7-Substituted 5-Amino-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines as A_{2A} Adenosine Receptor Antagonists: A Study on the Importance of Modifications at the Side Chain on the Activity and Solubility

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It was demonstrated in the early 1990s that adenosine exerts many physiological functions through the interaction with four different receptors, named A_1, A_{2A}, A_{2B}, and A_3. In the past few years, our group has been involved in the development of A_{2A} antagonists, which led to the synthesis of SCH 58261 (**1**), the first potent and selective adenosine A_{2A} antagonist, which has been widely used as a reference compound. In this paper, we present an extended series of pyrazolotriazolopyrimidines synthesized with the aim to investigate the influence of the substitutions on the pyrazole ring. The choice of the substituents was based on their capability to improve water solubility while retaining high affinity and selectivity at the human A_{2A} adenosine receptor subtype. In this series, some structural characteristics that are important for activity, i.e., tricyclic structure, free amino group at 5-position, furan ring, and substituent at 7-position on the pyrazole moiety, have been maintained. We focused our attention on the nature of the phenyl ring substituent to improve water solubility. Following this strategy, we developed new compounds with good affinity and selectivity for A_{2A} adenosine receptors, such as **8d** (K_i 0.12 nM; hA_1/hA_{2A} ratio = 1025; R_m = 2.8), **8h** (K_i 0.22; hA_1/hA_{2A} ratio = 9818; R_m = 3.4), **8i** (K_i 0.18 nM; hA_1/hA_{2A} ratio = 994; R_m = 2.8), **8k** (K_i 0.13 nM; hA_1/hA_{2A} ratio = 4430; R_m = 3.6), and **14b** (K_i 0.19 nM; hA_1/hA_{2A} ratio = 2273; R_m = 2.7). All the new synthesized compounds have no significant interaction with either A_{2B} or A_3 receptor subtypes. This new series of compounds deeply enlightens some structural requirements to display high affinity and selectivity for the A_{2A} adenosine receptor subtype, although our goal of identifying new compounds with increased water solubility was not completely achieved. On this basis, other strategies will be devised to improve this class of compounds with a profile that appears to be promising for treatment of neurodegenerative disorders, such as Parkinson's disease.

Introduction

Part of the biological activity of adenosine occurs through the activation of specific receptors located on cell membranes and belonging to the extensive family of G-protein coupled receptors.[1,2] Currently, four adenosine receptors have been cloned and characterized pharmacologically, namely, A_1, A_{2A}, A_{2B}, and A_3. The adenosine receptors are associated with different second messenger systems: A_1 and A_3 mediate adenylate cyclase inhibition, whereas A_{2A} and A_{2B} stimulate the adenylate cyclase activity controlling intracellular cyclic AMP levels.[1]

Recently, important progress has been made with the development of selective A_{2A} receptor antagonists having an interesting pharmacological profile.[3,4] In this field, there are a number of compounds belonging to different chemical classes possessing a common feature, namely, high A_{2A} receptor affinity combined with weak interactions with other adenosine receptors.[5,6]

One of the most important reference compound reported is the pyrazolotriazolopyrimidine SCH 58261 (**1**), (5-amino-7-(2-phenyl)ethyl-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine), which has been widely characterized in a variety of binding and functional assays.[7] From the initial work on SCH 58261 (**1**), we have prepared several different compounds series, bearing different substitution at the pyrazole nitrogen, such as SCH 63390 (**2**) and their oxygenated derivatives (**3**−**6**) (Chart 1).

The result of this effort has been an improvement of A_{2A} receptor affinity, separation from other receptors, and improved biological activity in pharmacological assays.[8,9] This synthetic strategy is relevant for the identification of compounds possessing a suitable pharmacological profile in models of neurodegenerative disorders for diagnostic[10] and therapeutic uses, a target area of medical research where A_{2A} receptor antagonists can have a highly interesting perspective.[11,2,3]

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Chart 1

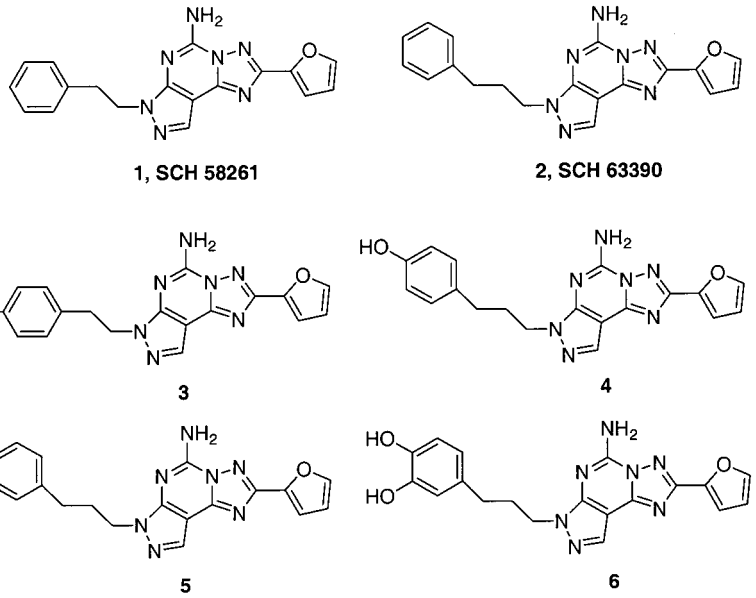
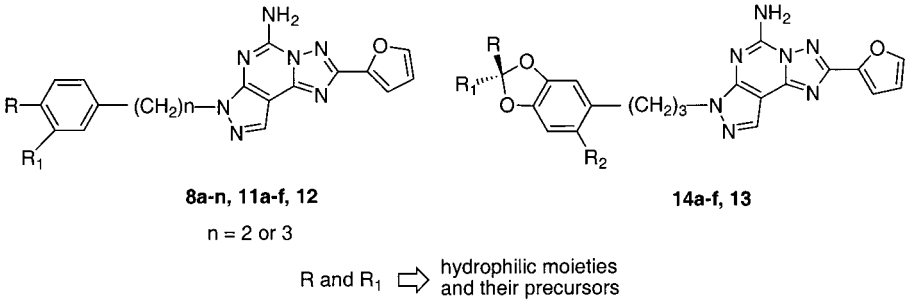


Chart 2



Some characteristics of the reference compounds have to be considered important from a structure−activity relationship point of view; mainly, the tricyclic structure of the pyrazolotriazolopyrimidine, the presence of the furan ring, the free amino group at the 5-position, and the arylalkyl substituent on the nitrogen at the 7-position seem to be essential for both affinity and selectivity. Thus, we decided to investigate the modifications on the phenyl ring of the side chain using different polar moieties, based upon the good results obtained with the oxygenated derivatives **3**−**6** (Chart 2).[7−9]

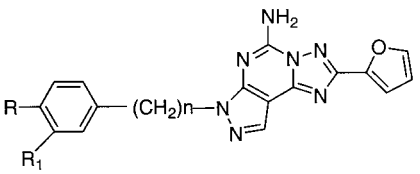
In particular, we conjectured to introduce different substituents, like basic or acidic moieties suitable for the preparation of salts or possible prodrugs, with the aim to modify the physicochemical properties of the compounds, maintaining the same affinity and selectivity for the adenosine A_{2A} receptor subtype. These modifications should be able to improve the water solubility of compounds that could be proposed as possible candidates for further development. In addition, a full biological characterization at all four cloned human adenosine receptor subtypes (hA_1, hA_{2A}, hA_{2B}, hA_3) of the synthesized derivatives is reported.

Chemistry. In previous papers, we reported the synthesis of compounds structurally related to SCH 58261 starting from the most appropriate hydrazines in order to obtain only the 7-substituted derivatives.[8,9] As mentioned above, to better evaluate the importance of the substituents on the phenyl ring in the side chain, an enlarged series of derivatives has been prepared (Table 1).

With this aim in mind, a common key intermediate that, after alkylation with an appropriate alkyl halide, could afford the desired final compounds has been prepared. This common intermediate has been identified in 2-furan-2-yl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]-pyrimidin-5-ylamine (**7**), which has been synthesized as previously reported.[8] This derivative could be easily alkylated, using anhydrous potassium carbonate as base in dry DMF, on the pyrazole nitrogen without competition by the amino group on the pyrimidine ring to afford a series of alkylated compounds. The two regioisomers at the 7- and the 8-positions of the pyrazolotriazolopyrimidine nucleus (ratio 3:1) were purified by flash chromatography, and only the alkylated derivatives at the 7-position were tested in biological assays (**8a**−**g**, Scheme 1). When the appropriate alkyl halides were not commercially available, they were synthesized by standard methods (Schemes 3 and 4).[12−15] All the other compounds **8h**−**n** were obtained by modifications of the former analogues (Scheme 2).[16]

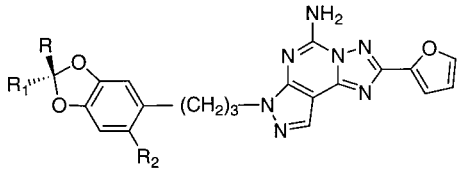
A different chemical pathway was utilized for the synthesis of the sulfo analogues of SCH 58261 (**1**) and its homologue SCH 63390 (**2**) due to the presence of the sulfonic moiety on the phenyl ring, which hampers the alkylation of **7** under basic conditions. To avoid these problems, the chlorosulfonic group was introduced directly onto the SCH 58261 (**1**) and SCH 63390 (**2**)

