutic potential.[17] 5′-*N*-Ethylcarboxamidoadenosine (NECA, **1**, Figure 1) has been considered one of the most useful ligands at the A_{2B} receptor subtype[18−20] (EC_{50} = 160 nM, Table 2), although it shows high affinity toward all other adenosine receptors (K_i from binding assays in the low nanomolar range; see Table 2). In a recent patent application by Rosentreter et al., a series of substituted 2-thio-4-aryl-3,5-dicyano-6-aminopyrimidine derivatives were claimed to behave as potent non-nucleosidic agonists for adenosine receptors.[21] An extension of this work[22] led to the identification of both partial (2-amino-4-(4-hydroxyphenyl)-6-(1*H*-imidazol-2-ylmethylsulfanyl)pyridine-3,5-dicarbonitrile, hA_1

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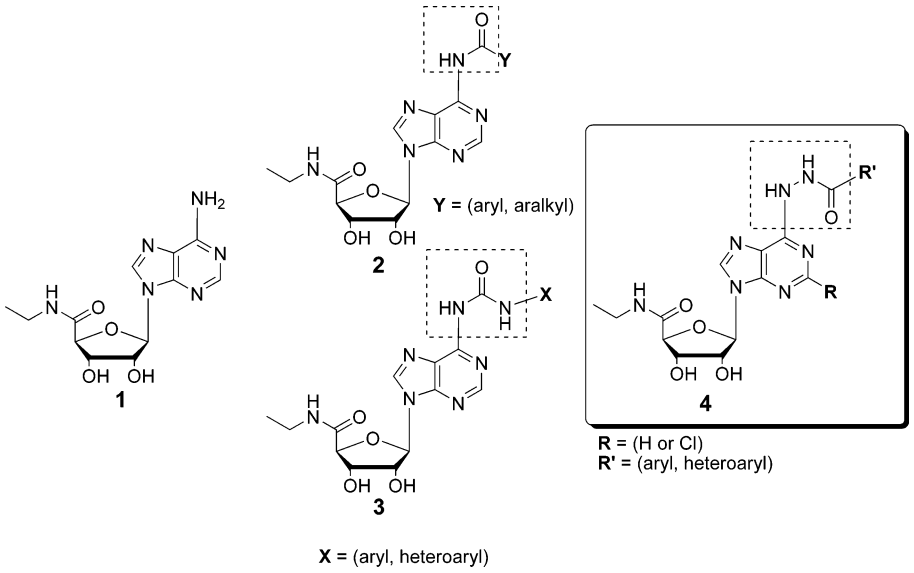
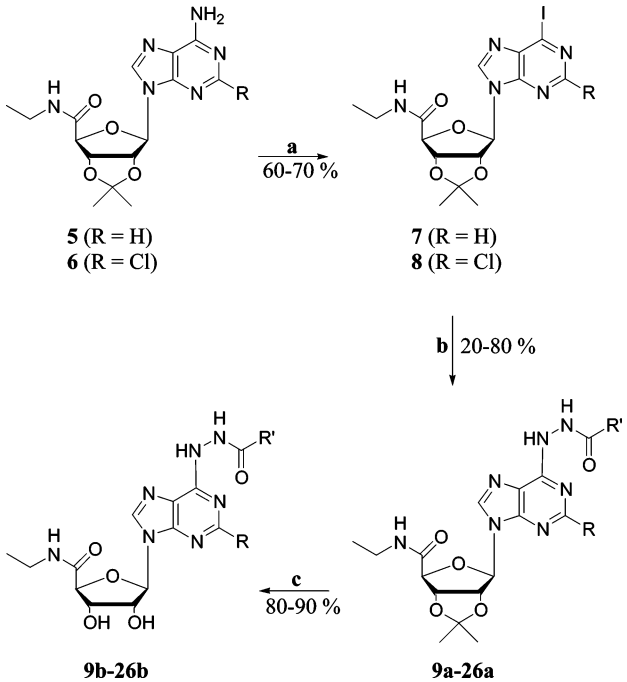


Figure 1. Representative structural correlation among 1-deoxy-1-[6-[((hetero)arylcarbonyl)hydrazino]-9*H*-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamides and N^6-arylcarbamoyl/carboxamido NECA analogues previously reported.

K_i = 2.6 nM, hA_{2A} K_i = 28 nM, hA_{2B} EC_{50} = 12 nM, hA_3 K_i = 538 nM) and full (2-amino-4-(3-hydroxyphenyl)-6-(1*H*-imidazol-2-ylmethylsulfanyl)pyridine-3,5-dicarbonitrile, hA_1 K_i = 4.4 nM, hA_{2A} K_i = 21 nM, hA_{2B} EC_{50} = 10 nM, hA_3 K_i = 104 nM) nonselective agonists at the A_{2B} adenosine receptor.

