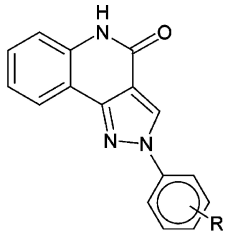
Journal of Medicinal Chemistry

Article

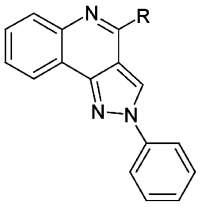
New 2-Arylpyrazolo[4,3-*c*]quinoline Derivatives as Potent and Selective Human A Adenosine Receptor Antagonists

Pier Giovanni Baraldi, Mojgan Aghazadeh Tabrizi, Delia Preti, Andrea Bovero, Francesca Fruttarolo, Romeo Romagnoli, Naser Abdel Zaid, Allan R. Moorman, Katia Varani, and Pier Andrea Borea

J. Med. Chem., **2005**, 48 (15), 5001-5008• DOI: 10.1021/jm050125k • Publication Date (Web): 06 July 2005



R = H, CH_3, F, Cl, OCH_3, ethyl, *iso*-propyl, *n*-butyl



R =NH_2, cyclohexylamine, benzylamine, N-methylpiperazine, OCH_3, phenylurea

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

New 2-Arylpyrazolo[4,3-*c*]quinoline Derivatives as Potent and Selective Human A_3 Adenosine Receptor Antagonists

Pier Giovanni Baraldi,*,† Mojgan Aghazadeh Tabrizi,† Delia Preti,† Andrea Bovero,† Francesca Fruttarolo,† Romeo Romagnoli,† Naser Abdel Zaid,§ Allan R. Moorman,|| Katia Varani,‡ and Pier Andrea Borea‡

Dipartimento di Scienze Farmaceutiche, Dipartimento di Medicina Clinica e Sperimentale-Sezione di Farmacologia, Università di Ferrara, 44100 Ferrara Italy, College of Pharmacy, An-Najah National University, Nablus, and King Pharmaceutical R & D, 4000 CentreGreen Way, Suite 300 Cary, North Carolina 27513

Received February 9, 2005

In this paper we report the synthesis and biological evaluation of a new class of 2-phenyl-2,5-dihydro-pyrazolo[4,3-*c*]quinolin-4-ones as A_3 adenosine receptor antagonists. We designed a new route based on the Kira-Vilsmeier reaction for the synthesis of this class of compounds. Some of the synthesized compounds showed A_3 adenosine receptor affinity in the nanomolar range and good selectivity as evaluated in radioligand binding assays at human (h) A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor subtypes. We introduced several substituents on the 2-phenyl ring. In particular substitution at the 4-position by methyl, methoxy, and chlorine gave optimal activity and selectivity **6c** (K_ihA_1, A_{2A}>1000 nM, EC_{50}hA_{2B}>1000 nM, K_ihA_3 = 9 nM), **6d** (K_ihA_1, A_{2A}>1000 nM, EC_{50}hA_{2B}>1000 nM, K_ihA_3 = 16 nM), **6b** (K_ihA_1, A_{2A} >1000 nM, EC_{50}-hA_{2B}>1000 nM, K_ihA_3 = 19 nM). In conclusion, the 2-phenyl-2,5-dihydro-pyrazolo[4,3-*c*]quinolin-4-one derivatives described herein represent a new family of *in vitro* selective antagonists for the adenosine A_3 receptor.

Introduction

Adenosine, an endogenous modulator of a wide range of biological functions in the nervous, cardiovascular, renal, and immune systems, interacts with at least four cell surface receptor subtypes classified as A_1, A_{2A}, A_{2B} and A_3.[1,2] All four adenosine receptor subtypes are coupled via G-protein to the enzyme adenylate cyclase in either an inhibitory (A_1 and A_3 subtypes) or stimulatory manner (A_{2A} and A_{2B} subtypes).[3] In addition, the A_3 adenosine receptor subtype, that is distributed in different organs (lung, liver, heart, kidney, and, in low density, in the brain)[4] also exerts its action through the stimulation of phospholipases C4 and D5.[5] The potential therapeutic applications of activating or antagonizing this receptor subtype have been investigated in recent years[6,7] and in particular, antagonists for the A_3 receptor may prove to be useful for the treatment of inflammation and in the regulation of cell growth.[8] Activation of A_3 adenosine receptors in the rat results in hypotension by promotion of release or inflammatory mediators from mast cells.[9] It has also been suggested that the A_3 receptor plays an important role in brain ischemia,[10,11] immunosuppression,[12] and bronchospasm in several animal models.[13] From these pharmacological observations, highly selective A_3 adenosine receptor antagonists are being sought as potential antiasthmatic,[14] antiinflammatory,[15] and cerebroprotective agents.[16] In the past few years, Colotta et al. directed much effort toward the study of adenosine receptor antagonists[17−21] and they focused their attention on 2-arylpyrazolo[3,4-c]-quinoline derivatives. More recently, they have focused on 2-aryl-1,2,4-triazolo[4,3-*a*]quinoxaline-1,4-diones, 2-aryl-1,2,4-triazolo[4,3-*a*]quinoxalin-4-amino-1-ones, finding potent and/or selective A_3 adenosine receptor antagonists.[22,23] In the past few years, different classes of nonxanthinic structures have been reported to be A_3 adenosine receptor antagonists.[24] In particular the pyrazolo-triazolo-pyrimidine structure has been extensively investigated by our research group.[25−28] Considering the activity profile of the 2-arylpyrazolo[3,4-*c*]quinoline derivatives synthesized by Colotta et al., we decided to synthesize and biologically evaluate the structural isomers of this class of compounds, a new series of 2-arylpyrazolo[4,3-*c*]quinoline. In this account we describe a new synthetic route for the synthesis of 2-arylpyrazolo[4,3-*c*]quinolines as potent and selective A_3 adenosine receptor antagonists. Our goal was to synthesize a series of 2-arylpyrazolo[4,3-*c*]quinolines variously substituted to evaluate its affinity toward A_1, A_{2A}, A_{2B} and A_3 adenosine receptor subtypes.

Initially we investigated the nature and the position of the substituents on the 2-phenyl ring. *Para*-substitution provided the most potent hA_3 compounds. From our studies it also emerged the relevance of a 4-oxo functional group for A_3 antagonist activity. In fact, substitution of the 4-oxo moiety with different functionalities, like amines, ureas, hydrazides, ethers, or thioethers induces a loss of affinity. Replacement of the 4-oxo functional group with a thione moiety also leads to a decrease in affinity. The N^5-methylated compound (**20**) shows no affinity at any of the adenosine receptor subtypes. Enlargement of the ring from a quinoline structure to a benzazocine structure (**23**) causes a loss of affinity.