Table 1. Structures and Physicochemical Parameters of Synthesized Compounds

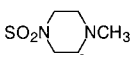


8a-n, 11a-f, 12

n = 2 or 3



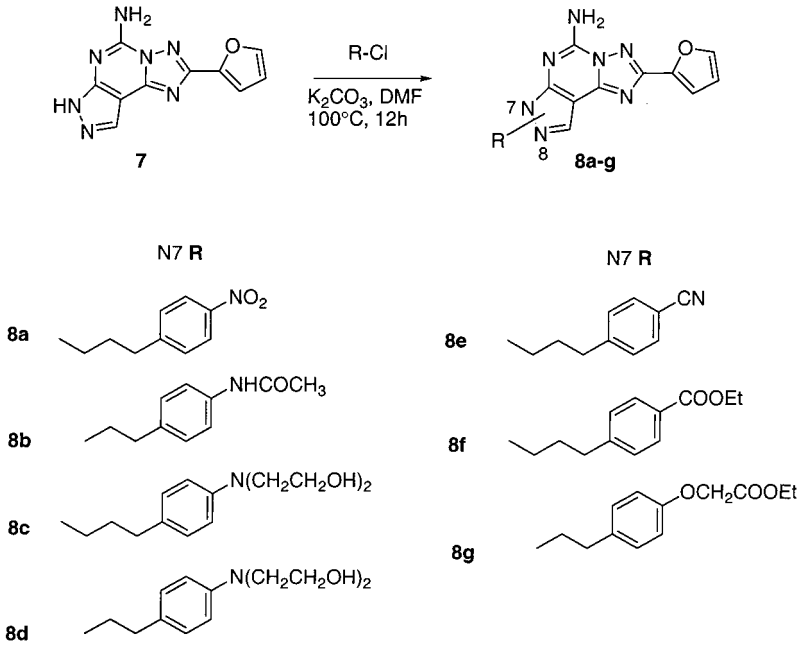
14a-f, 13

Compound	n	R	R_1	R_2	mp (°C)	MW	formula	anal.
8a	3	NO_2	H		245-247	404.38	$C_{19}H_{16}N_8O_3$	C, H, N
8b	2	$NHCOCH_3$	H		205 with dec.	402.41	$C_{20}H_{18}N_8O_2$	C, H, N
8c	3	$N(CH_2CH_2OH)_2$	H		176-177	462.50	$C_{23}H_{26}N_8O_3$	C, H, N
8d	2	$N(CH_2CH_2OH)_2$	H		180-182	448.48	$C_{22}H_{24}N_8O_3$	C, H, N
8e	3	CN	H		225-227	384.39	$C_{20}H_{16}N_8O$	C, H, N
8f	3	COOEt	H		194-195	431.45	$C_{22}H_{21}N_7O_3$	C, H, N
8g	2	OCH_2COOEt	H		190-192	447.45	$C_{22}H_{21}N_7O_4$	C, H, N
8h	3	NH_2	H		210-211	374.40	$C_{19}H_{18}N_8O$	C, H, N
8j	3	$C(NOH)NH_2$	H		218 with dec.	417.42	$C_{20}H_{19}N_9O_2$	C, H, N
8k	3	CH_2NH_2	H		>300	388.43	$C_{20}H_{20}N_8O$	C, H, N
8l	2	NH_2	H		262-264	360.37	$C_{18}H_{16}N_8O$	C, H, N
8m	3	$C(NH)NH_2$	H		>300	461.42	$C_{22}H_{23}N_9O_3$	C, H, N
8n	3	COOH	H		270	403.39	$C_{20}H_{17}N_7O_3$	C, H, N
8o	2	OCH_2COOH	H		>300	419.39	$C_{20}H_{17}N_7O_4$	C, H, N
11a	2	SO_3H	H		>300	425.42	$C_{18}H_{15}N_7O_4S$	C, H, N
11b	2	SO_2NH_2	H		260 with dec.	424.44	$C_{18}H_{16}N_8O_3S$	C, H, N
11c	2	$SO_2N(CH_2CH_2OH)_2$	H		183-185	512.54	$C_{22}H_{24}N_8O_5S$	C, H, N
11d	2		H		229-230	507.57	$C_{23}H_{25}N_9O_3S$	C, H, N
11e	2	$SO_2N(CH_2CH_2Cl)_2$	H		223-225	549.43	$C_{22}H_{22}Cl_2N_8O_3S$	C, H, N
11f	2	SO_2NHCH_2COOH	H		246-248	482.47	$C_{20}H_{18}N_8O_5S$	C, H, N
12	3	SO_3H	H		>300	439.45	$C_{19}H_{17}N_7O_4S$	C, H, N
13	3	H	H	SO_3H	>300	483.46	$C_{20}H_{17}N_7O_6S$	C, H, N
14a	3	COOEt	H	H	170-171	475.46	$C_{23}H_{21}N_7O_5$	C, H, N
14b	3	CH_2OH	CH_2OH	H	100-102	463.45	$C_{22}H_{21}N_7O_5$	C, H, N
14c	3	COOH	COOH	H	180-182	491.41	$C_{22}H_{17}N_7O_7$	C, H, N
14d	3	COOH	H	H	234-235	447.40	$C_{21}H_{17}N_7O_5$	C, H, N
14e	3	COONa	COONa	H	>300	535.38	$C_{22}H_{15}N_7Na_2O_7$	C, H, N
14f	3	COOEt	COOEt	H	158-160	547.52	$C_{26}H_{25}N_7O_7$	C, H, N

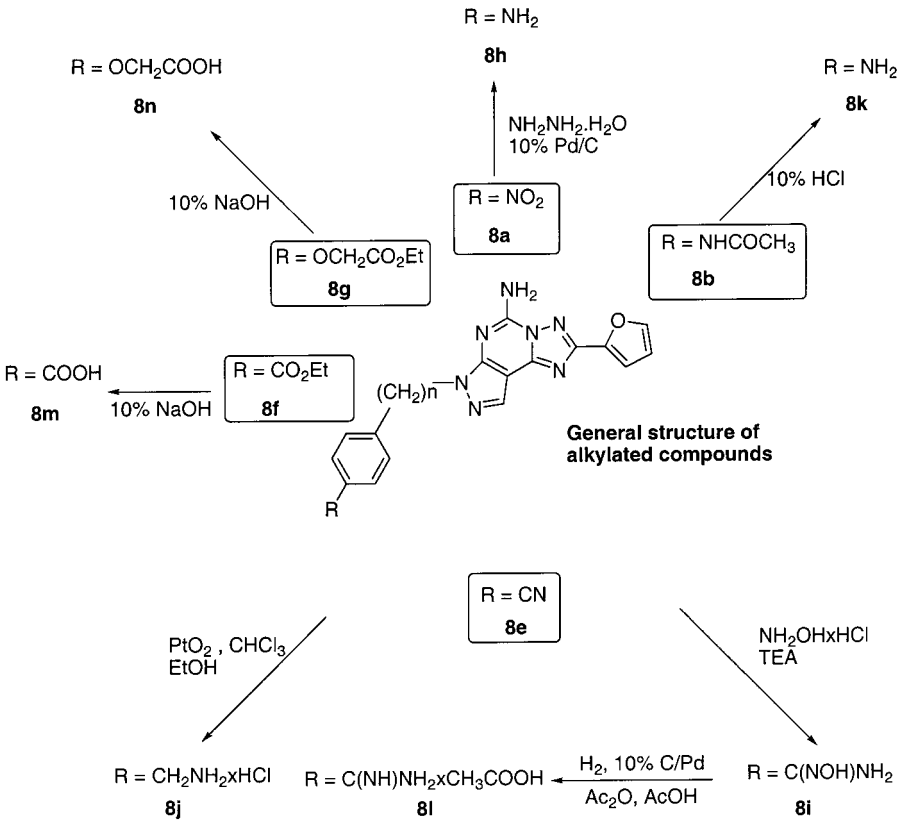
skeletons by chlorosulfonic acid to afford the corresponding derivatives **9** and **10**, respectively, in very good yield.[17] The latter could be easily transformed in the corresponding sulfonamido analogues **11b**−**f** through the reaction with the appropriate amines and the sulfonic acids **11a** and **12** by hydrolysis (Scheme 5).

In a previous paper, we reported 5-amino-7-[3-[3,4-(methylenedioxy)phenyl]propyl]-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine (**5**), which was of interest for its affinity and selectivity at the human A_{2A} adenosine receptors (hA_{2A} K_i = 3.3 nM, hA_1/hA_{2A} ratio = 658, hA_3/hA_{2A} ratio > 3000) and for the presence of a biologically common substituent on the pyrazole ring.[9] For these reasons, we decided to modify this compound to improve its affinity and, in particular, its water solubility (R_m = 3.6). The goal was achieved by the introduction of a sulfonic moiety to furnish **13**. A further improvement was performed by the introduction of functions, such as carboxylic and alcoholic moieties, starting from the dihydroxy derivative **6**[9] by reaction with diethyl dibromomalonate and subsequent reduction to the alcohol or decarboxylation (**14a**−**e**) (Scheme 6).

Scheme 1



Scheme 2

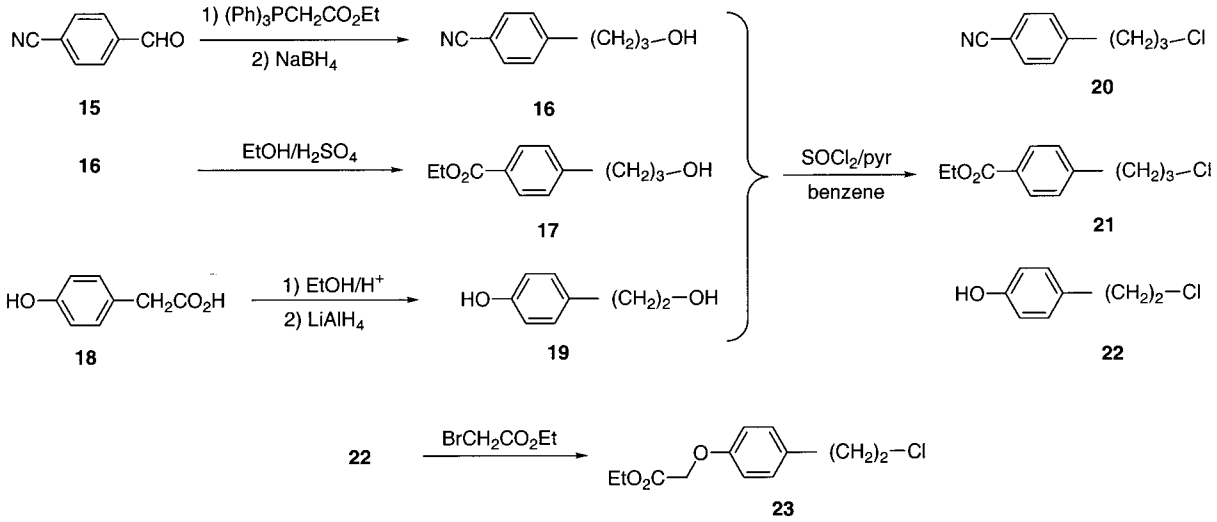


Results and Discussion

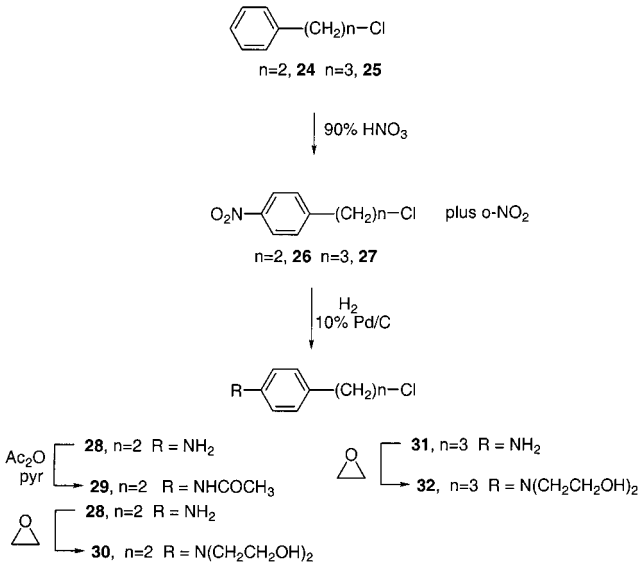
The binding data of the new series of pyrazolotriazolopyrimidines and their water solubility measured as R_m values are reported in Tables 2 and 3, respectively. Table 2 summarizes the receptor binding affinities of **8a**−**n**, **11a**−**f**, **12**, **13**, and **14a**−**e** determined at the human A_1, A_{2A}, A_{2B}, and A_3 receptors expressed in CHO (A_1, A_3) or HEK-293 (A_{2A}, A_{2B}) cells. [^{3}H]-1,3-Dipropyl-8-cyclopentyl xanthine ([^{3}H]-DPCPX)[18,19] (A_1 and A_{2B}), [^{3}H]-5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4triazolo[1,5-*c*]pyrimidine ([^{3}H]-SCH 58261) (A_{2A}),[20] and [^{3}H]-5-(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine ([^{3}H]-MRE3008-F20) (A_3)[19] have been used as radioligands in binding assays.