A few years ago, we reported the synthesis and the biological activity of a series of N^6-arylcarbamoyl and N^6-carboxamido derivatives of adenosine-5′-*N*-ethyluronamide (NECA) as A_1 and A_3 adenosine receptor agonists.[23−25] From the binding data we obtained, we observed that the carboxamido derivatives (general structure **2**, Figure 1) generally behaved as low selective A_1 receptors ligands, while some N^6-(substituted phenylcarbamoyl) derivatives (general structure **3**, Figure 1) were found to have affinity at rat A_3 receptors in the low nanomolar range with different degrees of selectivity versus A_1 and A_{2A} adenosine receptors. These results suggested that small modifications of the chain at the 6-position of the purine nucleus are able to produce significant changes in the selectivity pattern of such compounds. In particular, it appeared that the presence of an amide vs a urea functionality at the 6-position was generally detrimental in terms of affinity at rat A_3 receptors. Considering its fundamental importance for the modulation of both affinity and selectivity, we decided to further investigate the N^6-position, synthesising a new series of N^6-functionalized 5′-*N*-ethylcarboxamidoadenosine analogues. According to the principles of bioisosterism, we replaced the (hetero)arylurea function of the reported A_3 agonists with the isomeric (hetero)arylcarbonylhydrazino moiety and evaluated the effect on the binding and functional profile of the synthesized compounds (general structure **4**, Figure 1). This spacer is able to provide flexibility to the N^6-chain, being at the same time rich in potential hydrogen bond anchoring sites. The coexisting effect of substitution at the 2-position of the purine with a chlorine atom has been also evaluated. Surprisingly, the new class of 1-deoxy-1-[6-[((hetero)-arylcarbonyl)hydrazino]-9*H*-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide and 1-deoxy-1-[2-chloro-6-[((hetero)arylcarbonyl)-hydrazino]-9*H*-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide derivatives has been found to be the first examples of both potent and selective A_{2B} adenosine receptor agonists. Some N^6-substituted adenosine analogues containing cyclic hydrazines at the C^6 position have in the past been conceived as the aza isosteres of the known A_1 receptor agonist CPA (*N*-cyclopentyladenosine), and their A_{2A}/A_1 selectivity was estimated by means of radioligand binding assays.[26,27] These studies allowed the detection of interesting molecules exhibiting a high level of selectivity at the A_1 receptor versus A_{2A} receptor. Unfortunately no data were furnished with respect to the A_{2B} subtype. At any rate, with the exception of compound **20b**, none of the newly reported compounds were found to exert significant affinity at the A_1 adenosine receptor subtype.

Scheme 1[a]

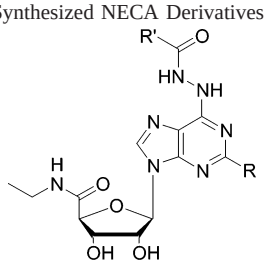


[a] Reagents: (a) CH_2I_2, isopentylnitrite, 85 °C, 1 h; (b) TEA, substituted (hetero)arylhydrazides, autoclave, 90−100 °C, 7−8 h (for the 2-chloro derivative **8**), or 120 °C, ON (for the 2-unsubstituted derivative **7**); (c) TFA/H_2O, 1:1, room temp, 3 h.

Chemistry

The synthetic strategy employed for the preparation of target compounds **9b**−**26b** is depicted in Scheme 1. 2′,3′-*O*-Isopro-

Table 1. Structures and Physicochemical Parameters of the Synthesized NECA Derivatives **9b**−**26b**



