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Synthesis and Biological Evaluation of Metabolites of 2-*n*-Butyl-9-methyl-8-[1,2,3]triazol-2-yl-9*H*-purin-6-ylamine (ST1535), A Potent Antagonist of the A_{2A} Adenosine Receptor for the Treatment of Parkinson's Disease

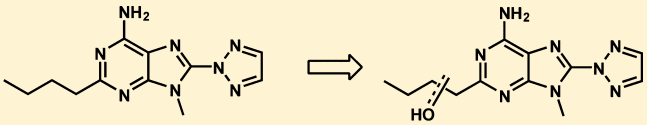
Giovanni Piersanti,*[,†] Francesca Bartoccini,[†] Simone Lucarini,[†] Walter Cabri,[‡] Maria Antonietta Stasi,[‡] Teresa Riccioni,[‡] Franco Borsini,[‡] Giorgio Tarzia,[†] and Patrizia Minetti*[,‡]

[†]Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino, Piazza Rinascimento 6, I-61029 Urbino (PU), Italy

[‡]Sigma-Tau Industrie Farmaceutiche Riunite S.p.A., Via Pontina Km 30,400, I-00040 Pomezia, Italy

Supporting Information

ABSTRACT: The synthesis and preliminary in vitro evaluation of five metabolites of the A_{2A} antagonist ST1535 (**1**) are reported. The metabolites, originating in vivo from enzymatic oxidation of the 2-butyl group of the parent compound, were synthesized from 6-chloro-2-iodo-9-methyl-9*H*-purine (**2**) by selective C−C bond formation via halogen/magnesium exchange reaction and/or palladium-catalyzed reactions. The metabolites behaved in vitro as antagonist ligands of cloned human A_{2A} receptor with affinities (K_i 7.5−53 nM) comparable to that of compound **1** (K_i 10.7 nM), thus showing that the long duration of action of **1** could be in part due to its metabolites. General behavior after oral administration in mice was also analyzed.



INTRODUCTION

The high attrition rate associated with drug discovery has prompted increasing attention to the metabolism of the early drug candidates as a relevant factor in successful drug development.[1] Although in most cases the metabolism of drugs leads to pharmacologically inactive compounds, sometimes active or toxic metabolites are found. Species-dependent metabolic transformation add further complexity to the development process, and for these reasons metabolite identification and examination have become an integral part of early drug discovery programs.[2] Knowledge of the metabolic pathway of drug candidates is useful to improve their safety and pharmacological profile. We recently reported a series of 8-(2*H*-1,2,3-triazol-2-yl)purine derivatives as potent A_{2A} adenosine receptor antagonists (Figure 1) for their potential utility in the treatment of Parkinson's disease (PD).[3] Compound **1** (ST1535), a member of this series, has been evaluated in various pharmacological models of Parkinson's disease.[4]

Investigation of the in vitro metabolic fate of **1** by liquid chromatography/mass spectrometry (LC/MS) indicated the presence of four metabolites with MW = MW_{ST1535} + 16, accompanied by small amounts of two other oxidized compounds with MW = MW_{ST1535} + 14 (data not shown).[5] In humans, the principal metabolite is a product with MW = MW_{ST1535} + 14.[6] In all cases, MS of the isolated metabolites indicated enzymatic oxidation of the 2-butyl group. Several possibilities for hydroxylation, oxidation, and allylic oxidation were considered, and we report here the synthesis, in vitro affinity, and intrinsic activity on adenosine receptors of compounds (Figure 1) that match the physicochemical properties of five of the isolated metabolites. Moreover, general behavior after oral administration in mice was evaluated.