All the new compounds show a good affinity and selectivity for hA_{2A} adenosine receptor with a different degree of selectivity versus hA_1. In contrast, all the synthesized compounds resulted to be almost inactive or inactive in binding hA_{2B} and hA_3 receptors. This

Scheme 3



Scheme 4

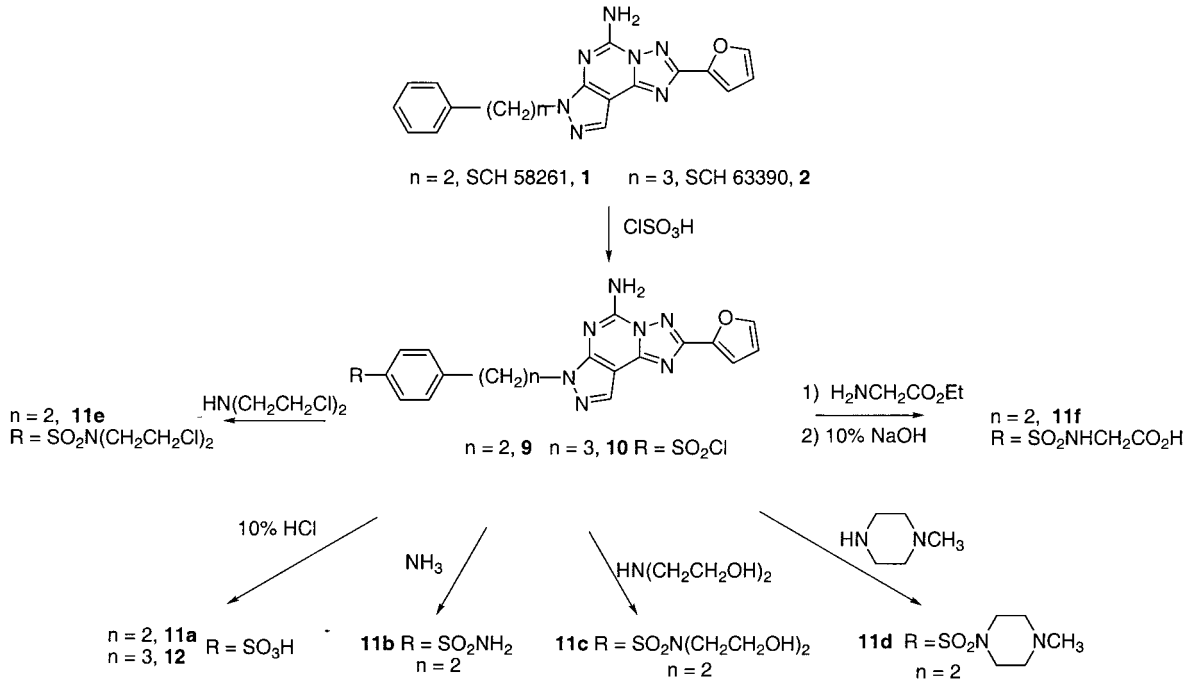


experimental observation further validates the efficiency and selectivity of pyrazolotriazolopyrimidine derivatives in comparison to the structurally related reference compound CGS 15943 (5-amino-9-chloro-2-(2-furyl)-[1,2,4]triazolo[1,5-*c*]quinazoline), which turned out to be nonselective showing binding in the nanomolar range of hA_1 (K_i = 4.4 nM), hA_{2A} (K_i = 0.43 nM), hA_{2B} (K_i = 23 nM), and hA_3 (K_i = 85 nM) receptors.[21] This finding strongly supports, as previously observed,[21] that the pyrazole nitrogens also play a fundamental role in receptor recognition and discrimination and that the substitutions on the pyrazolotriazolopyrimidine nucleus modulate affinity and selectivity vs adenosine receptor subtypes.

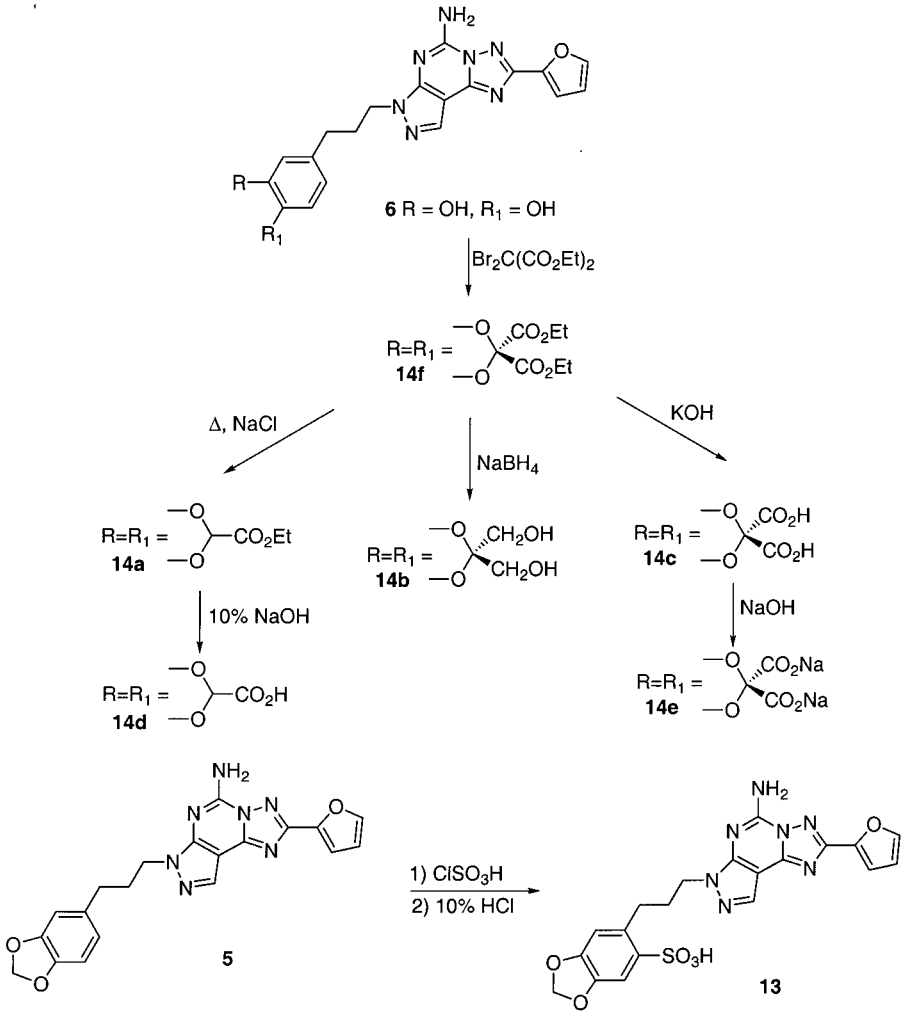
Unfortunately, our main goal, water solubility, was not completely achieved; in fact, the two sulfonic analogues **11a** and **12** was shown to be completely water-soluble, but a significant reduction of both the affinity and the selectivity was observed (**11a** hA_{2A} K_i = 100 nM; hA_1/hA_{2A} ratio = 1.9; R_m = 0.78; **12** hA_{2A} K_i = 140; hA_1/hA_{2A} ratio = 1; R_m = 0.77). The other sulfo derivatives (**11b**−**d**, **f**) are quite water-soluble, as indicated by the R_m values (Table 3). A good retention of the affinity for A_{2A} adenosine receptor was detected (**11b** hA_{2A} K_i = 1.31 nM; hA_1/hA_{2A} ratio = 2000; R_m = 2.5; **11c** hA_{2A} K_i = 0.8 nM; hA_1/hA_{2A} ratio = 3118, R_m = 2.7). These findings were not so surprisingly in view of our hypothesis previously reported.[9] Recently, on the basis of the thermodynamic studies, we hypothesized the presence of a lipophilic pocket where the side chain can take place and be able to form a hydrogen bond. Probably the lipophilic properties of this pocket hampered the adjustment of the substituent, possibly due to the anionic form of the sulfonic moieties at the physiological pH. The parameters of water solubility and receptor affinity suggest that compounds **11b** and **c** are the best compounds in terms of human A_{2A} affinity in the series of the sulfonic derivatives. Moreover, they support the hypothesis of a hydrogen bond, during the adjustment of the side chain in this pocket. In this family of compounds, **11e** was prepared in analogy with nitrogen mustards with the aim to obtain an irreversible A_{2A} antagonist to more deeply investigate the biochemical characteristics of this receptor subtype.[22] Surprisingly, **11e** showed a reversible antagonism similar to that of the other reported compounds, displaying an interesting affinity and selectivity for the human A_{2A} adenosine receptor (**11e** hA_{2A} K_i = 0.59 nM; hA_1/hA_{2A} ratio = 8977; R_m = 3.8), although the water solubility, measured by the R_m value, was shown to be poor as expected.

To retain the water solubility of **11a** and **12** and to restore the affinity and selectivity for the A_{2A} receptor subtype, a weaker acidic function on the phenyl ring, such as the carboxylic moiety or its ethyl ester, was introduced. The affinity of the **8m** for human A_{2A} adenosine receptor was acceptable and comparable to the corresponding unsubstituted reference compound SCH 63390 (**2**), but the water solubility was lower than that of the sulfonic derivative **12**, although the R_m value was better than that of reference compound **2** (**8m** hA_{2A} K_i = 4.4 nM; hA_1/hA_{2A} = 1128; R_m = 2.6; SCH 63390, **2**, hA_{2A} K_i = 1.2 nM; hA_1/hA_{2A} = 291; R_m = 3.6; **12** hA_{2A} K_i = 140 nM; hA_1/hA_{2A} = 1; R_m = 0.77). Moreover, the carboxylic function, when introduced on the skeleton of **5**, was successful in terms of the water solubility as shown by the R_m values of its analogues, i.e., the dicarboxylic derivative **14c** (R_m = 0.18) and its sodium salt **14e** (R_m = 0.16), but the affinity for the human A_{2A}

Scheme 5



Scheme 6



adenosine receptor strongly decreased (**14c** hA_{2A} K_i = 120 nM; hA_1/hA_{2A} = 78; **14e** hA_{2A} K_i = 120 nM; hA_1/hA_{2A} = 79), displaying K_i values comparable to the sulfonic derivatives **11a** and **12**. The monocarboxylated compound **14d** and mainly its ethyl ester **14a** showed a good affinity for human A_{2A} adenosine receptor, but

Table 2. Binding Affinity at hA_1, hA_{2A}, hA_{2B}, and hA_3 Adenosine Receptors of Synthesized Compounds

	K_i (nM)						
compd	hA_1[a]	hA_{2A}[b]	hA_{2B}[c]	hA_3[d]	hA_1/hA_{2A}	hA_{2B}/hA_{2A}	hA_3/hA_{2A}
1 SCH 58261	549 (322−987)	1.1 (0.75−1.6)	>10 000	>10 000	499	>9 090	>9 090
2 SCH 63390	350 (332−370)	1.2 (1.03−1.4)	>10 000	>10 000	291	>8 333	>8 333
8a	1026 (785−1341)	1.00 (0.94−1.27)	>10 000	>10 000	1026	>10 000	>10 000
8b	419 (374−470)	4.8 (4.58−5.03)	>10 000	>10 000	87	>2 083	>2 083
8c	558 (467−667)	1.1 (0.9−1.3)	>10 000	>10 000	507	>9 090	>9 090
8d	123 (98−154)	0.12 (0.1−0.16)	>10 000	>10 000	1025	≫10 000	≫10 000
8e	6496 (6024−7006)	86 (77−96)	>10 000	>10 000	75.5	>116	>116
8f	4494 (4040−5000)	4.0 (3.6−4.5)	>10 000	>10 000	1123	>2 500	>2 500
8g	4197 (3844−4581)	0.43 (0.4−0.47)	>10 000	>10 000	9760	≫10 000	≫10 000
8h	2160 (1816−2569)	0.22 (0.16−0.31)	>10 000	>10 000	9818	≫10 000	≫10 000
8i	179 (146−220)	0.18 (0.15−0.22)	>10 000	>10 000	994	≫10 000	≫10 000
8j	60 (55−70)	6.0 (5.2−7.6)	>10 000	>10 000	10	>1 666	>1 666
8k	576 (532−625)	0.13 (0.10−0.17)	>10 000	>10 000	4430	≫10 000	≫10 000
8l	75 (66−85)	55 (49−61)	>10 000	>10 000	1.36	>181	>181
8m	4965 (4691−5255)	4.4 (4.1−4.7)	>10 000	>10 000	1128	>2 272	>2 272
8n	4927 (4392−5528)	4.63 (4.28−5.01)	>10 000	>10 000	1064	>2 160	>2 160
11a	190 (172−209)	100 (89−112)	>10 000	>10 000	1.9	>100	>100
11b	2630 (2366−2924)	1.31 (1.18−1.43)	>10 000	>10 000	2 007	>7 633	>7 633
11c	2495 (2153−2891)	0.80 (0.69−0.92)	>10 000	>10 000	3 118	≫10 000	≫10 000
11d	369 (324−420)	3.8 (3.4−4.2)	>10 000	>10 000	97	>2 631	>2 631
11e	5297 (4943−5678)	0.59 (0.49−0.70)	>10 000	>10 000	8 977	≫10 000	≫10 000
11f	9330 (8783−9911)	50.0 (48.9−52.4)	>10 000	>10 000	186	>200	>200
12	139 (107−181)	140 (129−152)	>10 000	>10 000	1	>714	>714
13	6392 (5697−7171)	75 (66−85)	>10 000	>10 000	85	>133	>133
14a	1793 (1460−2201)	5.48 (4.95−6.08)	>10 000	>10 000	327	>1 824	>1 824
14b	432 (363−514)	0.19 (0.16−0.21)	>10 000	>10 000	2 273	≫10 000	≫10 000
14c	9399 (9038−9773)	120 (98−144)	>10 000	>10 000	78	>83	>83
14d	3599 (3420−3788)	59 (49−72)	>10 000	>10 000	61	>169	>169
14e	9533 (9257−9817)	120 (98−144)	>10 000	>10 000	79	>83	>83