* To whom correspondence should be addressed: Prof: Pier Giovanni Baraldi, Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17−19, 44100 Ferrara Italy. Tel: +39−0532−291293. Fax: +39-0532-291296. E-mail: pgb@dns.unife.it.

† Dipartimento di Scienze Farmaceutiche, Università di Ferrara.

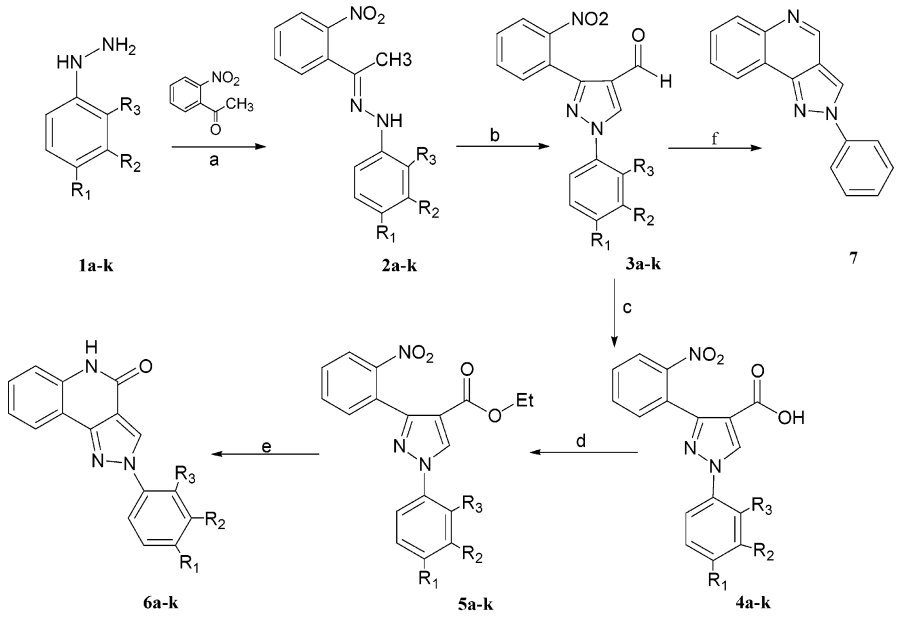
‡ Dipartimento di Medicina Clinica e Sperimentale-Sezione di Farmacologia, Università di Ferrara.

§ An-Najah National University.

|| King Pharmaceutical R & D.

10.1021/jm050125k CCC: $30.25
Published on Web 07/06/2005

Scheme 1



	R_1	R_2	R_3
a	H	H	H
b	Cl	H	H
c	CH_3	H	H
d	OCH_3	H	H
e	H	Cl	H
f	Cl	Cl	H
g	F	H	H
h	H	H	Cl
i	n-Butyl	H	H
j	Ethyl	H	H
k	Isopropyl	H	H

Reagents: a: Ethanol, acetic acid, reflux; b: 1) $POCl_3$, 0 °C, DMF 2) reflux; c: $KMnO_4$, KOH/H_2O, rt; d: ethanol, H_2SO_4, reflux; e: 1) H_2/C−Pd, ethanol, 2) reflux or 2-methoxy-ethanol/water, Fe/HCl conc, reflux; f: ethanol, H_2/Pd−C, reflux.

Chemistry

The synthetic pathways employed to obtain the pyrazolo[4,3-*c*]quinoline nucleus are illustrated in Scheme 1: phenyl-hydrazones (**2a**−**k**) were obtained according to Werber et al.[29] by reacting the corresponding phenylhydrazines with *o*-nitro-acetophenone. The pyrazole-4-carboxaldehydes (**3a**−**k**) were obtained by the Vilsmeier reaction, adding the corresponding phenylhydrazone to $POCl_3$ and DMF. The oxidation of the pyrazole-4-carboxaldehydes with $KMnO_4$ yielded the pyrazole-4-carboxylic acids (**4a**−**k**) that were transformed to the respective esters (**5a**−**k**) in a mixture of ethanol and sulfuric acid. Hydrogenation of **3a** and subsequently boiling the amine intermediate in ethanol affords compound **7**.[30] Reduction of **5a**, **5c**, **5d**, **5i**−**k** with hydrogen/Pd−C followed by refluxing of the amine intermediate in ethanol afforded **6a**, **6c**, **6d**, **6i**−**k**, while the reduction of **5b**, **e**, **f**, **g**, **h** containing the halogen atoms on the 2-phenyl ring, was realized with an alternative method[31] using iron powder and concentrated solution of HCl to yield **6b**, **e**, **f**, **g**, **h**.

Schemes 2 and 3 show the synthetic routes adopted to functionalize the 4-position of the pyrazoloquinoline nucleus. Refluxing **6a** in a mixture of $POCl_3$/PCl_5 gave **8** which was treated with the corresponding amines to obtain **9**−**12**. The urea derivative **14** was prepared by adding phenylisocyanate to amine **9**. Treatment of **8** with sodium methoxide provided **13**. Reaction of **8** with requisite 4-chlorobenzoic acid hydrazide at 70 °C afforded **17**. When the same reaction was performed at 120 °C with 2-furoic acid hydrazide or 4-chlorobenzoic acid hydrazide, the corresponding tetracyclic compounds **15** and **16** respectively, were produced. Treating **6a** with Lawesson's reagent afforded **18**, which was alkylated with methyl iodide to yield **19**. (Scheme 3) Compound **20** was obtained by alkylation of **6a** in the presence of methyl iodide and K_2CO_3.

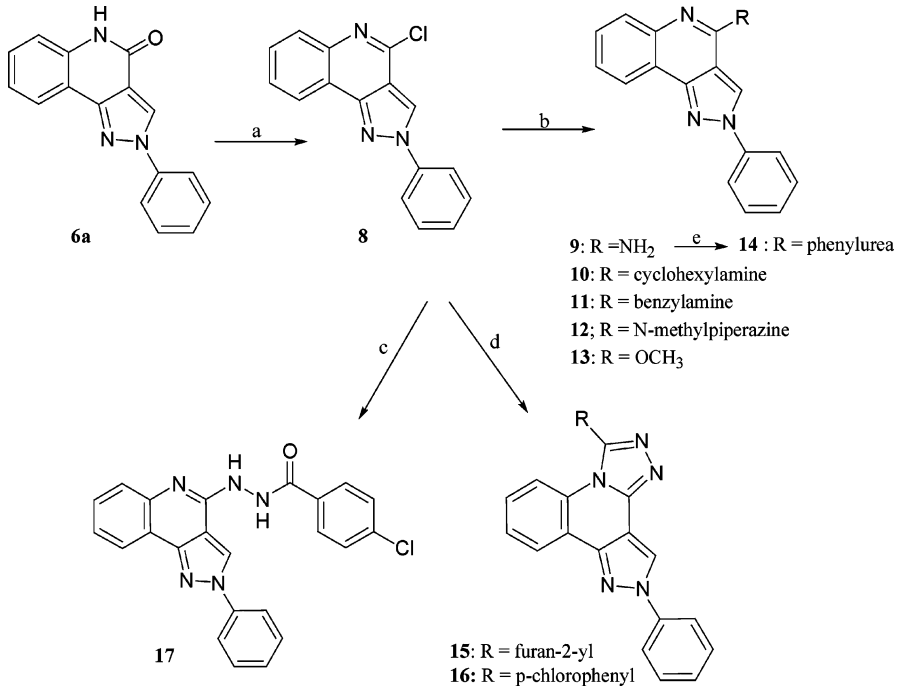
The synthesis of the benzazocine derivative **23** is displayed in Scheme 4. Aldehyde **3a** was reacted with triphenyl-λ^5-phosphanylidene-acetic acid ethyl ester (Wittig reaction) to obtain **21**, which was hydrogenated to obtain **22** and subsequently treated with NaH to afford **23**.