9b-26b

compd	R	R′	mp (°C)	MW	formula
9b	H	phenyl	174−175	427.16	$C_{19}H_{21}N_7O_5$
10b	H	4-chlorophenyl	188−189	461.86	$C_{19}H_{20}ClN_7O_5$
11b	H	3-pyridyl	170	428.40	$C_{18}H_{20}N_8O_5$
12b	H	2-furyl	233−234	417.38	$C_{17}H_{19}N_7O_6$
13b	H	5-bromofuran−2-yl	171	496.27	$C_{17}H_{18}BrN_7O_6$
14b	H	5-methylfuran−2-yl	154−155	431.4	$C_{18}H_{21}N_7O_6$
15b	H	1-methyl-4-nitro-1*H*-imidazol-2-yl	169−170	476.40	$C_{17}H_{20}N_{10}O_7$
16b	H	5-methylthiophen-2-yl	153−154	447.47	$C_{18}H_{21}N_7O_5S$
17b	Cl	phenyl	253−254	461.86	$C_{19}H_{20}ClN_7O_5$
18b	Cl	2-furyl	269−270	451.82	$C_{17}H_{18}ClN_7O_6$
19b	Cl	5-methylthiophen-2-yl	156	481.91	$C_{18}H_{20}ClN_7O_5S$
20b	Cl	(thiophen-2-yl)methyl	153−154	481.91	$C_{18}H_{20}ClN_7O_5S$
21b	Cl	thiophen-3-yl	249−250	467.89	$C_{17}H_{18}ClN_7O_5S$
22b	Cl	thiophen-2-yl	189−190	467.89	$C_{17}H_{18}ClN_7O_5S$
23b	Cl	3-methylthiophen-2-yl	160−161	481.91	$C_{18}H_{20}ClN_7O_5S$
24b	Cl	1*H*-pyrrol-2-yl	230−231	450.84	$C_{17}H_{19}ClN_8O_5$
25b	Cl	5-methylisoxazol-3-yl	182−183	466.11	$C_{17}H_{19}ClN_8O_6$
26b	Cl	5-phenylisoxazol-3-yl	169−170	528.91	$C_{22}H_{21}ClN_8O_6$

pylidene-5′-*N*-ethylcarboxamidoadenosine (**5**)[28] and 2′,3′-*O*-isopropylidene-2-chloro-5′-*N*-ethylcarboxamidoadenosine (**6**) were quite efficiently converted into the corresponding 6-iodo derivatives **7** and **8** by treatment with diiodomethane and isopentyl nitrite as reported by Nair (yield 60%).[26,29] Intermediates **7** and **8** proved to be useful key substrates for the subsequent substitution reactions with the appropriate substituted (hetero)arylcarboxylic acid hydrazides, which were performed in a steal bomb at 90−100 °C for 7−8 h in the case of the 2-chloro derivative **8** or at 120 °C overnight in the case of the 2-unsubstituted **7** to furnish derivative **9a**−**26a** (yield 20−80%). The 2-chloro intermediate **8** was shown to be visibly more reactive then the corresponding 2-dehalogenated intermediate **7** toward the SNAr reaction with the employed hydrazides, requiring milder reaction conditions and reduced reaction times. This is reflected in the differences observed in the reaction yields, which are about 20−30% for derivative **7** compared to 70−80% for 2-chloro intermediate **8**. Despite the drastic reaction conditions (steel bomb, 100−120 °C), in no case did we observe the byproducts deriving from the substitution of the 2-chloro atom by the hydrazide nucleophilic species. The (hetero)-arylcarboxylic acid hydrazides of our interest were found to be commercially available or readily synthesized from the corresponding carboxylic acids or carboxylic acid ethyl esters, according to well-known procedures.[30]

Protected N^6-substituted nucleoside derivatives **9a**−**26a** were stirred for about 3 h at room temperature in a 1:1 mixture of water and trifluoroacetic acid to give unprotected final compounds **9b**−**26b** in a nearly quantitative yield.

Biological Activity. Results and Discussion

Competition binding experiments[31] were performed to evaluate the affinity of the synthesized compounds **9b**−**26b** to hA_1, hA_{2A}, and hA_3 receptors expressed in CHO cells using as radioligands [^{3}H]CHA, [^{3}H]CGS 21680, and [^{125}I]AB-MECA, respectively. The compounds were also evaluated in functional assays,[32] measuring their capacity to modulate cAMP levels in CHO cells expressing hA_{2B} receptors. Structures, chemical properties, and biological data of the synthesized compounds are listed in Tables 1 and 2.

The series has been developed introducing different aromatic nuclei on the N^6-hydrazide chain. We have chosen phenyl (**9b**, **17b**), 4-chlorophenyl (**10b**), a six-membered heterocycle (pyridine) (**11b**), and several five-membered heterocycles, such as furan (**12**−**14b**, **18b**), thiophene (**16b**, **19**−**23b**), imidazole (**15b**), pyrrole (**24b**), and isoxazole (**25**−**26b**). Replacement of the hydrogen at the 2-position of the purine ring with a chlorine atom has also been evaluated.

From the analysis of binding and functional data reported in Table 2 it is apparent that of the synthesized compounds **9b**−**26b** some of the heteroarylcarbonylhydrazino derivatives here described show considerable potency in activating A_{2B} adenosine receptors, with EC_{50} values ranging from 82 to 450 nM. The most innovative finding rests in the analysis of the selectivity information emerging from the comparison between affinity and functional data related to the four adenosine receptors subtypes. Of the examined molecules, the ones showing the capability to activate A_{2B} adenosine receptors were inactive at the hA_3AR ($K_i > 5000$) and showed high nanomolar to micromolar affinity at the A_1 and A_{2A} subtypes (K_i varying from 700 to 5000 nM). Thus, we have identified, to the best of our knowledge, the first examples of A_{2B} adenosine receptor agonists endowed with good selectivity.