[a] Displacement of specific [^{3}H]-DPCPX binding at human A_1 receptors expressed in CHO cells (n = 3−6). [b] Displacement of specific [^{3}H]SCH 58261 binding at human A_{2A} receptors expressed in HEK-293 cells. [c] Displacement of specific [^{3}H]-DPCPX binding at human A_{2B} receptors expressed in HEK-293 cells (n = 3−6). [d] Displacement of specific [^{3}H]-MRE3008-F20 binding at human A_3 receptors expressed in CHO cells. Data are expressed as geometric means, with 95% confidence limits.

Table 3. R_m Values of Synthesized Compounds Measured at pH 7.0 by TLC in Methanol−Water System

compd	R_m (0)[a]	compd	R_m (0)[a]
8a	3.9 ± 0.2	**11a**	0.78 ± 0.1
8b	2.7 ± 0.2	**11b**	2.5 ± 0.1
8c	2.4 ± 0.2	**11c**	2.7 ± 0.2
8d	2.8 ± 0.2	**11d**	1.9 ± 0.2
8e	2.9 ± 0.2	**11e**	3.8 ± 0.1
8f	3.0 ± 0.1	**11f**	2.2 ± 0.2
8g	3.3 ± 0.1	**12**	0.77 ± 0.2
8h	3.4 ± 0.2	**13**	1.2 ± 0.1
8i	2.8 ± 0.2	**14a**	3.4 ± 0.2
8j	2.5 ± 0.2	**14b**	2.7 ± 0.1
8k	3.6 ± 0.2	**14c**	0.18 ± 0.1
8l	2.9 ± 0.2	**14d**	2.1 ± 0.1
8m	2.6 ± 0.2	**14e**	0.16 ± 0.1
8n	2.1 ± 0.2		

[a] The R_m values were measured with a mobile phase of different concentrations of CH_3OH/H_2O. R_m values are reported as theoretical at 0% organic solvent in the mobile phase (R_m(0)).

their R_m values were not encouraging (**14d** hA_{2A} K_i = 59 nM, hA_1/hA_{2A} = 61; R_m = 2.1; **14a** hA_{2A} K_i = 5.48 nM; hA_1/hA_{2A} = 327; R_m = 3.4). On the whole, none of these derivatives showed an interesting profile at the human A_{2A} adenosine receptor, strongly supporting the negative influence of acidic moieties for the hA_{2A} affinity, which seems strictly correlated to the nature of the function on the phenyl ring. Better results were obtained with the corresponding dihydroxy derivative **14b**, which shows again a good affinity but lower water solubility (**14b** hA_{2A} K_i = 0.19 nM; hA_1/hA_{2A} ratio = 2,273; R_m = 2.7). Also in this case, we tried to introduce the sulfonic moiety on **5** but, as expected, both the affinity and the selectivity at the human A_{2A} adenosine receptor were significantly reduced (**13** hA_{2A} K_i = 75 nM; hA_1/hA_{2A} ratio = 85; R_m = 1.2).

The cyano derivative **8e** showed a poor affinity for the A_{2A} receptor subtype, but it was a useful key-intermediate for obtaining the aminomethyl analogue **8j**, the N-hydroxyamidine **8i** and the amidine **8l**, which showed a restored affinity for this receptor subtype. In particular, the compound **8i** displayed a good affinity for the A_{2A} receptor and a better water solubility with respect to the reference compound SCH 63390 (**2**) (**8i** hA_{2A} K_i = 0.18 nM hA_1/hA_{2A} ratio = 994; R_m = 2.8; SCH 63390, **2**, R_m = 3.6).

Considering the importance of a moiety on the phenyl ring of the side chain capable of a hydrogen bond, we also investigated the modification of the hydroxy group of **3** previously described.[9] In particular, we synthesized **8g** and **8n** structurally related to the selective A_{2A} agonist CGS 21680 (2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamido adenosine).[23] **8g** showed a high affinity but a low water solubility differently from the derivative **8n**, which had exactly an opposite behavior, confirming a common trend (**8g** hA_{2A} K_i = 0.43 nM; hA_1/hA_{2A} ratio = 9760; R_m = 3.3; **8n** hA_{2A} K_i = 4.63 nM; hA_1/hA_{2A} ratio = 1064; R_m = 2.1).

Finally, we decided to investigate the modification of the amino group on the phenyl ring, with the aim to obtain soluble salts, even though the basicity of an aromatic amino group is not significantly high. This choice was fruitful mainly in regards of affinity for the adenosine A_{2A} receptor subtype, as shown in Table 2. The two derivatives **8h** and **8k** possess high affinity and selectivity for the human A_{2A} adenosine receptor, even

though the water solubility was not significantly increased (**8h** hA_{2A} $K_i = 0.22$ nM; hA_1/hA_{2A} ratio = 9818; $R_m = 3.4$; **8k** hA_{2A} $K_i = 0.13$ nM; $hA_1/hA_{2A} = 4430$; $R_m = 3.6$). However, from these results, the latter compounds appear to be more promising for further pharmacological studies, possibly as prodrugs.

Conclusions

In conclusion, the present study confirmed the importance of some common structural parameters required to maintain affinity and selectivity for the human A_{2A} adenosine receptor in the SCH 58261 series. Therefore, the series of compounds (retaining the pyrazolotriazolopyrimidine nucleus, a free amino group at the 5-position, the furan ring, and the substituent at the 7-position) still displays good affinity and selectivity for this receptor subtype. Unfortunately, our rationale of designing a new compound endowed with both water solubility and affinity was not achieved, which can be explained in part by Lipinski's rules.[24] Anyway, the study herein presented provides new useful information about the structural requirements necessary for A_{2A} antagonist receptor recognition.

Experimental Section

Chemistry. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) and visualized with iodine or aqueous potassium permanganate. Infrared spectra (IR) were measured on a Perkin-Elmer 257 instruments. ^{1}H NMR were determined in $CDCl_3$ or DMSO-d_6 solutions with a Bruker AC 200 spectrometer; peaks 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 with Merck 230−400 mesh silica gel. All products reported showed IR and ^{1}H NMR spectra in agreement with the assigned structures. 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.

Synthesis of 3-(4-Cyanophenyl)propanol (16). 4-Cyanobenzaldehyde (**15**, 5 g, 0.038 mol) and triphenylphosphoranylidene acetic acid ethyl ester (16 g, 0.045 mol) were dissolved in dry benzene (70 mL), and the solution was stirred at room temperature for 12 h. Then the solvent was removed under vacuum, and the residue was taken up with light petroleum; the solid that formed was removed by filtration, and the organic phase was evaporated to give 3-(4-cyanophenyl)-3-propenoic acid ethyl ester as a yellow oil. The oil (4.59 g, 0.022 mol), without any further purification, was dissolved in absolute ethanol (100 mL) and added dropwise to a suspension of sodium borohydride (8.55 g, 0.22 mol) in ethanol (100 mL) cooled at 0 °C. The mixture was allowed to reach room temperature, and stirring was maintained for 18 h after which time the mixture was poured onto water and extracted with ethyl acetate several times. The combined organic solutions were washed twice with aqueous 2 N HCl (20 mL) and finally once with water (30 mL). The organic phase was dried, and subsequent evaporation of the solvent gave **16** as a light yellow oil (3.22 g, 53% yield from **15**). ^{1}H NMR ($CDCl_3$): 1.86−1.93 (m, 2H), 2.76 (t, 2H, $J = 8$), 3.65−3.71 (m, 2H), 7.24−7.28 (m, 3H), 7.96 (d, 2H, $J = 8$). IR (neat): 3403, 2228, 1607. Anal. ($C_{10}H_{11}NO$) C, H, N.

Synthesis of Ethyl 4-(3-Hydroxypropyl)benzoate (17). A stirred solution of **16** (0.2 g, 1.2 mmol) in 5 mL of 95% ethanol and concentrated H_2SO_4 (30 μL) was refluxed for 24 h. Workup consisted of adding CH_2Cl_2 (5 mL) and water (3 mL) to the cooled reaction mixture and separating the layers. The organic layer was washed with water, 5% aqueous $NaHCO_3$ solution, and saturated NaCl solution; dried; and evaporated to obtain **17** as an oil (0.19 g, 76% yield). ^{1}H NMR ($CDCl_3$): 1.39 (t, 3H, $J = 8$), 2.06−2.13 (m, 2H), 2.80−2.88 (m, 2H), 3.31 (t, 2H, $J = 6$), 4.36 (q, 2H, $J = 8$), 7.25−7.29 (m, 3H), 7.97 (d, 2H, $J = 6$). IR (neat): 3415, 1715. Anal. ($C_{12}H_{16}O_3$) C, H.

Synthesis of 2-(4-Hydroxyphenyl)ethanol (19). 4-Hydroxyphenylacetic acid (**18**, 5 g, 0.032 mol) was refluxed in ethanol (50 mL) in the presence of H_2SO_4 (50 μL) for 3 h, then the solvent was removed, and the residue (5.58 g, 0.031 mol) was dissolved in dry THF (15 mL). This solution was added dropwise to a suspension of $LiAlH_4$ (1.76 g, 0.046 mol) in dry THF (30 mL) cooled at 0 °C. The mixture was allowed to reach room temperature and was stirred for 3 h. The solution was cooled again to 0 °C, and water was added slowly to precipitate salts that were filtered off. The filtrate was dried and evaporated to furnish the desired compound as an oil (4.2 g, 95% yield). ^{1}H NMR ($CDCl_3$): 2.74 (t, 2H, $J = 8$), 3.79 (bs, 1H), 3.86 (t, 2H, $J = 8$), 6.68 (d, 2H, $J = 6$), 6.95 (d, 2H, $J = 6$), 10.51 (bs, 1H). IR (neat): 3435. Anal. ($C_8H_{10}O_2$) C, H.

General Procedure for the Synthesis of Chloro Derivatives (20−22). A solution of alcohol (0.066 mol) and pyridine (5.5 mL) in dry benzene (40 mL) cooled at 0 °C was added dropwise of a solution of thionyl chloride (9.5 mL) in dry benzene (20 mL) and stirred at room temperature for 2 h. At the end of this time, the mixture was cooled again at 0 °C, and water (50 mL) was added. The organic layer was first washed twice with saturated $NaHCO_3$ (30 mL), then dried, and evaporated. The residue was purified by flash chromatography (EtOAc/light petroleum 20%).