4-substituted-phenylhydrazines **1a**−**h** are available commercially, the 4-alkyl-phenylhydrazines **1i**−**k** were prepared according to the procedure reported in the literature.[32]

Results and Discussion

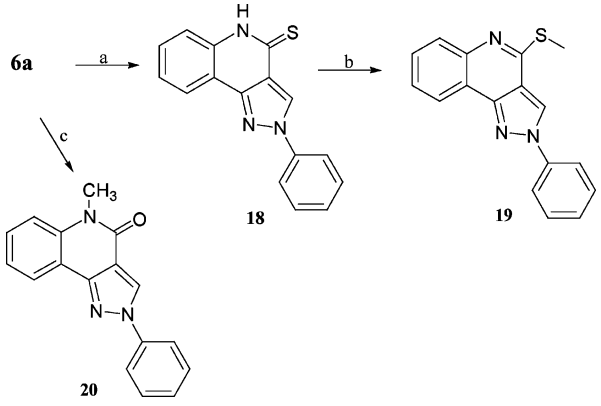
Affinities of the pyrazolo[4,3-*c*]quinoline derivatives in radioligand binding assays at hA_1, hA_{2A}, and hA_3 receptors are reported in Table 1. Receptor binding assays were performed using Chinese Hamster Ovary cells (CHO) transfected with human A_1, A_{2A}, or A_3 adenosine receptors. Potency of the compounds versus hA_{2B} adenosine receptors was studied by evaluating their capability to inhibit (100 nM) NECA stimulated cAMP levels. Moreover, the three compounds that showed high affinity to hA_3 adenosine receptors were also studied to calculate their potencies. cAMP experi-

Scheme 2



Reagents: a: $POCl_3$, PCl_5, reflux; b: NH_3 liq, ethanol/ethyl ether, 120 °C (for compound 9), corresponding amines, 120 °C, sealed tube, sodium methylate, methanol, reflux (for compound 13); c: 4-chlorobenzoic acid hydrazide, ethanol, TEA, 70 °C; d: 2-furoic acid hydrazide or 4-chlorobenzoic acid hydrazide, 120 °C, sealed tube, TEA, ethanol; e: phenylisocyanate, dioxane, reflux.

Scheme 3



Reagents: a: Lowesson's reagent, toluene, reflux; b: sodium acetate, ethanol, CH_3I, reflux; c: K_2CO_3, CH_3I, DMF, rt.

ments were performed evaluating their capability to block the inhibitory effect mediated by Cl−IB-MECA (100 nM). (Figure 1).

All the examined compounds proved to be almost inactive in rA_3CHO membranes showing different percentage of inhibition in the range 1−22% of specific binding at a concentration of 10 μM. These results are in agreement with low degree of sequence homology (72%) of the A_3 adenosine receptor subtypes.

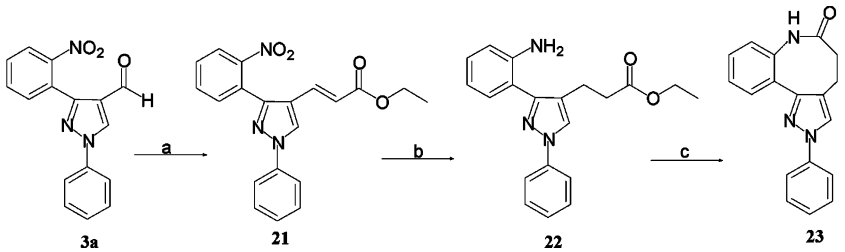
The synthesis of these pyrazolo[4,3-*c*]quinolines has produced some potent and selective A_3 antagonists (**6a**−**d, 6g**). No compound showed affinity at A_1, A_{2A} and A_{2B} adenosine receptor subtypes, while the structurally isomeric pyrazolo[3,4-*c*]quinoline derivatives reported in the literature showed good affinity toward both the A_1 and A_3 adenosine receptors.

The parent unsubstituted 2-phenyl (compound **6a)** showed good affinity and selectivity for the adenosine A_3 receptor. Introduction of an electron-donating group, such as methyl (**6c**) or methoxy (**6d**) at the 4-position of the phenyl ring enhanced affinity. The presence of an electron-withdrawing chlorine (**6b**) or fluorine (**6g**) atom at the *p*-position of the phenyl ring produced comparable affinity at the adenosine receptor subtypes. The presence of a chlorine atom at the *m*-position of the phenyl ring induced a loss of affinity, such as was seen with the 2-(3-chlorophenyl) (**6e**) and 2-(3,4-dichlorophenyl) (**6f**) derivatives. The presence of a chlorine atom at the *o*-position (**6h**) did not affect affinity relative to the parent (**6a**).

Considering the activity of compound **6c**, we thought to explore the effect of chain elongation at the *p*-position of the 2-phenyl ring, creating an homologous series of derivatives (*p*-ethyl, **6j**; *p*-isopropyl, **6k**; *p*-*n*-butyl, **6i**). We observed that these bulky groups decrease the affinity. The *p*-ethyl derivative maintains a slight activity, but further increases in the number of carbon atoms affords a loss of activity.

Investigation of the amide function on the pyrazoloquinoline nucleus provided interesting information about the importance of this moiety for the activity of this class of compounds. Transformation of the amide function of the quinoline nucleus into an imine moiety (compound **7**) led to a significant decrease of affinity for A_3 adenosine receptor. Replacement of the 4-oxo functional with the 4-amino group (**9**) yielded compounds with no significant affinity for A_3 adenosine receptor. In fact the pyrazoloquinoline-4-amine (**9**), pyrazoloquinoline-4-cyclohexylamine (**10**) and pyrazoloquinoline-4-(4-methyl)-piperazine (**12**) were completely inactive. Only the pyrazoloquinoline-4-benzylamine (**11**) showed a slight affinity toward A_3 adenosine receptor. The presence of an urea moiety at the 4-position (**14**) determined a loss of affinity. Substitution of the 4-oxo functional group with the corresponding thio-derivative (**18**) or methoxy

Scheme 4



Reagents: triphenyl-λ^5-phosphanylidene-acetic acid ethyl ester, $CHCl_3$, 70 °C; b: ethanol, C−Pd, H_2, c: NaH 60%, toluene, reflux.