Figure 2 reports the dose response curves of NECA and compound **12b** in hA_{2B} CHO cells showing that this new ligand is a full A_{2B} agonist. Similar behavior is observed for the other examined compounds.

Cristalli and co-workers have explored the SAR of 2-substituted 5′-uronamide adenosine derivatives, such as *S*-PHPNECA ((*S*)-2-phenylhydroxypropynyl-NECA), as potent but nonselective agonists at the $A_{2B}AR$.[33−35] Comparable marks have been attained with the cited 2-thio-4-aryl-3,5-dicyano-6-aminopyri-

Table 2. Binding, Functional Data, and R_M Values of the Synthesized NECA Derivatives **9b**−**26b**[a]

compd	[^{3}H]CHA binding hA_1 CHO K_i (nM)	[^{3}H]CGS21680 binding hA_{2A} CHO K_i (nM)	cAMP assay hA_{2B} CHO EC_{50} (nM)	[^{125}I]AB-MECA binding hA_3 CHO K_i (nM)	R_M (0)
1 (NECA)	18.3 ± 2.5	12.5 ± 2.8	160 ± 20	34.6 ± 3.3	0.94 ± 0.09
9b	>5000 (35%)	>5000 (18%)	>5000 (10%)	>5000 (8%)	1.65 ± 0.15
10b	>5000 (5%)	>5000 (8%)	>5000 (6%)	>5000 (20%)	2.10 ± 0.18
11b	>5000 (11%)	>5000 (7%)	>5000 (9%)	>5000 (17%)	0.62 ± 0.05
12b	1050 ± 132	1550 ± 165	82 ± 10	>5000 (23%)	0.84 ± 0.09
13b	780 ± 34	1200 ± 135	369 ± 42	>5000 (13%)	1.45 ± 0.13
14b	700 ± 25	1600 ± 147	227 ± 18	>5000 (15%)	1.05 ± 0.09
15b	>5000 (7%)	>5000 (12%)	>5000 (14%)	>5000 (11%)	0.35 ± 0.04
16b	1100 ± 124	2100 ± 185	273 ± 12	>5000 (19%)	1.52 ± 0.14
17b	>5000 (37%)	>5000 (40%)	>5000 (5%)	>5000 (45%)	2.21 ± 0.18
18b	3500 ± 275	4950 ± 356	210 ± 13	>5000 (26%)	1.37 ± 0.11
19b	2600 ± 194	4100 ± 390	175 ± 20	>5000 (17%)	2.02 ± 0.20
20b	62 ± 4	633 ± 60	603 ± 31	25 ± 3	2.15 ± 0.22
21b	933 ± 76	3300 ± 315	450 ± 29	>5000 (18%)	1.89 ± 0.19
22b	737 ± 46	1700 ± 180	200 ± 20	>5000 (12%)	1.84 ± 0.16
23b	1600 ± 140	3800 ± 305	340 ± 35	>5000 (9%)	2.26 ± 0.24
24b	610 ± 32	3200 ± 330	359 ± 36	>5000 (11%)	1.54 ± 0.16
25b	>5000 (7%)	>5000 (3%)	>5000 (21%)	>5000 (16%)	0.86 ± 0.09
26b	>5000 (5%)	>5000 (2%)	>5000 (24%)	>5000 (15%)	2.41 ± 0.25

[a] The data are expressed as the mean ± SEM. The percentages in parentheses indicate the % of displacement of the new tested compounds in the binding experiments or the % of stimulation of cAMP levels in functional experiments.

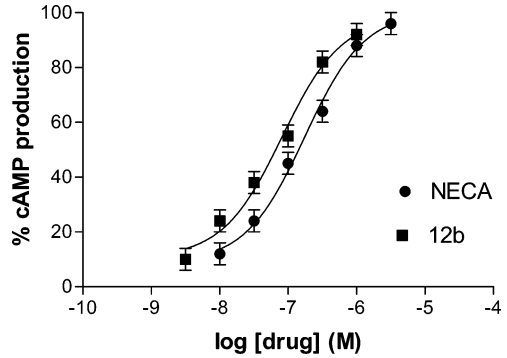


Figure 2. Dose response curve of NECA and **12b** on cAMP assays in hA_{2B} CHO cells.

midines,[21,22] an interesting example of non-adenosine receptor partial and full agonists, which can be considered a model for the development of very potent, but as yet not selective, ligands.