3-(4-Cyanophenyl)propyl Chloride (20). 89% yield; ^{1}H NMR ($CDCl_3$): 2.05−2.15 (m, 2H), 2.84 (t, 2H, $J = 7$), 3.52 (t, 2H, $J = 7$), 7.32 (d, 2H, $J = 8$), 7.56 (d, 2H, $J = 8$). IR (neat): 2261. Anal. ($C_{10}H_{10}ClN$) C, H, N.

Ethyl 4-(3-Chloropropyl)benzoate (21). 91% yield; ^{1}H NMR ($CDCl_3$): 1.38 (t, 3H, $J = 8$), 2.05−2.12 (m, 2H), 2.83 (t, 2H, $J = 8$), 3.51 (t, 2H, $J = 8$), 4.36 (q, 2H, $J = 8$), 7.27 (d, 2H, $J = 8$), 7.96 (d, 2H, $J = 8$). IR (neat): 1716. Anal. ($C_{12}H_{15}ClO_2$) C, H.

2-(4-Hydroxyphenyl)ethyl Chloride (22). 88% yield; ^{1}H NMR ($CDCl_3$): 3.01 (t, 2H, $J = 7$), 3.67 (t, 2H, $J = 7$), 6.86 (d, 2H, $J = 8$), 7.14 (d, 2H, $J = 8$), 10.3 (bs, 1H). IR (neat): 3355. Anal. (C_8H_9ClO) C, H.

Synthesis of Ethyl 4-(2-Chloroethyl)phenoxyacetate (23). Compound **22** (0.86 g, 5.5 mmol) and K_2CO_3 (0.76 g, 5.5 mmol) were suspended in dry DMF (10 mL), stirred at room temperature for 20 min, and then ethyl bromoacetate (0.6 mL, 5.5 mmol) was added. The mixture was stirred at room temperature for 12 h, then the solvent was removed under reduced pressure, and the residue was suspended in water (20 mL) and extracted with EtOAc (20 × 3 mL). The organic layer, dried and evaporated, afforded **23** as an oil (1.23 g, 92% yield). ^{1}H NMR ($CDCl_3$): 1.3 (t, 3H, $J = 7$), 3.0 (t, 2H, $J = 8$), 3.67 (t, 2H, $J = 8$), 4.26 (q, 2H, $J = 7$), 4.6 (s, 2H), 6.87 (d, 2H, $J = 8$), 7.14 (d, 2H, $J = 8$). IR (neat): 1752. Anal. ($C_{12}H_{15}ClO_3$) C, H.

Synthesis of 2-(4-Nitrophenyl)ethyl Chloride and 3-(4-Nitrophenyl)propyl Chloride (26, 27). The chloro derivatives **24** or **25** (0.076 mol) were cooled at 0 °C, and 90% HNO_3 (20 mL) was added dropwise, and the mixture was stirred at the same temperature for 20 min. Then water (100 mL) was added slowly and extracted with CH_2Cl_2 (40 × 3 mL). The organic layer was dried and evaporated. The residue was purified by flash chromatography (Et_2O/light petroleum 15%; 30% ortho and 70% para nitro derivatives).

2-(4-Nitrophenyl)ethyl Chloride (26). Yellow solid (68% yield), mp 44−45 °C. ^{1}H NMR ($CDCl_3$): 3.21 (t, 2H, $J = 7$), 3.8 (t, 2H, $J = 7$), 7.42 (d, 2H, $J = 8$), 8.19 (t, 2H, $J = 8$). IR (neat): 1495, 1324, 839. Anal. ($C_8H_8ClNO_2$) C, H.

3-(4-Nitrophenyl)propyl Chloride (27). Yellow oil (67% yield); ^{1}H NMR ($CDCl_3$): 2.31−2.36 (m, 2H), 3.2 (t, 2H, $J = 7$), 3.81 (t, 2H, $J = 7$), 7.42 (d, 2H, $J = 8$), 8.22 (d, 2H, $J = 8$). IR (neat): 1505, 1335, 840. Anal. ($C_9H_{10}ClNO_2$) C, H.

Synthesis of 2-(4-Aminophenyl)ethyl Chloride (28). 2-(4-Nitrophenyl)ethyl chloride **26** (1.24 g, 6.7 mmol) in CH_3OH (30 mL) in the presence of 10% Pd/C (65 mg) was reducted in a Parr apparatus under pressure (40 psi) for 10 min. The catalyst was removed by filtration on a pad of Celite, and the filtrate was evaporated to give **28** (1 g, 96% yield) as an oil, which was used in the next step without further purification.

Synthesis of 2-[(4-Acetylamino)phenyl]ethyl Chloride (29). The 2-(4-aminophenyl)ethyl chloride **28** (1 g, 6.45 mmol) in pyridine (20 mL) and acetic anhydride (2.45 mL, 25.8 mmol) were stirred at room temperature for 24 h. Then, the solvent was removed under reduced pressure, and the residue was suspended in water (70 mL) and extracted with EtOAc (20 × 3 mL). The organic phase, dried and evaporated, furnished the compound as white solid (0.8 g, 68% yield); mp 117−119 °C. ^{1}H NMR ($CDCl_3$): 2.16 (s, 3H), 3.02 (t, 2H, J = 7), 3.68 (t, 2H, J = 7), 7.16 (d, 2H, J = 8), 7.43 (bs, 1H), 7.47 (d, 2H, J = 8). IR (KBr): 3425, 1654. Anal. ($C_{10}H_{12}ClO$) C, H.

Synthesis of 2-[(4-*N*,*N*-Bis-*β*-hydroxyethylamino)phenyl]ethyl Chloride (30). To a suspension of 2-(4-aminophenyl)-ethyl chloride **28** (1 g, 6.4 mmol) in 25% CH_3COOH (15 mL), ethylene oxide (3.6 mL) was added with swirling. The mixture was stirred at room temperature for 24 h, after which the clear solution was made slightly basic with $NaHCO_3$ and extracted with EtOAc (30 × 3 mL). After being dried, the solvent was removed in a vacuum to afford **30** as an oil (1.48 g, 95% yield). ^{1}H NMR ($CDCl_3$): 2.95 (t, 2H, J = 8), 3.52 (t, 4H, J = 4), 3.65 (t, 2H, J = 8), 3.79 (t, 4H, J = 4), 4.05 (bs, 2H), 6.61 (d, 2H, J = 8), 7.1 (d, 2H, J = 8). IR (neat): 3413, 786. Anal. ($C_{12}H_{18}ClNO_2$) C, H, N.

Synthesis of 3-(4-Aminophenyl)propyl Chloride (31). 3-(4-Nitrophenyl)propyl chloride **27** (1 g, 5 mmol) in CH_3OH (25 mL), in the presence of 10% Pd/C (50 mg), was reducted in a Parr apparatus under pressure (40 psi) for 10 min. The catalyst was removed by filtration on a pad of Celite, and the filtrate was evaporated to give the compound as an oil, which was used in the next step without further purification (0.82 g, 97% yield).

Synthesis of 3-[(4-*N*,*N*-Bis-*β*-hydroxyethylamino)phenyl]propyl Chloride (32). To a suspension of 3-(4-aminophenyl)propyl chloride **31** (0.5 g 2.9 mmol) in 25% CH_3COOH (7 mL), ethylene oxide (1.8 mL) was added with swirling. The mixture was stirred at room temperature for 24 h, after which the clear solution was made slightly basic with $NaHCO_3$ and extracted with EtOAc (15 × 3 mL). After being dried, the solvent was removed in a vacuum to afford **32** as an oil (0.69 g, 92% yield). ^{1}H NMR ($CDCl_3$): 1.98−2.06 (m, 2H), 2.67 (t, 2H, J = 8), 3.48−3.55 (m, 6H), 3.79 (t, 4H, J = 4), 3.91 (bs, 2H), 6.62 (d, 2H, J = 8), 7.5 (d, 2H, J = 8). IR (neat): 3388, 851. Anal. ($C_{13}H_{20}ClNO_2$) C, H, N.

General Procedure for Alkylation of 2-Furan-2-yl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-ylamine (8a−g). To a mixture of **7**[8] (100 mg, 0.41 mmol) and K_2CO_3 (62 mg, 0.45 mol) in dry DMF (10 mL), the opportune halide (0.45 mmol) was added, and the mixture was heated at 100 °C for 6−8 h. Then the solvent was removed under reduced pressure, and the residue was suspended in water (20 mL) and extracted with EtOAc (15 × 3 mL). The organic phase was dried, evaporated, and purified by flash chromatography (EtOAc/light petroleum 50−80%).

8a. Light yellow solid (56% yield); ^{1}H NMR (DMSO-d_6): 2.03−2.07 (m, 2H), 2.42 (t, 2H, J = 8), 4.23 (t, 2H, J = 8), 6.49 (d, 2H, J = 8), 6.72−6.74 (m, 1H), 6.88 (d, 2H, J = 8), 7.22 (d, 1H, J = 4), 7.94 (s, 1H), 8.09 (bs, 2H), 8.16 (s, 1H). IR (KBr): 3397, 1551, 1349, 723.

8b. White solid (61% yield); ^{1}H NMR (DMSO-d_6): 1.99 (s, 3H), 3.11 (t, 2H, J = 8), 4.45 (t, 2H, J = 8), 6.72−6.74 (m, 1H), 7.07 (d, 2H, J = 8), 7.22 (d, 1H, J = 4), 7.43 (d, 2H, J = 8), 7.94 (s, 1H), 8.09 (bs, 2H), 8.16 (s, 1H), 9.85 (bs, 1H). IR (KBr): 3423, 3354, 1655, 678.

8c. White solid (61% yield); ^{1}H NMR (DMSO-d_6): 2.05−2.09 (m, 2H), 2.46 (t, 2H, J = 8), 3.34 (t, 4H, J = 6), 3.49 (t, 4H, J = 6), 4.25 (t, 2H, J = 8), 4.72 (t, 2H, J = 6), 6.58 (d, 2H, J = 8), 6.72−6.74 (m, 1H), 6.98 (d, 2H, J = 8), 7.22 (d, 1H, J = 4), 7.94 (s, 1H), 8.08 (bs, 2H), 8.16 (s, 1H). IR (KBr): 3470, 3378, 729.

8d. White solid (63% yield); ^{1}H NMR (DMSO-d_6): 3.01 (t, 2H, J = 8), 3.31−3.35 (m, 4H), 3.47−3.49 (m, 4H), 4.39 (t, 2H, J = 8), 4.72 (bs, 2H), 6.56 (d, 2H, J = 8), 6.71−6.74 (m, 1H), 6.95 (d, 2H, J = 8), 7.22 (d, 1H, J = 4), 7.94 (s, 1H), 8.08 (bs, 2H), 8.16 (s, 1H). IR (KBr): 3451, 3354, 814.

8e. Off-white solid (65% yield); ^{1}H NMR (DMSO-d_6): 2.22−2.29 (m, 2H), 2.71 (t, 2H, J = 8), 4.33 (t, 2H, J = 8), 6.62−6.65 (m, 1H), 7.2 (d, 1H, J = 4), 7.38 (d, 2H, J = 8), 7.61 (d, 2H, J = 8), 7.7 (bs, 2H), 7.91 (s, 1H), 8.07 (s, 1H). IR (KBr): 3351, 2262, 842.