Table 1. Adenosine Receptor Affinities of Derivatives **6a**−**d**, **6g**, **h**, **j**, **7**, **11**, **13**

Compd	hA_1[a] K_i (nM)	hA_{2A}[b] K_i (nM)	hA_{2B}[c] IC_{50} (nM)	hA_3[d] K_i (nM)
6a	>1000 (5%)	>1000 (2%)	>1000 (1%)	27 (23−32)
6b	>1000 (4%)	>1000 (1%)	>1000 (1%)	19 (14−25)
6c	>1000 (3%)	>1000 (1%)	>1000 (2%)	9 (7−11)
6d	>1000 (1%)	>1000(1%)	>1000 (2%)	16 (12−23)
6g	>1000 (10%)	>1000 (10%)	>1000 (10%)	27 (24−31)
6h	>1000 (3%)	>1000 (1%)	>1000 (8%)	44 (36−54)
6j	>1000 (12%)	>1000 (1%)	>1000 (13%)	157 ± 18
7	>1000 (5%)	>1000 (3%)	>1000 (1%)	212 (188−240)
11	>1000 (4%)	>1000 (2%)	>1000 (1%)	125 (98−159)
13	>1000 (16%)	>1000 (5%)	>1000 (10%)	166 (140−197)
18	>1000 (14%)	>1000(8%)	>1000 (11%)	220 (202−239)

[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]ZM241385 binding at human A_{2A} receptors expressed in CHO cells (n = 3−6). [c] Potency (IC_{50}) of examined compounds to inhibit 100 nM-NECA stimulation cAMP levels in hA_{2B} CHO cells. In parentheses are reported the % of inhibition to hA_1, A_{2A} and A_{2B} CHO cells. [d] Displacement of specific [^{3}H]MRE3008-F20 binding at human A_3 receptors expressed in CHO cells (n = 3−6). Data are expressed as geometric means with 95% confidence limits. For compounds **6e**, **f, i, k**, **9**, **10**, **12**, **14**−**17**, **19**, **20**, **23**: hA_1, A_{2A}, A_{2B}, A_3 > 1000 nM.

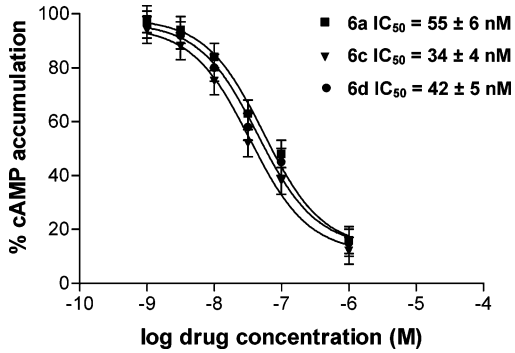


Figure 1. Inhibitory curves of cAMP accumulation in human A_3 adenosine receptors by adenosine antagonists blocking the effect of 100 nM Cl−IB-MECA.

group (**13**), caused a decrease of A_3 adenosine receptor affinity, while the presence of a methyl-thio moiety in this position (**19**) led to a complete loss of affinity. Replacement of the 4-oxo moiety with an hydrazide (**17**) or the corresponding tetracyclic compounds obtained by cyclization (**15** and **16**), afforded inactive ligands at the adenosine receptors.

Alkylation of the nitrogen at the 5-position (**20**) caused a loss of affinity. Indeed the presence of an hydrogen atom in this position appears essential for the activity of this class of compounds.

Finally, we investigated the effects of the cycle enlargement, switching from a quinoline nucleus to a benzazocine nucleus (**23**). This compound was inactive.

In conclusion, to delineate the SAR of the 2-arylpyrazolo[4,3-*c*]quinolin-4-one class as A_3 adenosine receptor antagonist, we have investigated different portions of this structure, modifying the substituents. It is emerged from this study that within the pyrazoloquinolone nucleus certain functionalities, such as the amide linkage, are important for the activity of these compounds. Modification of the 2-phenyl ring at the *para*-position with sterically small groups is permitted, and may improve the A_3 adenosine receptor affinity.

Experimental Section

Chemistry General. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{245} Merck plates). Products were visualized with iodine or potassium permanganate solution. ^{1}H NMR spectra 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 Hz. Light petroleum ether refers to the fractions boiling at 40−60 °C. Melting points were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatography was performed using Merck 60−200 mesh silica gel. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. Organic solutions were dried over anhydrous magnesium sulfate. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Ferrara, and were within 0.4% of the calculated values.

General Procedure for the Preparation of Phenylhydrazones (2a−k). Classic method for the synthesis of phenyl-hydrazones was applied: To a solution of *o*-nitroacetophenone (50 mmol) in ethanol (50 mL), substituted phenylhydrazines (50 mmol) and acetic acid (2 mL) were added. The reaction mixture was refluxed for 8 h. The solvent was removed and the residue was taken up with chloroform and washed with a saturated solution of $NaHCO_3$. Organic phases were dried over anhydrous $MgSO_4$, filtered and concentrated at reduced pressure to afford a residue that was used without purification in the next reaction.

General Procedure for the Preparation of 3-(2-Nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes (3a−k). A variant of the Vilsmeyer reaction was used: $POCl_3$ (100 mmol) was added dropwise to anhydrous DMF (100 mmol) at 0 °C and stirring was continued for 20 min untill the formation of Vilsmeyer complex. The corresponding phenylhydrazone (50 mmol) dissolved in DMF (20 mL) was added and the reaction mixture was stirred for 30 min at room temperature and then refluxed for 10 h. The reaction mixture was portioned in water/ice (100 mL) and was allowed to cool to 0 °C, 2 N NaOH was added and stirred for 30 min. The precipitated product was filtered and the solid obtained was purified by crystallization from ethanol (yield: 65−80%).