Compounds **9b**-**11b** and **17b**, containing six-membered aromatic rings at the N^6-position, did not show any significant affinity or activity at the four adenosine receptors (K_i and EC_{50} values of >5000 nM). Comparable results have been accomplished with some of the derivatives functionalized with five-membered heterocycles, such as the imidazole (**15b**) and the isoxazole (**25b**, **26b**). Interesting levels of receptor affinity and selectivity have been otherwise reached as a result of the introduction of thiophene (**16b**, **19b**−**23b**), furan (**12b**−**14b**, **18b**) and pyrrole (**24b**) moieties. The pyrrole **24b** (hA_{2B} EC_{50} = 359 nM) is 2-fold less active than the corresponding unsubstituted thiophene **22b** (hA_{2B} EC_{50} = 200 nM) and furan derivative **18b** (hA_{2B} EC_{50} = 210 nM). The presence of a hydrogen bond acceptor (O or S) is therefore preferred to a hydrogen bond donor (NH). By comparison of the pharmacological behavior of compounds containing the furan ring, **14b** (hA_{2B} EC_{50} = 227 nM) and **18b**, with the related thiophene derivatives **16b** (hA_{2B} EC_{50} = 273 nM) and **22b**, it is possible to assert that both furan and thiophene are able to exert similar receptor interactions that are favorable for receptor activation. Extraordinarily, in our series of compounds, thiophene did not behave as a bioisoster of the phenyl moiety.

The effect of introducing several substituents at the 5-position of the furan nucleus has been evaluated. Introduction of the small lipophilic methyl group is sufficient to produce a loss of activity of about 3-fold (**14b**, hA_{2B} EC_{50} = 227 nM) in comparison with the unsubstituted derivative **12b**, while the introduction of bromine atom determined a 4-fold loss of activity (**13b**, hA_{2B} EC_{50} = 369 nM). The unsubstituted thiophene derivative **22b** showed K_i and EC_{50} values similar to those of the 5-methylthiophene analogue **19b**, while the substitution at the 3-position with a methyl group was able to slightly affect the results of the functional assay (compound **23b**, hA_{2B} EC_{50} = 340 nM). This preliminary SAR investigation seems to suggest that the steric hindrance at the N^6 chain could play a primary role for the receptor−ligand interaction. A loss of activity has in fact been observed for those derivatives in which the heterocycle has been expanded from five to six members or when a small substituent, such as a bromine atom or a methyl group, has been introduced into five-membered rings.

No correlation can be established between the biological activity and the lipophilic character of the molecules. Table 2 reports R_M values of the examined compounds showing that the major part of the new ligands show lipophilicity parameters ranging from 0.36 to 2.19. The reference compound NECA, with a R_M value of 0.94, is located among the most hydrophilic studied molecules. The calculated R_M value of our most potent compound **12b** was comparable to that of NECA. An evident index that the lipophilic nature of the aromatic ring is not so important in influencing the potency of this class of compounds is derived from a comparison of R_M values. For example, compounds **12b**, **11b**, and **25b** showed comparable R_M values while exerting opposite biological properties.

The linkage position of the thiophene ring is also important. The thiophen-3-yl derivative **21b** (hA_{2B} EC_{50} = 450 nM) was 2-fold less active than the thiophen-2-yl positional isomer **22b** (hA_{2B} EC_{50} = 200 nM).

The introduction of a methylene spacer between the thiophene and the hydrazide function led us to identify compound **20b**. The examined structural modulation appeared to address the binding toward human A_1 and A_3 adenosine receptors subtypes, decreasing the capability of the molecule to interact with the hA_{2B} receptor. This confirms our previous results[23−25] indicating that small modifications of the N^6 chain can lead to dramatic shifts in the corresponding ligand activity; compound **22b** showed a hA_3 K_i value higher than 5μM, while **20b** acquires low nanomolar affinity for the same target (hA_3, K_i = 25 nM).

The presence of the chlorine atom at the 2-position of the purine nucleus does not seem to affect the ability of the tested compounds to activate $hA_{2B}AR$, as is clear from the comparison of chlorinated derivatives **18b** (hA_{2B} EC_{50} = 210 nM) and **19b** (hA_{2B} EC_{50} = 175 nM) with the corresponding nonchlorinated **12b** (hA_{2B} EC_{50} = 82 nM) and **16b** (hA_{2B} EC_{50} = 273 nM). Even though the chlorine atom at the 2-position did not allow us to improve the pharmacological profile, most of the synthesized compounds show this structural element for chemical reasons, in light of the relevant improvement in the substitution reaction yields.