8f. Off-white solid (56% yield); ^{1}H NMR ($CDCl_3$): 1.37 (t, 3H, J = 7), 2.27−2.31 (m, 2H), 2.72 (t, 2H, J = 8), 4.33−4.42 (m, 4H), 5.93 (bs, 2H), 6.59−6.62 (m, 1H), 7.23−7.27 (m, 3H), 7.64 (s, 1H), 7.94 (d, 2H, J = 8), 8.21 (s, 1H). IR (KBr): 3346, 1758, 789.

8g. White solid (58% yield); ^{1}H NMR (DMSO-d_6): 3.1 (t, 2H, J = 8), 4.43 (t, 2H, J = 8), 4.71 (s, 2H), 6.71−6.72 (m, 1H), 6.79 (d, 2H, J = 8), 7.07 (d, 2H, J = 8), 7.21 (d, 1H, J = 4), 7.93 (s, 1H), 8.08 (bs, 2H), 8.15 (s, 1H). IR (KBr): 3458, 1747, 1112, 616.

Synthesis of 7-[3-(4-Aminophenyl)propyl]-2-furan-2-yl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-ylamine (8h). Compound **8a** (40 mg, 0.1 mmol) was suspended in dry dioxane (3 mL) and 10% Pd/C (3 mg) and hydrazine monohydrate (0.3 mL, 6.17 mmol) were added. The mixture was refluxed for 45 min, then the catalyst was removed by filtration over a bed of Celite, and the filtrate was dried and evaporated to afford the compound in quantitative yield as a white solid. ^{1}H NMR (DMSO-d_6): 2.03−2.07 (m, 2H), 2.42 (t, 2H, J = 8), 4.23 (t, 2H, J = 8), 4.84 (d, 2H, J = 4), 6.47 (d, 2H, J = 8), 6.72−6.74 (m, 1H), 6.86 (d, 2H, J = 8), 7.22 (d, 1H, J = 4), 7.94 (s, 1H), 8.09 (bs, 2H), 8.16 (s, 1H). IR (KBr): 3435, 3379, 757.

Synthesis of 7-[3-(4-Aminophenyl)ethyl]-2-furan-2-yl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-ylamine (8k). Compound **8b** (60 mg, 0.145 mmol) was dissolved in a mixture of dioxane (10 mL) and aqueous 10% HCl (8 mL) and refluxed for 2 h. The solution was concentrated under reduced pressure, the pH was adjusted around 7 with aqueous 5% NaOH, and it was extracted with EtOAc (10 × 3 mL). The organic layer was dried and evaporated to give the compound as a pale yellow solid (50 mg, 96% yield). ^{1}H NMR (DMSO-d_6): 2.97 (t, 2H, J = 8), 4.38 (t, 2H, J = 8), 4.86 (bs, 2H), 6.44 (d, 2H, J = 8), 6.72−6.74 (m, 1H), 6.62 (d, 2H, J = 8), 7.21 (d, 1H, J = 4), 7.94 (s, 1H), 8.04 (bs, 2H), 8.15 (s, 1H). IR (KBr): 3415, 3386, 754.

General Procedure for Synthesis of {4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)ethyl]phenoxy}acetic Acid (8n) and 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzoic Acid (8m). The ester, **8f** or **8g**, (0.082 mmol) was suspended in aqueous 10% NaOH (4 mL) and refluxed for 30 min. The solution was cooled, and the pH was adjusted to around 7 with aqueous 5% HCl. The precipitate was filtered and dried under vacuum.

8n. White solid (98% yield); ^{1}H NMR (DMSO-d_6): 3.1 (t, 2H, J = 8), 4.44 (t, 2H, J = 8), 4.58 (s, 2H), 6.72−6.74 (m, 1H), 6.78 (d, 2H, J = 8), 7.08 (d, 2H, J = 8), 7.22 (d, 1H, J = 4), 7.94 (s, 1H), 8.08 (bs, 2H), 8.16 (s, 1H), 13.01 (bs, 1H). IR (KBr): 3437, 1702, 616.

8m. White solid (97% yield); ^{1}H NMR (DMSO-d_6): 2.27−2.31 (m, 2H), 2.73 (t, 2H, J = 8), 4.32 (t, 2H, J = 8), 6.59−6.72 (m, 1H), 7.23−7.27 (m, 3H), 7.64 (s, 1H), 7.94 (d, 2H, J = 8), 8.07 (bs, 2H), 8.15 (s, 1H), 12.56 (bs, 1H). IR (KBr): 3478, 1712, 658.

Synthesis of 7-[3-(4-Aminomethylphenyl)propyl]-2-furan-2-yl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-ylamine Hydrochloride (8j). The hydrogenation of **8e** (10 mg, 0.026 mmol), dissolved in EtOH (20 mL) and $CHCl_3$ (5 mL), was carried out on a Parr apparatus (PtO_2 0.65 mg, 45 psi, 2.5 h) followed by filtration through Celite and evaporation of the solvent under reduced pressure. The residue

was crystallized from MeOH to give the compound as a white solid (95% yield). ^{1}H NMR (DMSO-d_6): 2.08−2.16 (m, 2H), 2.61 (t, 2H, J = 8), 3.95 (bs, 2H), 4.27 (t, 2H, J = 8), 6.72−6.74 (m, 1H), 7.21−7.22 (m, 1H), 7.24 (d, 2H, J = 8), 7.38 (d, 2H, J = 8), 7.95 (s, 1H), 8.1 (bs, 2H), 8.16 (s, 1H), 8.39 (bs, 3H). IR (KBr): 3402, 2924, 1596, 764.

Synthesis of 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)ethyl]-*N*-hydroxybenzamidine (8i). A suspension of **8e** (20 mg, 0.052 mmol), hydroxylamine hydrochloride (18 mg, 0.26 mmol), and triethylamine (0.036 mL, 0.26 mmol) in methanol (3 mL) was stirred with heating at 50 °C for 18 h. The right compound was filtered after precipitation from the cooled mixture as a white solid (MeOH, 96% yield). ^{1}H NMR (DMSO-d_6): 2.12−2.21 (m, 2H), 2.61 (t, 2H, J = 8), 4.27 (t, 2H, J = 8), 5.76 (bs, 2H), 6.72−6.74 (m, 1H), 7.2−7.24 (m, 3H), 7.58 (d, 2H, J = 8), 7.94 (s, 1H), 8.08 (bs, 2H), 8.16 (s, 1H), 9.56 (s, 1H). IR (KBr): 3457, 3245, 1678, 689.

Synthesis of 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzamidine Acetic Acid Salt (8l). To a solution of **8i** (20 mg, 0.048 mmol) in acetic acid (10 mL), acetic anhydride (5 μL, 1.1 equiv) and 10% Pd/C (0.5 mg) were added, and the mixture was poured in a Parr apparatus (40 psi, 24 h). Then the catalyst was removed by filtration through a bed of Celite, and the filtrate was evaporated under reduced pressure to afford the desired compound as a white solid (MeOH, 69% yield). ^{1}H NMR (DMSO d_6): 1.81 (s, 3H), 2.01−2.03 (m, 2H), 2.5 (t, 2H, J = 8), 3.39 (bs, 1H), 4.18−4.28 (m, 3H), 6.72−6.74 (m, 1H), 7.22 (d, 1H, J = 4), 7.44 (d, 2H, J = 8), 7.71−7.73 (m, 4H), 7.94 (s, 1H), 8.19 (bs, 3H). IR (KBr): 3342, 2989, 1678, 685.

General Procedure for Synthesis of 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)ethyl]benzenesulfonyl Chloride (9) and 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzenesulfonyl Chloride (10). The tricyclic derivative, SCH 58261 **1** or SCH 63390 **2** (0.35 mmol), was added in small portions to the chlorosulfonic acid (2 mL) cooled at 0 °C. The mixture was stirred at this temperature for 1 h, and then water (15 mL) was added carefully keeping the temperature at 0 °C. The white precipitate was filtered off and immediately used for the next step (98% yield).

General Procedure for Synthesis of 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)ethyl]benzenesulfonic acid (11a) and 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzenesulfonic Acid (12). To a solution of the chlorosulfonyl derivative, **9** or **10**, (0.32 mmol) in dioxane (2 mL), aqueous 10% HCl (2 mL) was added. The mixture was stirred at room temperature for 2 days. Then the solvent was removed under reduced pressure, and the residue was taken up several times with benzene and methanol. **11a** or **12** were purified by preparative HPLC in 25 min at a flow rate of 30 mL/min (reversed phase column; gradient elution 0−60% solvent B, where solvent A is 10% (v/v) acetonitrile in 0.1% TFA and solvent B is 60% (v/v) acetonitrile in 0.1% TFA).

11a. White solid (60% yield); ^{1}H NMR (D_2O): 3.01 (t, 2H, J = 6), 4.14 (t, 2H, J = 6), 6.36−6.37 (m, 1H), 6.75−6.76 (m, 1H), 7.12 (d, 2H, J = 8), 7.39 (s, 1H), 7.58 (d, 2H, J = 8), 7.69 (s, 1H). IR (KBr): 3040, 1667, 1350, 1165.

12. White solid (65% yield); ^{1}H NMR (D_2O): 2.19−2.22 (m, 2H), 2.66 (t, 2H, J = 6), 4.16 (t, 2H, J = 6), 6.64−6.65 (m, 1H), 7.09−7.11 (m, 1H), 7.32 (d, 2H, J = 8), 7.68 (d, 2H, J = 8), 7.71 (s, 1H), 8.0 (s, 1H). IR (KBr): 3050, 1667, 1352, 1160.

Synthesis of 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)ethyl]benzenesulfonamide (11b). In a solution of the crude chlorosulfonyl derivative **9** (0.338 mmol) in dry dioxane (20 mL) cooled at 0 °C was bubbled NH_3 till saturation of the solvent. The precipitate that formed was filtered off, and the filtrate was purified by flash chromatography (EtOAc 100%) to afford **11b** as a white solid (yield 63%). ^{1}H NMR (DMSO-d_6): 3.27−3.39 (m, 4H), 4.53 (t, 2H, J = 6), 6.71−6.73 (m, 1H), 7.21−7.23 (m, 1H), 7.35 (d, 2H, J = 8), 7.69 (d, 2H, J = 8), 7.94 (bs, 3H), 8.15 (s, 1H). IR (KBr): 3339, 1667, 1330, 1157.

Synthesis of 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)ethyl]-*N*,*N*-bis-(2-hydroxyethyl)benzenesulfonamide (11c). The diethanolamine (47 mg, 0.45 mmol) was added to a solution of the crude **9** (0.38 mmol) in dry dioxane (10 mL), and the mixture was stirred at room temperature for 3 h. Then the solvent was removed under reduced pressure, and the residue was purified by flash chromatography (EtOAc 100%) to furnish **11c** as a white solid (65% yield). ^{1}H NMR (DMSO-d_6): 3.04 (t, 4H, J = 6), 3.27 (t, 2H, J = 6.6), 3.41−3.50 (m, 4H), 4.54 (t, 2H, J = 6.6), 4.81 (t, 2H, J = 6), 6.72−6.73 (m, 1H), 7.21 (d, 1H, J = 4), 7.35 (d, 2H, J = 8), 7.64 (d, 2H, J = 8), 7.94 (s, 1H), 8.08 (bs, 2H), 8.16 (s,1H). IR (KBr): 3200, 1667, 1340, 1160.