General Procedure for the Preparation of 3-(2-Nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carboxylic acids (4a−k). To a stirred solution of the corresponding 1*H*-pyrazole-4-carboxaldehydes **3a**−**k** (5 mmol) in dioxane (20 mL) and water (5 mL), was added KOH (7.5 mmol) in water (3 mL), followed by $KMnO_4$ (7.5 mmol). The reaction mixture was stirred at

room temperature and monitored by TLC. The excess of $KMnO_4$ was decomposed by addition of diluted H_2O_2. The reaction mixture was filtered through a Celite pad, the filtrate was evaporated under reduced pressure, and the residue was acidified with HCl solution 10% (100 mL). The white solid precipitate that formed was filtered and washed with water. Recrystallization of the crude product from ethanol provided the title compounds. (yield: 70−80%)

General Procedure for the Preparation of Ethyl 3-(2-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carboxylates (5a−k). To a solution of the corresponding 3-(2-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carboxylic acid (5 mmol) in absolute ethanol (30 mL), H_2SO_4 (2 mL) was added and refluxed for 8 h. The reaction mixture was concentrated in vacuo, the residue was neutralized with a saturated solution of $NaHCO_3$. The white solid precipitated was filtered and purified by crystallization from ethanol. (yield: 90−95%)

General Procedure for the Preparation of 2-Phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-4(5*H*)-ones (6a, 6c, 6d, 6i−k). To a solution of the corresponding ethyl 3-(2-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carboxylate (5 mmol) in absolute ethanol (100 mL), 10% Pd−C (0.5 g) was added portion wise. The reaction mixture was hydrogenated (50 psi) at room temperature for 2 h, then filtered through a Celite pad and the filtrate was stirred at reflux for 8 h. Ethanol was evaporated under reduced pressure and the oily residue was purified by crystallization from ethanol to give a white solid. (yield: 60−65%)

General Procedure for the Preparation of 2-Phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-4(5*H*)-ones (6b, 6e, 6f−h). To a solution of the appropriate 3-(2-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carboxylate (0.2 mmol) in 2-methoxyethanol (10 mL) and water (2.5 mL), iron powder (100 mg) was added followed by concentrated HCl (0.1 mL). The mixture was stirred at reflux for 1 h, then cooled and filtered through a Celite pad. The filter cake was washed well with hot 2-methoxyethanol and the filtrate was diluted with water to precipitate the product that was purified by crystallization from ethanol. (yield: 65−70%)

2-Phenyl-2*H*-pyrazolo[4,3-*c*]quinoline (7). To a solution of **3a** (5 mmol) in absolute ethanol (100 mL) was added Pd−C (0.5 g) portion wise. The reaction mixture was hydrogenated (50 psi) at room temperature for 2 h, then was heated to reflux with stirring for 30 min. After filtration through a Celite pad, the filtrate was evaporated under reduced pressure and the oily residue was purified by crystallization from ethanol/water to give a white solid. (yield: 90%)

4-Chloro-2-phenyl-2*H*-pyrazolo[4,3-c]quinoline (8). A mixture of **6a** (1.15 mmol), $POCl_3$ (10.5 mL) and PCl_5 (0.35 mmol) was refluxed with stirring for 2 h. The solvent was evaporated and the crude solid obtained was suspended in water/ice (30 mL) and filtered to afford the product as a white solid. (Yield: 90%).

2-Phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-4-amine (9). A solution of **8** (1 mmol) and liquid NH_3 (5 mL) in 20 mL of ethanol/ethyl ether 9:1 was heated in a sealed tube at 120 °C for 7 h. The reaction mixture was evaporated in vacuo and the solid residue was dissolved in ethyl ether, heated and filtered to afford a white solid. (yield: 83%)

N−Cyclohexyl-2-phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-4-amine (10), N-benzyl-2-phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-4-amine (11) and 4-(4-methylpiperazin-1-yl)-2-phenyl-2*H*-pyrazolo[4,3-*c*]quinoline (12). A solution of **8** (0.3 mmol) in 1 mL of cyclohexylamine, benzylamine or *N*-methyl-piperazine was heated in a sealed tube at 120 °C for 8 h. The reaction mixture was cooled, ethyl ether (5 mL) was added and the product was filtered. The title compounds **10** and **12** were purified by crystallization respectively from ethyl-ether/hexane and ethanol/water, while the compound **11** was purified by column chromatography (ethyl acetate/petroleum ether 1:1). (yield: 70−80%)

4-Methoxy-2-phenyl-2*H*-pyrazolo[4,3-*c*]quinoline (13). To a solution of compound **8** in methanol (10 mL) was added sodium methoxide (1.75 mmol). The reaction mixture was refluxed for 4 h under stirring. Water (10 mL), was added and the reaction mixture was stirred for 10 min at room temperature and filtered. (yield 86%)

1-Phenyl-3-(2-phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-4-yl)-urea (14). To a solution of compound 9 (0.17 mmol) in anhydrous dioxane (50 mL) was added phenyisocyanate (0.1 mL). The reaction mixture was refluxed for 10 h with stirring, then allowed to cool to room temperature. The solvent was evaporated in vacuo to yield a solid. Recrystallization of the crude product from dioxane provided the title compound as a white solid. (yield: 75%).

2-Phenyl-6-(furan-2-yl)-2*H*-pyrazolo[4,3-*c*][1,2,4]triazolo-[4,3-*a*]quinoline (15) and 2-Phenyl-6-(4-chlorophenyl)-2*H*-pyrazolo[4,3-*c*][1,2,4]triazolo[4,3-*a*]quinoline (16). A solution of **8** (0.36 mmol), furan-2-carbohydrazide or 4-chlorobenzohydrazide (0.54 mmol) and triethylamine (0.36 mmol) in ethanol (5 mL) was heated in a sealed tube at 120 °C for 10 h. The reaction mixture was concentrated in vacuo, the residue was suspended in ethyl acetate and filtered. The product was purified by column chromatography (ethyl acetate/petroleum ether 7:3) to afford a white solid. (yield 70%).

4-Chloro-N′-(2-phenyl-2*H*-pyrazolo[4,3-c]quinolin-4-yl)-benzohydrazide (17). A solution of **8** (0.50 mmol), 4-chlorobenzohydrazide (0.75 mmol) and triethylamine (0.5 mmol) in ethanol (10 mL) was heated at 70 °C for 1 h. The precipitate was filtered, washed with water and purified by crystallization from DMF/water to provide compound **17** as a yellow solid. (yield: 60%).