Conclusions

In conclusion, we have designed and synthesized a new class of nucleoside adenosine ligands structurally related to NECA that appear to be the first example of potent and rather selective A_{2B} adenosine receptor agonists. Compound 1-deoxy-1-{6-[*N*′-(furan-2-carbonyl)hydrazino]-9*H*-purin-9-yl}-*N*-ethyl-*β*-D-ribofuranuronamide (**12b**, hA_1,hA_{2A} K_i > 1000 nM; hA_{2B} EC_{50} = 82 nM, hA_3 K_i > 5000 nM) was the most potent of the series, and it was confirmed to be a full agonist in a functional assay based on the measurement of its capacity to modulate cAMP levels in CHO cells expressing the hA_{2B} receptor. The examined molecules can be considered valuable tools for the design and development of new and even more selective and potent ligands. Furthermore, this study could provide useful foundations for the attainment of a detailed pharmacological and physiological characterization of the adenosine A_{2B} receptor. A potent and selective radiolabeled agonist at the hA_{2B} adenosine receptor is thus far unavailable; only antagonist radioligands have been identified with the aim to perform binding studies at the hA_{2B} receptor subtype.[36] The present report can contribute to the identification of the first useful agonist radioligand for the characterization of the human A_{2B} adenosine receptor.

Experimental Section

Chemistry. Reaction progress and product mixtures were monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) and visualized with aqueous potassium permanganate or a methanolic solution of H_2SO_4. ^{1}H NMR data were determined in $CDCl_3$ or DMSO-d_6 solutions with a Varian VXR 200 spectrometer or a Varian Mercury Plus 400 spectrometer. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and *J* values are given in hertz. light petroleum refers to the fractions boiling at 40−60 °C. Melting points were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatography was performed on Merck 230−400 mesh silica gel. Organic solutions were dried over anhydrous sodium sulfate. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Ferrara, and were within ±0.4% of the theoretical values for C, H, and N.

General Procedure for the Preparation of 1-Deoxy-1-(6-iodo-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-ethyl-*β*-D-ribofuranuronamide (7) and 1-Deoxy-1-(2-chloro-6-iodo-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-ethyl-*β*-D-ribofuranuronamide (8).[29] A suspension of NECA (**5**) or 2-chloro-NECA (**6**) (2.85 mmol) in isopentyl nitrite (8.25 mL) and CH_2I_2 (21.55 mL) was heated at 85 °C for 1 h. The reagents were evaporated, and the residue was dissolved with CH_2Cl_2 (100 mL). The organic phase was washed with H_2O (2 × 50 mL) and dried with anhydrous Na_2SO_4, and the solvent was evaporated under vacuum to obtain a crude oil, which was washed with light petroleum (3 × 20 mL).

1-Deoxy-1-(6-iodo-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-ethyl-*β*-D-ribofuranuronamide (7). The product was purified by column chromatography with silica gel, eluting with a mixture of CH_2Cl_2/CH_3OH, 9.8:0.2, and crystallizing with a mixture of CH_2Cl_2/light petroleum, 1:2. Yellow solid; 60% yield; mp 79−80 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 0.49 (t, 3 H, *J* = 7.2), 1.32 (s, 3H), 1.52 (s, 3H), 2.70 (m, 2H), 4.50−4.60 (m, 1H), 5.43 (s, 2H), 6.42 (s, 1H), 7.47 (bt, 1H), 8.43 (s, 1H), 8.47 (s, 1H).

1-Deoxy-1-(2-chloro-6-iodo-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-ethyl-*β*-D-ribofuranuronamide (8). The product was purified by column chromatography with silica gel, eluting with a mixture of EtOAc/light petroleum, 3:7, and crystallizing with a mixture of EtOAc/Et_2O/light petroleum, 1:1:1. White solid; 70% yield; mp 95 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 0.55 (t, 3 H, *J* = 7.2), 1.35 (s, 3H), 1.53 (s, 3H), 2.80 (m, 2H), 4.61 (s, 1H), 5.43 (m, 2H), 6.42 (s, 1H), 7.58 (bt, 1H), 8.77 (s, 1H).

General Procedure for the Preparation of Compounds 9a−26a. A mixture of **7** or **8** (0.17 mmol), TEA (30 μL, 0.21 mmol), and the appropriate (hetero)arylhydrazide (0.21 mmol) in absolute EtOH (3 mL) was heated in a steal bomb at 90−100 °C for 7−8 h in the case of the 2-chloro derivative **8** or at 120 °C overnight in the case of the 2-unsubstituted **7**. The solvent was evaporated, and the residue was suspended with EtOAc. The organic layer was washed with H_2O (2 × 20 mL) and dried with anhydrous Na_2SO_4. After filtration, the solvent was evaporated under vacuum, and the products were purified by crystallization or by column chromatography on silica gel.