Synthesis of 2-Furan-2-yl-7-{3-[4-(4-methylpiperazine-1-sulfonyl)phenylethyl}-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo-[1,5-*c*]pyrimidin-5-ylamine (11d). The crude **9** (0.52 mmol) in dry dioxane (10 mL) at 0 °C was added to 1-methylpiperazine (104 mg, 1.04 mmol, 2 equiv), and the mixture was stirred at room temperature for 2 h. Then the solvent was removed, and the residue was purified by flash chromatography (EtOAc 100%) to give **11d** as a pale yellow solid (64% yield). ^{1}H NMR (DMSO-d_6): 2.24 (s, 3H), 2.39 (t, 4H, J = 4.2), 2.79 (t, 4H, J = 4.2), 3.3 (t, 2H, J = 6), 4.59 (t, 2H, J = 6), 6.62−6.63 (m, 1H), 7.17 (d, 1H, J = 3.2), 7.23 (d, 2H, J = 8), 7.5 (d, 2H, J = 8), 7.63 (bs, 2H), 7.93 (s, 1H), 8.08 (s, 1H). IR (KBr): 3265, 1667, 1350, 1160.

Synthesis of 4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)ethyl]-*N*,*N*-bis-(2-chloroethyl)benzenesulfonamide (11e). To a solution of crude **9** (0.38 mmol) and TEA (73 μL, 0.53 mmol, 1.4 equiv) in dry dioxane (10 mL) cooled at 0 °C, bis(2-chloroethyl)amine hydrochloride (80 mg, 0.45 mmol, 1.2 equiv) was added, and the mixture was stirred at room temperature for 3 h. Then the solvent was removed under vacuum, and the residue was purified by flash chromatography (EtOAc/light petroleum 50%) to afford the desired **11e** as an off-white solid (68% yield). ^{1}H NMR (DMSO-d_6): 3.32−3.41 (m, 6H), 3.64 (t, 4H, J = 6.8), 4.54 (t, 2H, J = 6), 6.71−6.72 (m, 1H), 7.20 (d, 1H, J = 3), 7.34 (d, 2H, J = 8), 7.68 (d, 2H, J = 8), 7.93 (s, 1H), 8.05 (bs, 2H), 8.15 (s, 1H). IR (KBr): 3210, 1667, 1355, 1170.

Synthesis of {4-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzenesulfonylamino}acetic Acid (11f). A solution of crude **9** (0.5 mmol) in dry dioxane (10 mL) was added slowly to a solution of glycine ethyl ester hydrochloride (69 mg, 0.5 mmol) and TEA (0.2 mL, 1.5 mmol) in dry dioxane (4 mL), and the mixture was stirred at room temperature for 2 h. Then the solvent was removed, and the residue was dissolved in EtOAc (15 mL) and washed with saturated NaCl solution (2 × 5 mL). The organic phase was dried and evaporated. The residue was dissolved in a mixture of THF/CH_3OH/H_2O (4:1:1, 3 mL), and lithium hydroxide monohydrate (10.8 mg) was added. The mixture was stirred for 12 h at room temperature, and then it was washed with CH_2Cl_2 (2 × 3 mL). The aqueous layer was acidified to pH 3 with 10% HCl, and the precipitate was filtered off. The compound was crystallized (dioxane/Et_2O) to afford a pale yellow solid (63% yield, two steps). ^{1}H NMR (DMSO-d_6): 3.07 (t, 2H, J = 7), 3.49−3.51 (m, 3H), 4.52 (t,2H, J = 7), 6.72−6.74 (m, 1H), 7.21 (d, 1H, J = 3.6), 7.37 (d, 2H, J = 8), 7.67 (d, 2H, J = 8), 7.95 (bs, 2H), 8.1 (bs, 2H), 8.16 (s, 1H). IR (KBr): 3265, 1667, 1350, 1155.

Synthesis of 5-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzo[1,3]-dioxole 2,2-Dicarboxylic Acid Ethyl Ester (14f). To a suspension of **6** (100 mg, 0.25 mmol) and K_2CO_3 (120 mg, 3.5 equiv) in acetone (4 mL), diethyl dibromomalonate (80 mg, 1 equiv) in acetone (2 mL) was added dropwise, and the mixture was stirred at room temperature for 24 h. Then EtOAc (20 mL) was added and washed with water (2 × 10 mL); the organic phase was dried and evaporated under vacuum to furnish **14f** as off-white solid (72% yield; acetone/DMF). ^{1}H NMR ($CDCl_3$): 1.34 (t, 6H, J = 7), 2.19−2.28 (m, 2H), 2.57 (t,

2H, $J = 7.4$), 4.27 (t, 2H, $J = 7.4$), 4.35 (q, 4H, $J = 7$), 6.15 (bs, 2H), 6.59−6.61 (m, 1H), 6.7−6.86 (m, 3H), 7.24 (d, 1H, $J = 5$), 7.64 (s, 1H), 8.2 (s, 1H). IR (KBr): 3278, 1745, 677.

Synthesis of 5-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzo[1,3]-dioxole 2-Carboxylic Acid Ethyl Ester (14a). To a solution of **14f** (0.09 mmol) in DMSO (2 mL) and 1 drop of H_2O, NaCl (5 mg, 1 equiv) was added, and the mixture was heated at 160 °C for 12 h. Then water was added (20 mL), and the solution was extracted with EtOAc (4 × 10 mL). The organic phase was dried and evaporated under reduced pressure to give **14a**. Off-white solid (42% yield; EtOAc/light petroleum); ^{1}H NMR ($CDCl_3$): 1.34 (t, 3H, $J = 7$), 2.17−2.27 (m, 2H), 2.58 (t, 2H, $J = 8$), 4.31−4.37 (m, 4H), 6.03 (bs, 2H), 6.27 (s, 1H), 6.61−6.62 (m, 1H), 6.69−6.75 (m, 3H), 7.25 (d, 1H, $J = 3.4$), 7.63 (s, 1H), 8.2 (s, 1H). IR (KBr): 3220, 1745, 667.

Synthesis of 5-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzo[1,3]-dioxole 2,2-Dicarboxylic Acid (14c). To a solution of **14f** (50 mg, 0.09 mmol) in dioxane (5 mL), aqueous 2 N KOH (1 mL) was added, and the mixture was refluxed for 6 h. Then the pH was adjusted to around 3 with aqueous 10% HCl, and the solution was extracted with EtOAc (2 × 10 mL). The organic layer was dried and evaporated under reduced pressure to give **14c** as a pale yellow solid (89% yield; EtOAc/light petroleum). ^{1}H NMR (DMSO-d_6): 1.98−2.07 (m, 2H), 2.54 (t, 2H, $J = 6$), 3.52 (bbs, 2H), 4.28 (t, 2H, $J = 6$), 6.71−6.75 (m, 2H), 6.9−6.94 (m, 2H), 7.16 (d, 1H, $J = 4.2$), 7.94 (s, 1H), 8.15 (bs, 2H), 8.17 (s, 1H). IR (KBr): 3189, 1698, 677.

Synthesis of 5-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzo[1,3]-dioxole 2,2-Dicarboxylic Disodium Salt (14e). The compound **14c** (20 mg, 0.04 mmol) was dissolved in aqueous 0.04 M NaOH (2 mL), and the mixture was stirred at room temperature for 12 h. Then the solvent was removed in vacuo to afford **14e** as white solid (97% yield). ^{1}H NMR (D_2O): 2.01−2.11 (m, 2H), 2.45 (t, 2H, $J = 6$), 4.06 (t, 2H, $J = 6$), 6.62−6.72 (m, 4H), 7.06 (d, 1H, $J = 5$), 7.79 (s, 1H), 7.83 (s, 1H). IR (KBr): 2540−3350, 1640, 667.

Synthesis of 5-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzo[1,3]-dioxole 2-Carboxylic Acid (14d). Aqueous 1 N NaOH (2 mL) was added to a solution of **16a** (20 mg, 0.042 mmol) in THF (2 mL), and the mixture was refluxed for 30 min. Then the solution was cooled, and the pH was adjusted to around 3 with acqueous 1 N HCl and extracted with EtOAc (4 × 10 mL). The organic layer was dried and evaporated under reduced pressure to afford **14d**. Pale yellow solid (98% yield; EtOAc/light petroleum); ^{1}H NMR (DMSO-d_6): 2.11 (t, 2H, $J = 6$), 3.34 (bs, 1H), 4.25 (t, 2H, $J = 6$), 6.45 (s, 1H), 6.69−6.72 (m, 2H), 6.83−6.87 (m, 2H), 7.22 (d, 1H, $J = 3.2$), 7.94 (s, 1H), 8.09 (bs, 2H), 8.17 (s, 1H). IR (KBr): 3230, 1710, 687.

Synthesis of {5-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]-2-hydroxymethylbenzo[1,3]dioxol-2-yl}methanol (14b). To a solution of **14f** (50 mg, 0.09 mmol) in distilled THF (10 mL), $NaBH_4$ (70 mg, 20 equiv) was added in small portions, and the mixture was stirred at room temperature for 2 h. Then EtOAc (15 mL) and water (10 mL) were added, and the organic phase was separated and washed with water (2 × 10 mL) and saturated NaCl solution. The organic layer was dried and evaporated to give **14b** as white solid (70% yield). ^{1}H NMR (DMSO-d_6): 2.18−2.25 (m, 2H), 2.49 (t, 2H, $J = 7$), 3.86 (d, 4H, $J = 6.4$), 4.2 (t, 2H, $J = 6.4$), 4.32 (t, 2H, $J = 7$), 6.40−6.53 (m, 4H), 6.73 (bs, 2H), 7.24 (d, 1H, $J = 5$), 7.64 (s, 1H), 8.15 (s, 1H). IR (KBr): 3345, 3256, 1610, 677.

Synthesis of 6-[3-(5-Amino-2-furan-2-ylpyrazolo[4,3-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-7-yl)propyl]benzo[1,3]-dioxole 5-Sulfonic Acid (13). To a suspension of **5** (50 mg, 0.12 mmol) in distilled CH_2Cl_2 (10 mL) cooled at 0 °C was added chlorosulfonic acid (3 drops), and the mixture was stirred at this temperature for 1 h (till complete dissolution). The solvent was removed, and the residue was taken up with EtOAc (7 mL), and the chlorosulfonic derivative was precipitated by the addition of light petroleum (15 mL). Aqueous 10% HCl (0.5 mL) was added to the precipitate suspended in dioxane (2 mL), and the mixture was stirred at room temperature for 3 d. The solvent was removed under vacuum, and the residue was purified by flash chromatography (MeOH/EtOAc 20%) to give **13** as a white solid (52% yield). ^{1}H NMR (DMSO-d_6): 2.14 (m, 2H), 2.95 (t, 2H, $J = 7$), 4.25 (t, 2H, $J = 7$), 5.97 (s, 2H), 6.70 (s, 1H), 6.71−6.72 (m, 1H), 7.22 (s, 1H), 7.23 (d, 1H, $J = 4$), 7.94 (s, 1H), 8.15 (bs, 2H), 8.16 (s, 1H). IR (KBr): 3432, 1668, 1178, 629.

Determination of R_m Values by C_{18} RP−HPTLC. The HPTLC determinations were carried out on Whatman $KC_{18}F$ plates as previously described.[25] Solvent mixtures of methanol−water buffer at pH 7.0 were used as mobile phase. The methanol concentration ranged from 60% to 90%. The solutes were detected under UV 254-nm light.

Biology. CHO Membranes Preparation. The expression of the human adenosine receptors in CHO cells is described elsewhere.[26,27] The cells were grown adherently and maintained in Dulbecco's modified Eagle's medium with nutrient mixture F12 without nucleosides at 37 °C in 5% CO_2/95% air. Cells were split two or three times weekly, and then the culture medium was removed for membrane preparations. The cells were washed with phosphate-buffered saline and scrapped off flasks in ice cold hypotonic buffer (5 mM Tris-HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with Polytron, and the homogenate was centrifuged for 30 min at 48000*g*. The membrane pellet was resuspended in 50 mM Tris-HCl buffer at pH 7.4 for A_1 adenosine receptors; in 50 mM Tris-HCl and 10 mM $MgCl_2$ at pH 7.4 for A_{2A} adenosine receptors; and in 50 mM Tris-HCl, 10 mM $MgCl_2$, and 1 mM EDTA at pH 7.4 for A_3 adenosine receptors. They were utilized for binding assay. HEK-293 cells transfected with the human recombinant A_{2B} adenosine receptor were obtained from Receptor Biology, Inc. (Beltsville, MD).