2-Phenyl-2*H*-pyrazolo[4,3-*c*]quinoline-4(5*H*)-thione (18). To a solution of **6a** (0.20 mmol) in anhydrous toluene (5 mL) was added Lawesson's reagent (0.10 mmol), and the reaction mixture was heated at reflux under a nitrogen atmosphere for 15 min. The solvent was evaporated under reduced pressure, the residue was dissolved in ethanol, heated and filtered to remove Lawesson's reagent. The filtrate was evaporated under reduced pressure and the residue was crystallized from ethanol. (yield: 90%)

4-(Methylthio)-2-phenyl-2*H*-pyrazolo[4,3-*c*]quinoline (19). To a solution of **18** (0.35 mmol) in ethanol (10 mL) was added anhydrous sodium acetate (0.70 mmol) and methyl iodide (0.70 mmol). The reaction mixture was heated at reflux for 2 h. Then the solvent was evaporated under reduced pressure, to the residue was added 10 mL of water, and the precipitate was collected by filtration to afford the title compound. (yield: 65%)

5-Methyl-2-phenyl-2*H*-pyrazolo[4,3-c]quinolin-4(5*H*)-one (20). A solution of **6a** (0.35 mmol), K_2CO_3 (0.40 mmol) and iodomethane (0.50 mmol) in DMF (5 mL) was stirred for 24 h at room temperature. The solvent was evaporated and the reaction mixture was suspended in water, filtered and purified by crystallization from DMF/water. (yield: 75%)

(E)-ethyl 3-(3-(2-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)acrylate (21). To a solution of **3a** (30 mmol) in chloroform (20 mL) triphenyl-λ^5-phosphanylidene)-acetic acid ethyl ester (30 mmol) was added, and the reaction mixture was heated at 70 °C with stirring for 3 h. The solvent was evaporated and the residue was purified by crystallization from ethyl ether/petroleum ether to afford a yellow solid. (yield: 80%).

Ethyl 3-(3-(2-aminophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-propanoate (22). To a solution of **21** (0.50 mmol) in absolute ethanol (10 mL), Pd−C (0.26 g) was added portion wise. The reaction mixture was hydrogenated at room temperature at 50 psi for 3 h. The suspension was filtered through a Celite pad and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (ethyl ether/petroleum ether 1:1), to afford an oil (yield: 90%).

4-Phenyl-3,4,10-triaza-tricyclo[9.4.0.2,6]pentadeca-1(15),2,5,11,13-pentaen-9-one (23). A solution of 22 (0.30 mmol) and NaH 60% (300 mg) in anhydrous toluene (10 mL) was heated at reflux for 6 h and allowed to cool to room temperature. The mixture was diluted with water, the organic phase was separated, washed with water (10 mL), and evaporated. Compound 23 was purified by crystallization from ethyl acetate/petroleum ether. (yield: 90%)

Biology Experiments

CHO membranes preparation. The human A_1, A_{2A}, A_{2B} and A_3 receptors has been transfected in CHO cells according to the method previously described.[33] The cells were grown adherently and maintained in Dulbecco's modified Eagles 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 2 or 3 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 with Polytron and the homogenate was spun for 10 min at 1000 × g. The supernatant was then centrifuged for 30 min at 100000 × g. The membrane pellet was suspended in 50 mM Tris HCl buffer pH 7.4 (for A_3 adenosine receptors: 50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EDTA) and incubated with 3 IU/ml of adenosine deaminase for 30 min at 37 °C. Then the cell suspension was frozen at −80 °C.

Human cloned A_1, A_{2A} and A_3 Adenosine Receptor Binding Assay. All synthesized compounds have been tested for their affinity at human A_1, A_{2A} and A_3 adenosine receptors. Displacement experiments of [^{3}H]-DPCPX to CHO cells transfected with the human recombinant A_1 adenosine receptor were performed for 120 min at 25 °C in 0.2 mL of 50 mM Tris HCl buffer pH 7.4 containing 1 nM [^{3}H]-DPCPX, diluted membranes (50 μg of protein/assay) and at least 6−8 different concentrations of antagonists studied. Non specific binding was determined in the presence of 10 μM of CHA and this was always ≤ 10% of the total binding.[34]

Binding of [^{3}H]-ZM 241385 to CHO cells transfected with the human recombinant A_{2A} adenosine receptors (50 μg of protein/assay) was performed using 0.2 mL 50 mM Tris HCl buffer, 10 mM $MgCl_2$ pH 7.4 and at least 6−8 different concentrations of antagonists studied for an incubation time of 60 min at 4 °C. Nonspecific binding was determined in the presence of 1 μM ZM 241385 and was about 20% of total binding.[35]

Binding of [^{3}H]-MRE 3008F20 to CHO cells transfected with the human recombinant A_3 adenosine receptors was previously performed.[36] Competition experiments were carried out in duplicate in a final volume of 250 μL in test tubes containing 1 nM [^{3}H]-MRE 3008F20, 50 mM Tris HCl buffer, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4 and 100 μL of diluted membranes (50 μg of protein/assay) and at least 6−8 different concentrations of examined ligands for 120 min at 4 °C. Nonspecific binding was defined as binding in the presence of 1 μM MRE 3008F20 and was about 25% of total binding. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass filters which were washed three times with ice-cold buffer. Filter bound radioactivity was measured by scintillation spectrometry (LS-1800 Beckman) after addition of Aquensure liquid.

Rat cloned A_3 Adenosine Receptor Binding Assay. All synthesized compounds have been tested for their affinity to rat A_3 adenosine receptors. Binding of [^{125}I]-AB-MECA to CHO cells transfected with the rat recombinant A_3 adenosine receptors was previously performed.[37] Competition experiments were carried out in duplicate in a final volume 250 μl in test tubes containing 1 nM [^{125}I]-AB-MECA, 50 mM Tris HCl buffer, 10 mM MgCl2, pH 7.4 and 20 μl of diluted membranes (12 μg of protein/assay) and at least 6−8 different concentration of examined ligands for 60 min at 37 °C. Nonspecific binding was defined as binding in the presence of 50 μM R−PIA and was about 30% of total binding. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass fiber which were washed three times with ice-cold buffer. Filter bound radioactivity was measured by scintillation spectrometry (SL-1800 Beckman) after addition of Aquensure liquid.

Measurement of cyclic AMP levels in CHO cells transfected with human A_{2B} and A_3 adenosine receptors. CHO cells transfected with human A_{2B} or A_3 adenosine receptors were washed with phosphate-buffered saline, diluted trypsin and centrifuged for 10 min at 200 g. The pellet containing the CHO cells (1×10^6 cells/assay) was suspended in 0.5 mL of incubation mixture (mM): NaCl 15, KCl 0.27, NaH_2PO_4 0.037, $MgSO_4$ 0.1, $CaCl_2$ 0.1, Hepes 0.01, $MgCl_2$ 1, glucose 0.5, pH 7.4 at 37 °C, 2 IU/ml adenosine deaminase and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20−1724) as phosphodiesterase inhibitor and preincubated for 10 min in a shaking bath at 37 °C. The potencies of antagonists studied versus hA_{2B} adenosine receptors were determined by antagonism of NECA (100 nM)-induced stimulation of cyclic AMP levels. The potencies of antagonists examined versus hA_3 adenosine receptors were determined by antagonism of Cl−IB-MECA (100 nM)-induced inhibition of cyclic AMP levels. The reaction was terminated by the addition of cold 6% trichloroacetic 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. Samples of cyclic AMP standard (0−10 pmoles) were added to each test tube containing the incubation buffer (trizma base 0.1 M, aminophylline 8.0 mM, 2 mercaptoethanol 6.0 mM, pH 7.4) and [^{3}H] cyclic AMP in a total volume of 0.5 mL. The binding protein previously prepared from beef adrenals, was added to the samples previously incubated at 4 °C for 150 min, and after the addition of charcoal were centrifuged at 2000 g for 10 min. The clear supernatant was counted in a LS-1800 Beckman scintillation counter.