1-Deoxy-1-[6-(*N*′-benzoylhydrazino)-9*H*-purin-9-yl]-2,3-*O*-isopropylidene-*N*-ethyl-*β*-D-ribofuranuronamide (9a). The product was purified by crystallization with a mixture of Et_2O/petroluem ether 1:2. Pale-yellow solid; 25% yield; mp 124−125 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 0.64 (t, 3H, *J* = 7.2), 1.32 (s, 3H), 1.52 (s, 3H), 2.83 (bm, 2H), 4.53 (bs, 1H), 5.38 (bs, 2H), 6.35 (bs, 1H), 7.20 (bm, 1H), 7.49−7.57 (m, 3H), 7.90−7.95 (m,2H), 8.21 (s, 1H), 8.40(bs, 1H), 10.00−11.00 (bs, 2H).

1-Deoxy-1-{6-[*N*′-(4-chlorobenzoyl)hydrazino]-9*H*-purin-9-yl}-2,3-*O*-isopropylidene-*N*-ethyl-*β*-D-ribofuranuronamide (10a). The product was purified by column chromatography on silica gel, eluting with a mixture of EtOAc/petroleum ether, 1:1. White solid; 20% yield; mp 116−117 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 0.80 (t, 3H, *J* = 7.2), 1.32 (s, 3H), 1.54 (s, 3H), 3.04 (m, 2H), 4.57 (s, 1H), 5.30 (s, 2H), 6.17 (s, 1H), 7.18 (bt, 1H), 7.38 (d, 2H, *J* = 8), 7.92 (d, 2H, *J* = 8), 8.01 (s, 1H), 8.26 (s, 1H), 9.30 (bs, 1H), 10.56 (bs, 1H).

General Procedure for the Preparation of Compounds 9b−26b. The appropriate protected derivative, **9a−26a** (0.6 mmol), was dissolved in a mixture of trifluoroacetic acid/H_2O, 1:1 (4 mL), and the solution was stirred at room temperature for 3 h. The solvents were evaporated to dryness, and the residue was suspended with EtOAc. The organic layer was washed with H_2O (2 × 15 mL) and dried with Na_2SO_4, and after filtration, the solvent was evaporated.

1-Deoxy-1-[6-(*N*′-benzoylhydrazino)-9*H*-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (9b). The product was purified by column chromatography on silica gel, eluting with EtOAc. White solid; 85% yield; mp 173−174 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 1.07 (t, 3H, *J* = 7.2), 3.21 (m, 2H), 4.17 (bs, 1H), 4.32 (s, 1H), 4.61 (bm, 1H), 5.61 (bm, 1H), 5.75 (bm, 1H), 6.00 (bm, 1H), 7.56 (m, 3H), 7.94 (m, 2H), 8.34 (s, 1H), 8.45 (bs, 1H), 8.74 (bt, 1H), 10.00 (bs, 1H), 10.60 (bs, 1H). Anal. ($C_{19}H_{21}N_7O_5$) C, H, N.

1-Deoxy-1-[6-(*N*′-(4-chlorobenzoyl)hydrazino)-9*H*-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (10b). The product was purified by column chromatography on silica gel, eluting with a mixture of CH_2Cl_2/CH_3OH, 9.5:0.5. White solid; 80% yield; mp 188−190 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm) 1.10 (t, 3H, *J* = 7.2), 3.17 (m, 2H), 4.02 (bm, 1H), 4.31 (m, 1H), 4.63 (bs, 1H), 4.68 (bm, 2H), 6.00 (d, 1H, *J* = 7.3), 7.61 (d, 2H, *J* = 8), 7.96 (d, 2H, *J* = 8), 8.34 (s, 1H), 8.51 (bs, 1H), 8.75 (bt, 1H), 10.00 (bs, 1H), 10.80 (bs, 1H). Anal. ($C_{19}H_{20}ClN_7O_5$) C, H, N.

Determination of Binding (K_i Values) and Functional Parameters (EC_{50} Values). All synthesized compounds have been tested, by radioligand binding assay, for their affinity to human A_1, A_{2A}, and A_3 adenosine receptors and for their potency, in a cAMP assay, to human A_{2B} subtypes. The expression of the human A_1, A_{2A}, A_{2B}, and A_3 receptors in CHO cells has been previously