Binding Assays of the Human Cloned A_1, A_{2A}, A_{2B}, and A_3 Adenosine Receptor. Receptor Binding Assays. Binding of [^{3}H]-DPCPX to CHO cells transfected with the human recombinant A_1 adenosine receptor was performed according to the method previously described by Lohse et al.[18]

Displacement experiments were performed for 120 min at 25 °C in 0.20 mL of buffer containing 1 nM [^{3}H]-DPCPX, 20 μL of diluted membranes (50 μg of protein/assay) and at least 6−8 different concentrations of examined compounds. Nonspecific binding was determined in the presence of 10 μM of CHA, and this is always ≤10% of the total binding.

Binding of [^{3}H]-SCH58261 to HEK-293 cells transfected with the human recombinant A_{2A} adenosine receptors (Research Biomedical International, Natick, MA) (20 μg of protein/assay) was performed according to Zocchi et al.[20] In competition studies, at least 6−8 different concentrations of compounds were used, and nonspecific binding was determined in the presence of 50 μM NECA for an incubation time of 60 min at 25 °C.

Binding of [^{3}H]-DPCPX to HEK-293 cells (Receptor Biology Inc., Beltsville, MD) transfected with the human recombinant A_{2B} adenosine receptors was performed as already described by Varani et al.[19] In particular, assays were carried out for 60 min at 25 °C in 0.1 mL of 50 mM Tris-HCl buffer, 10 mM $MgCl_2$, 1 mM EDTA, and 0.1 mM benzamidine, pH 7.4, 2 IU/mL adenosine deaminase containing 40 nM [^{3}H]-DPCPX, diluted membranes (20 μg of protein/assay), and at least 6−8 different concentration of tested compounds. Nonspecific binding was determined in the presence of 100 μM NECA and was always ≤30% of the total binding.

Binding of [^{3}H]-MRE3008-F20 to CHO cells transfected with the human recombinant A_3 adenosine receptors was performed according to Varani et al.[19] Competition experiments were carried out in duplicate in a finale volume of 100 μL in test tubes containing 1 nM [^{3}H]-MRE3008-F20, 50 mM Tris-HCl buffer, 10 mM $MgCl_2$, pH 7.4, and 100 μL of diluted membranes (50 μg of protein/mL) and at least 6−8 different concentrations of examined ligands. Incubation time was 120 min at 4 °C, according to the results of previous time-course

experiments.[19] Nonspecific binding was defined as binding in the presence of 1 μM MRE3008-F20 and was about 25% of total binding. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass fiber filters using a Micro-Mate 196 cell harvester (Packard Instrument Company). The filter-bound radioactivity was counted on Top Count (efficiency 57%) with Micro-Scint 20. The protein concentration was determined according to a Bio-Rad method[28] with bovine albumin as reference standard.

A weighted nonlinear least-squares curve-fitting program LIGAND was used for computer analysis of inhibition experiments.[29] Inhibitory binding constant, K_i, values were calculated from the IC_{50} values according to the Cheng and Prusoff equation, $K_i = IC_{50}/(1 + [C^*]/K_d^*)$, where $[C^*]$ is the concentration of the radioligand and K_d^* is its dissociation constant.[30]

References

(1) Olah, M.; Stiles, G. L. The role of receptor structure in determining adenosine receptor activity. *Pharmacol. Ther.* **2000**, *85*, 55−75.

(2) Impagnatiello, F.; Bastia, E.; Ongini, E.; Monopoli, A. Adenosine receptors in neurological disorders. *Emerging Ther. Targets* **2000**, *4*, 635−663.

(3) Svenningson, P.; Le Moine, C.; Fisone, G.; Fredholm, B. B. Distribution, biochemistry and function of striatal adenosine A_{2A} receptors. *Prog. Neurobiol.* **1999**, *59*, 355−396.

(4) Ongini, E.; Fredholm, B. B. Pharmacology of adenosine A_{2A} receptors. *Trends Pharmacol. Sci.* **1996**, *17*, 364−372.

(5) (a) Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Borioni, A.; Viziano, M.; Dionisotti, S.; Ongini, E. Current developments of A_{2A} adenosine receptor antagonists. *Curr. Med. Chem.* **1995**, *2*, 707−722. (b) Jacobson, K. A.; Knutsen, L. J. S. P1 and P2 Purine and pyrimidine receptor ligands. In *Handbook of Experimental Pharmacology, Vol. 151/1, Purinergic and Pyrimidinergic Signalling I*; Abbracchio, M. P., Williams, M., Eds.; Springer-Verlag: Berlin, 2001; pp 129−175.

(6) Ongini, E.; Dionisotti, S.; Gessi, S.; Irenius, E.; Fredholm, B. B. Comparison of CGS 15943, ZM 241385 and SCH 58261 as antagonists at human adenosine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1999**, *359*, 7−10.

(7) (a) Baraldi, P. G.; Manfredini, S.; Simoni, D.; Zappaterra, L.; Zocchi, C.; Dionisotti, S.; Ongini, E. Synthesis of new pyrazolo-[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine and 1,2,3-triazolo[1,5-c]-pyrimidine displaying potent and selective activity as A_{2A} adenosine receptor antagonists. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2539−2544. (b) Ongini, E. SCH 58261: a selective A_{2A} adenosine receptor antagonists. *Drug Dev. Res.* **1997**, *42*, 63−70.

(8) Baraldi P. G.; Cacciari, B.; Spalluto, G.; Pineda de las Infantas y Villatoro, M. J.; Zocchi, C.; Dionisotti, S.; Ongini, E. Pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives: potent and selective A_{2A} adenosine antagonists. *J. Med. Chem.* **1996**, *39*, 1164−1171.

(9) Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Bergonzoni, M.; Dionisotti, S.; Ongini, E.; Varani, K.; Borea, P. A. Design, synthesis, and biological evaluation of a second generation of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines as potent and selective A_{2A} adenosine receptor antagonists. *J. Med. Chem.* **1998**, *41*, 2126−2133.

(10) Todde, S.; Moresco, R. M.; Simonelli, P.; Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Varani, K.; Monopoli, A.; Matarrese, M.; Carpinelli, A.; Magni, F.; Galli Kienle, M.; Fazio, F. Design, radiosynthesis, and biodistribution of a new potent and selective ligand for in vivo imaging of the adenosine A_{2A} receptor system using positron emission tomography. *J. Med. Chem.* **2000**, *43*, 4359−4362.

(11) Richardson, P. J.; Kase, H.; Jenner, P. G. Adenosine A_{2A} receptor antagonists as new agents for the treatment of Parkinson's disease. *Trends Pharmacol. Sci.* **1997**, *18*, 338−344.

(12) Weichert, J. P.; Longino, M. A.; Bakan, D. A.; Spigarelli, M. G.; Chou, T.; Schwendner, S. W.; Counsell, R. E. Polyiodinated triglyceride analogs as potential computed tomography imaging agents for the liver. *J. Med. Chem.* **1995**, *38*, 636−646.

(13) Akaboshi, S.; Ikegami, S. Synthesis of the heterocyclic compounds by Pschorr cyclization. III. Synthesis of 10,11-methylenedioxy-7,8-dihydro-6*H*-benzo[*c*]pyrid[1,2-a]azepinium salts and their derivatives. *Chem. Pharm. Bull.* **1966**, *14*, 622−627.

(14) Meschino, J. A.; Bond, C. H. 2-Amino-5,6-dihydro-1,3-oxazines. The reduction of carboxylic esters with sodium borohydride. *J. Org. Chem.* **1963**, *28*, 3129−3134.

(15) Anzalone, L.; Hirsch, J. A. Substituent effects on hydrogenation of aromatic rings: hydrogenation vs. hydrogenolysis in cyclic analogues of benzyl ethers. *J. Org. Chem.* **1985**, *50*, 2128−2133.

(16) Baraldi, P. G.; Guarneri, M.; Moroder, F.; Simoni, D.; Vicentini, C. B. Synthesis of 3-methyl-6-phenylamino-substituted-1*H*-pyrazolo[4,3-d]pyrimidine-7(6*H*)-ones. *Synthesis* **1982**, 70−71.

(17) Baker, B. R.; Cory, M. Irreversible enzyme inhibitors. 180. Irreversible inhibitors of the C′1a component of complement derived from *m*-(phenoxypropoxy)benzamidine and phenoxyacetamide. *J. Med. Chem.* **1971**, *14*, 119−125.

(18) Lohse, M. J.; Klotz, K.-N.; Lindernborn-Fotinos, J.; Reddington, M.; Schwabe, U.; Olsson, R. A. 8-Cyclopentyl 1,3-dipropylxanthine DPCPX a selective high affinity antagonist radioligand for A_1 adenosine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *336*, 204−210.

(19) Varani, K.; Merighi, S.; Gessi, S.; Klotz, K. N.; Leung, E.; Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Borea, P. A. [^{3}H]MRE3008-F20: a novel antagonist radioligand for the pharmacological and biochemical characterization of human A_3 adenosine receptors. *Mol. Pharmacol.* **2000**, *57*, 968−975.

(20) Zocchi, C.; Ongini, E.; Ferrara, S.; Baraldi, P. G.; Dionisotti, S. Binding of the radioligand [^{3}H]-SCH 58261, a new non-xanthine A_{2A} adenosine receptor antagonist, to rat striatal membranes. *Br. J. Pharmacol.* **1996**, *117*, 1381−1386.

(21) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Moro, S.; Klotz, K. N.; Leung, E.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. Pyrazolo[4,3-*e*]-1,2,4,-triazolo[1,5-*c*]pyrimidine derivatives as highly potent and selective human A_3 adenosine receptor antagonists: influence of the chain at N8 pyrazole nitrogen. *J. Med. Chem.* **2000**, *43*, 4768−4780.

(22) Shryock, J. C.; Snowdy, S.; Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Monopoli, A.; Ongini, E.; Baker, S. P.; Belardinelli, L. A_{2A}-adenosine receptor reserve for coronary vasodilation. *Circulation* **1998**, *98*, 711−718.

(23) Jarvis, M. F.; Schulz, R.; Hutchinson, A. J.; Do, U. H.; Sills, M. A.; Williams, M. [^{3}H]CGS 21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888−893.

(24) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development setting. *Adv. Drug Delivery Rev.* **2001**, *46*, 3−26.

(25) Pietrogrande, M. C.; Borea, P. A.; Biagi, G. L. Lipophilicity measurement of benzodiazepine-receptor ligands by reversed-phase liquid chromatography. Comparison between high performance liquid and thin-layer chromatography. *J. Chromatogr.* **1988**, *447*, 404−409.

(26) Olah, M. E.; Stiles, G. L. Adenosine receptor subtypes: characterization and therapeutic regulation. *Annu. Rev. Pharmacol. Toxicol* **1995**, *35*, 581−606.

(27) Abbracchio, M. P.; Brambilla, R.; Kim, H. O.; von Lubitz, D. K. J. E.; Jacobson, K. A.; Cattabeni, F. G-protein dependent activation of phospholipase-C by adenosine A_3 receptor in rat brain. *Mol. Pharmacol.* **1995**, *48*, 1038−1040.

(28) Bradford, M. M. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(29) Munson, P. J.; Rodbard, D. LIGAND: a versatile computerized approach for the characterization of ligand binding system. *Anal. Biochem.* **1980**, *107*, 220−239.

(30) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibitory constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

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