Data Analysis. The protein concentration was determined according to a Bio-Rad method[38] with bovine albumin as a standard reference. Inhibitory binding constants, K_i values, were calculated from those of IC_{50} according to Cheng & Prusoff equation[39] $K_i = IC_{50}/(1+[C^*]/K_D^*)$, where [C*] is the concentration of the radioligand and K_D* its dissociation constant. A weighted nonlinear least-squares curve fitting program LIGAND[40] was also used for computer analysis of inhibition experiments. Potency values (IC_{50}) obtained in cyclic AMP assays were calculated by nonlinear regression analysis using the equation for a sigmoid concentration−response curve (Graph PAD Prism, San Diego, CA). Affinity values are expressed as geometric mean

with 95% or 99% confidence limits in parentheses and IC_{50} values are expressed as the arithmetic mean ± s.e.mean.

Acknowledgment. We thank King Pharmaceutical Research and Development, Inc., 4000 CentreGreen Way, Suite 300 Cary, NC 27513, for financial support. We also thank Prof. Karl-Norbert Klotz for cDNA encoding the human adenosine receptors.

Supporting Information Available: Spectroscopic and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Fredhol, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J. International Union of Pharmacology XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev*. **2001**, *53*, 527−552.

(2) Jacobson, K. A.; Knutsen, L. J. S. P_1 and P_2 purine and pyrimidine receptor ligands. In: Purinergic and Pyrimidinergic Signaling; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology*, Vol 151/1; Springer: Berlin. 2001, 129−175.

(3) Poulsen, S. A.; Quinn, R. J. Adenosine receptors: new opportunities for future drugs. *Bioorg. Med. Chem.* **1998**, *6*, 619−641.

(4) Salvatore, C. A.; Jacobson, M. A.; Taylor, H. E.; Linden, J.; Johnson, R. G. Molecular cloning and characterization of the human A_3 adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A*. **1993**, *90*, 10365−9.

(5) Ali, H.; Choi, O. H.; Fraundorfer, P. F.; Yamada, K.; Gonzaga, H. M. S.; Beaven, M. A. Sustained activation of phospholipase D via adenosine A_3 receptors is associated with enhancement of antigen- and Ca(2+)-ionophore-induced secretion in a rat mast cell line. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 837−45.

(6) Murphree, L. J.; Linden, J. Adenosine Receptors. *Encyclopedia of Biological Chemistry* **2004**, *1*, 34−39.

(7) Fredholm, B. B. Adenosine receptors as targets for drug development. *Drug News & Perspectives* **2003**, *16* (5), 283−289.

(8) Brambilla, R.; Cattabeni, F.; Ceruti, S.; Barbieri, D.; Franceschi, C.; Kim, Y.; Jacobson, K. A.; Klotz, K. N.; Lohse, M. J.; Abbracchio, M. P. Activation of the A_3 adenosine receptor affects cell cycle progression and cell growth. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *361*, 225−34.

(9) Van Schaick, E. A.; Jacobson, K. A.; Kim, H. O.; IJzerman, A. P.; Danhof, M. Haemodynamic effects and histamine release elicited by the selective adenosine A3 receptor agonists 2-Cl−IB-MECA in conscious rats. *Eur. J. Pharm*. **1996**, *308*, 311−314.

(10) Von Lubitz, D. K. J.; Carter, M. F.; Deutsch, S. I.; Lin, R. C. S.; Mastropaolo, J.; Meshulam, J.; Jacobson, K. A. The effects of adenosine A_3 receptor stimulation on seizures in mice. *Eur. J. Pharmacol.* **1995**, *275*, 23−29.

(11) Von Lubitz, D. K. J. V.; Lin, R. C. S.; Popik, P.; Carter, M. F.; Jacobson, K. A. Adenosine A_3 receptor stimulation and cerebral ischemia. *Eur. J. Pharmacol.* **1994**, *263*, 59−67.

(12) Mackenzie, W. M.; Hoskin, D. W.; Blay, J. Adenosine inhibits the adhesion of anti-CD3-activated killer lymphocytes to adenocarcinoma cells through an A_3 receptor. *Cancer Res.* **1994**, *54*, 3521−3526.

(13) Meade, C. J.; Mierau, J.; Leon, I.; Ensinger, H. A. In vivo role of the adenosine A_3 receptor-N6−2-(4-aminophenyl)ethyladenosine induces bronchospasm in BDE rats by a neurally mediated mechanism involving cells resembling mast cells. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 1148−1156.

(14) Forsythe, P.; Ennis, M. Adenosine, mast cells and asthma. *Inflamm. Res.* **1999**, *48*, 301−307.

(15) Jacobson, K. A.; Tchilibon, S. J.; Bhalchandra, V.; Gao, Z.-G. A3 Adenosine Receptors. *Annual Reports in Medicinal Chemistry* **2003**, *38*, 121−130.

(16) Nieber, K.; Lewerenz, A.; Hentschel, S.; Vissiennon, Z. Adenosine A_1 and A_3 receptors: Neuroprotective targets with perspectives. *Bioforum* **2002**, *25* (4), 237−240.

(17) Colotta, V.; Cecchi, L.; Catarzi, D.; Filacchioni, G.; Martini, C.; Tacchi, P.; Lucacchini, A. Synthesis of some tricyclic heteroaromatic systems and their A_1 and A_{2a} adenosine binding activity. *Eur. J. Med. Chem*. **1995**, *30*, 133−139.

(18) Catarzi, D.; Cecchi, L.; Colotta, V.; Filacchioni, G.; Martini, C.; Tacchi, P.; Lucacchini, A. Tricyclic heteroaromatic systems. Synthesis and A_1 and A_{2A} adenosine binding activities of some 1-aryl-1,4-dihydro-3-methyl[1]benzopyrano[2,3-*c*]pyrazol-4-ones, 1-aryl- 4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-ones, and 1-aryl-1*H*-imidazo[4,5-*b*]quinoxalines. *J. Med. Chem.* **1995**, *38*, 1330−1336.

(19) Colotta, V.; Catarzi, D.; Varano, F.; Cecchi, L.; Filacchioni, G.; Martini, C.; Trincavelli, L.; Lucacchini, A. 4-Amino-6-benzylamino-1,2-dihydro-2-phenyl-1,2,4-triazolo[4,3*a*] quinoxalin-1-one: a new A_{2A} adenosine receptor antagonist with high selectivity versus A1 receptors. *Arch. Pharm. (Weinheim, Ger.* **1999**, *332*, 39−41.