described.[37] The cells were grown adherently and maintained in Dulbecco's modified Eagle's medium with nutrient mixture F12 (DMEM/F12) without nucleosides, containing 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 μg/mL), L-glutamine (2 mM), and Geneticin (G418, 0,2 mg/mL) at 37 °C in 5% CO_2/95% air. Cells were split two or three times weekly at a ratio between 1:5 and 1:20. For membrane preparation the culture medium was removed and the cells were washed with PBS and scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris-HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized and centrifuged for 30 min at 100000*g*. The membrane pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4, and incubated with 2 IU/mL of adenosine deaminase for 30 min at 37 °C. Then the suspension was frozen at −80 °C, and the protein concentration was determined according to a Bio-Rad method[38] with bovine albumin as a standard reference. Binding of 1 nM [^{3}H]CHA to hA_1 CHO cells (50 μg of protein/assay) was performed using 50 mM Tris-HCl buffer, pH 7.4, and at least six to eight different concentrations of agonists studied for an incubation time of 150 min at 25 °C.[39] Nonspecific binding was determined in the presence of 10 μM CHA and was about 20% of the total binding. Inhibition binding experiments of [^{3}H]CGS 21680 to hA_{2A} CHO cells (100 μg of protein/assay) was performed using 50 mM Tris-HCl buffer, 10 mM $MgCl_2$, pH 7.4, and at least six to eight different concentrations of agonists studied for an incubation time of 180 min at 25 °C.[40] Nonspecific binding was determined in the presence of 10 μM CGS 21680 and was about 25% of the total binding. Binding of [^{125}I]ABMECA to hA_3 CHO cells (50 μg of protein/assay) was performed using 0.2 mL of 50 mM Tris-HCl buffer, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4, and at least six to eight different concentrations of tested compounds for an incubation time of 60 min at 37 °C.[31] Nonspecific binding was determined in the presence of 1 μM ABMECA and was about 20% of total binding. Separation of bound from free radioligand was performed by rapid filtration through Whatman GF/B filters, which were washed three times with ice-cold buffer. Filter bound radioactivity was measured by scintillation spectrometry after addition of 5 mL of Aquassure. For cell preparation CHO cells transfected with human A_{2B} receptors were washed with PBS and diluted trypsine and centrifuged for 10 min at 200*g*. The pellet containing the CHO cells (1×10^6 cells/assay) was resuspended in an incubation mixture of 15 mM NaCl, 0.27 mM KCl, 0.037 mM NaH_2PO_4, 2 IU/mL adenosine deaminase, and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidone (Ro 20-1724) as phosphodiesterase inhibitor and preincubated for 10 min in a shaking bath at 37 °C. After the preincubation time at 37 °C the examined ligands (1 nM to 10 μM) were added to the mixture and incubated for a further 5 min. The reaction was terminated by the addition of cold 6% thrichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000*g* for 10 min at 4 °C, and the supernatant was extracted four times with water saturated diethyl ether. The final aqueous solution was tested for cyclic AMP levels by a competition protein binding assay.[41] Samples of cyclic AMP standard (0−10 pmol) were added to each test tube containing the incubation buffer (0.1 mM trizma base , 8.0 mM aminophylline, 6.0 mM 2-mercaptoethanol, pH 7.4) and [^{3}H]cAMP in a total volume of 0.5 mL. The binding protein, previously prepared from beef adrenals, was incubated at 4 °C for 150 min, and after the addition of charcoal was centrifuged at 2000*g* for 10 min. The clear supernatant was counted in a Beckman scintillation counter. Inhibitory binding constants, K_i, were calculated from those of IC_{50} according to the Cheng and Prusoff equation,[42]

$$K_i = \frac{IC_{50}}{1 + [C^*]/K_D^*}$$

where [C*] is the concentration of the radioligand and K_D* its dissociation constant. A weighted nonlinear least-squares curve-fitting program LIGAND[43] was used for computer analysis of inhibition binding experiments. The EC_{50} values obtained in cyclic AMP assay were calculated by nonlinear regression analysis using the equation for a sigmoid concentration−response curve (GraphPad Prism, San Diego, CA). All experimental data are expressed as the mean ± standard error of the mean (SEM) of three or four independent experiments performed in duplicate.

Determination of R_M Values by C_{18} RP-HPTLC. A reversed-phase TLC technique for measuring R_M values as an expression of the lipophilic character of molecules was performed. The HPTLC determinations were carried out on Whatman $KC_{18}F$ plates as previously described.[44] Solvent mixtures of methanol−water buffer at pH 7.0 were used as mobile phase. The methanol concentration ranged from 60% to 90%. The test compounds were dissolved in DMSO, and 1 μL of a 10 μM solution was spotted on the plates. The developed plates were dried and the spots detected under UV light (254 nm). R_M values were calculated by means of the equation

$$R_M = \log[(1/R_F) - 1]$$

For each R_F measurement at least three to four replications were carried out. For each compound, there was a linear relationship between the R_M values and composition of the mobile phase. R_M values represented the theoretical value at 0% methanol in the mobile phase, expressed as the mean ± SEM.

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Supporting Information Available: Detailed experimental procedures for the synthesis of the reported compounds, elemental analysis data, and ^{1}H NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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