(20) Colotta, V.; Catarzi, D.; Varano, F.; Cecchi, L.; Filacchioni, G.; Martini, C.; Trincavelli, L.; Lucacchini, A. Synthesis and structure−activity relationships of a new set of 2-arylpyrazolo[3,4-*c*]quinoline derivatives as adenosine receptor antagonists. *J. Med. Chem.* **2000**, *43*, 3118−3124.

(21) Colotta, V.; Catarzi, D.; Varano, F.; Calabri, F. R.; Lenzi, O.; Filacchioni, G.; Martini, C.; Trincavelli, L.; Delforian, F.; Moro, S. 1,2,4-triazolo[4,3-*a*]quinoxalin-1-one moiety as an attractive scaffold to develop new potent and selective human A_3 adenosine receptor antagonists: synthesis, pharmacological, and ligand−receptor modelling studies. *J. Med. Chem.* **2004**, *47*, 3580−3590.

(22) Colotta, V.; Catarzi, D.; Varano, F.; Cecchi, L.; Filacchioni, G.; Martini, C.; Trincavelli, L.; Lucacchini, A. 1,2,4-Triazolo[4,3-*a*]-quinoxalin-1-one: a versatile tool for the synthesis of potent and selective adenosine receptor antagonists. *J. Med. Chem.* **2000**, *43*, 1158−1164.

(23) Colotta, V.; Catarzi, D.; Varano, F.; Filacchioni, G.; Martini, C.; Trincavelli, L.; Lucacchini, A. Synthesis and structure−activity relationships of a new set of 1,2,4-Triazolo[4,3-*a*]quinoxalin-1-one Derivatives as Adenosine Receptor Antagonists. *Bioorg. Med. Chem*. **2003**, *11*, 3541−3550.

(24) Baraldi, P. G.; Aghazadeh Tabrizi, M.; Fruttarolo, F.; Bovero, A.; Avitabile, B.; Preti, D.; Romagnoli, R.; Merighi, S.; Gessi, S.; Varani, K.; Borea, P. A. Recent Developments in the Field of A_3 Adenosine Receptor Antagonists. *Drug Dev. Res*. **2003**, *58*, 315−329.

(25) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Klotz, K. N.; Leung, E.; Varani, K.; Gessi, S.; Meighi, 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. *J. Med. Chem.* **1999**, *42*, 4473−4478.

(26) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, S.; 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 the N8 Pyrazole Nitrogen. *J. Med. Chem*. **2000**, *43*, 4768−4780.

(27) Baraldi, P. G.; Cacciari, B.; Moro, S.; Romagnoli, R.; Ji, X. D.; Jacobson, K. A.; Gessi, S.; Borea, P. A.; Spalluto, G. Fluorosulfonyl- and Bis-(β-chloroethyl)amino-phenylamino Functionalized Pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine Derivatives: Irreversible Antagonists at the Human A_3 Adenosine Receptor and Molecular Modelling Studies. *J. Med. Chem*. **2001**, *44*, 2735−2742.

(28) Baraldi, P. G.; Cacciari, B.; Moro, S.; Spalluto, G.; Pastorin, G.; Da Ros, T.; Klotz, K. N.; Varani, K.; Gessi, S.; Borea, P. A. Synthesis, Biological Activity, and Molecular Modeling Investigation of New Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine Derivatives as Human A_3 Adenosine Receptor Antagonists. *J. Med. Chem.* **2001**, *45*, 770−780.

(29) Werbel, L. M.; Kesten, S. J.; Turner, W. R. Structure−activity relationship of antimalarial indolo[3,2-*c*]qiunolines[1,2]. *Eur. J. Med. Chem*. **1993**, *28*, 837−852.

(30) Sangwan, N. K.; Kelkar, K.; Prabhakar, M.; Rastogi, S. N.; Anand, N. Antifertility agents: Part XLIII. Synthesis of 3-aryl-4,5-dihydro-2-substituted-5-tosyl-2H-pyrazolo[4,3-c]quinolines and 2,4-dihydro-3-phenyl[1]benzopyrano[4,3-c]pyrazoles and their derivatives. *Indian journal of Chemistry, Section B: Organic Chemistry Including Medicinal Chemistry* **1985**, *24B* (6), 639−44.

(31) Baraldi, P. G.; Manfredini, S.; Periotto, V.; Simoni, D.; Guarneri, M.; Borea, P. A. Synthesis and Interaction of 5-(Substituted-phenyl)-3-methyl-6,7-dihydropyrazolo[4,3-*e*][1,4]diazepin-8(7*H*)-ones with Benzodiazepine Receptors in Rat Cerebral Cortex. *J. Med. Chem.* **1985**, *28*, 683−685.

(32) Xu, Y.-C.; Schaus, J. M.; Walker, C.; Krushinski, J.; Adham, N.; Zgombick, J. M.; Liang, S. X.; Kohlman, D. T.; Audia, J. E. *N*-Methyl-5-*tert*-butyltryptamine: A Novel, Highly Potent 5-HT1D Receptor Agonist. *J. Med. Chem.* **1999**, *42*, 526−531.

(33) Klotz, K. N.; Hessling, J.; Hegler, J.; Owman, C.; Kull, B.; Fredholm, B. B.; Lohse, M. J. Comparative pharmacology of human adenosine receptor subtypes − characterization of stably transfected receptors in CHO cells. *Naunyn Schmiedeberg's Arch Pharmacol.* **1998**, *357*, 1−9.

(34) Borea, P. A.; Dalpiaz, A.; Varani, K.; Gessi, S.; Gilli, G. Binding thermodynamics at A_1 and A_{2A} adenosine receptors. *Life Sciences* **1996**, *59*, 1373−1388.

(35) Borea, P. A.; Dalpiaz, A.; Varani, K.; Gessi, S.; Gilli, G. Binding thermodynamics of adenosine A_{2A} receptor ligands. *Biochem. Pharmacol.* **1995**, *49*, 461−469.
(36) Varani, K.; Merighi, S.; Gessi, S.; Klotz, K. N.; Leung, E.; Araldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Borea, P. A. [^{3}H]-MRE 3008-F20: a novel antagonist radioligand for the pharmacological and biochemical characterization of human A_3 adenosine receptors. *Mol. Pharmacol.* **2000**, *57*, 968−975.
(37) Varani, K.; Cacciari, B.; Baraldi, P G.; Dionisotti, S.; Ongini, E.; Borea, P. A. Binding affinità of adenosine receptor agonists and antagonists at human A_3 adenosine receptors. *Life Sciences* **1998**, *63*, 81−87.
(38) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *7*, 248−54.
(39) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *1*, 3099−108.
(40) Munson, P. J.; Rodbard, D. Ligand: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **1980**, *107*, 220−39.

JM050125K