CHEMISTRY

The procedure that was selected for the synthesis of the 6-amino-2-(hydroxyalkyl)-9-methyl-8-(2*H*-1,2,3-triazol-2-yl)-9*H*-purines is based on the general I−Mg exchange reaction of 2,6-dihalopurines proposed by Dvořák[7] to yield **3**, which was then transformed into compound **14** or on the C2-selective palladium-catalyzed cross-coupling of terminal alkynes with 2,6-dihalopurines to give **4**, **5a−c**, and **6**, which are then transformed into **15**, **16a−c**, **17**, and **18** (Scheme 1), respectively. In the case of halogen/Mg exchange, it is known that chlorine cannot be exchanged in the reaction of 6-chloropurine derivatives with *i*PrMgCl,[8] whereas selective exchange of iodine for magnesium in mixed 6-chloro-2-iodopurine derivatives are reported.[7] In the case of palladium-catalyzed cross-coupling reaction, some reports[9] corroborate our hypothesis that cross-coupling reactions with 6-chloro-2-iodopurine intermediates would show selectivity for alkynylation[9a] in the 2-carbon position. Products of such reactions would carry a chlorine atom suitable for further synthetic transformations such as cross-coupling reactions or nucleophilic substitution.

The synthesis of the purine derivatives **14−18** was pursued according to the method adopted for the synthesis of **1**[10] from the readily available 6-chloro-2-iodopurine (**2**) (Scheme 1).

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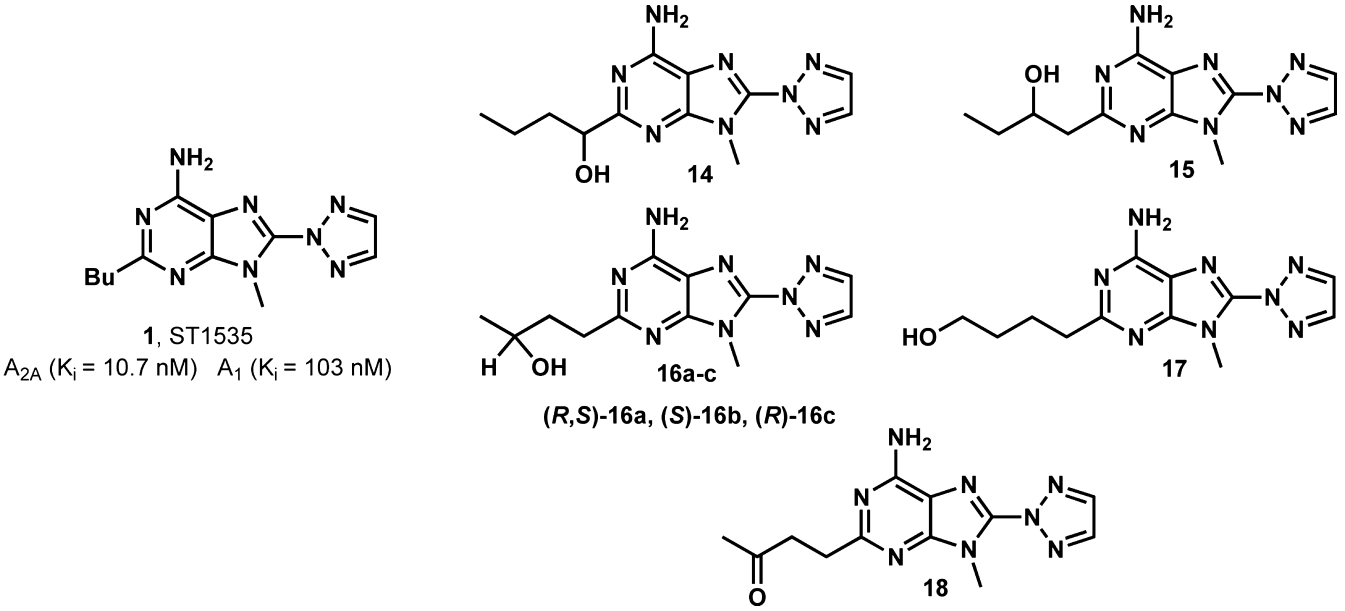
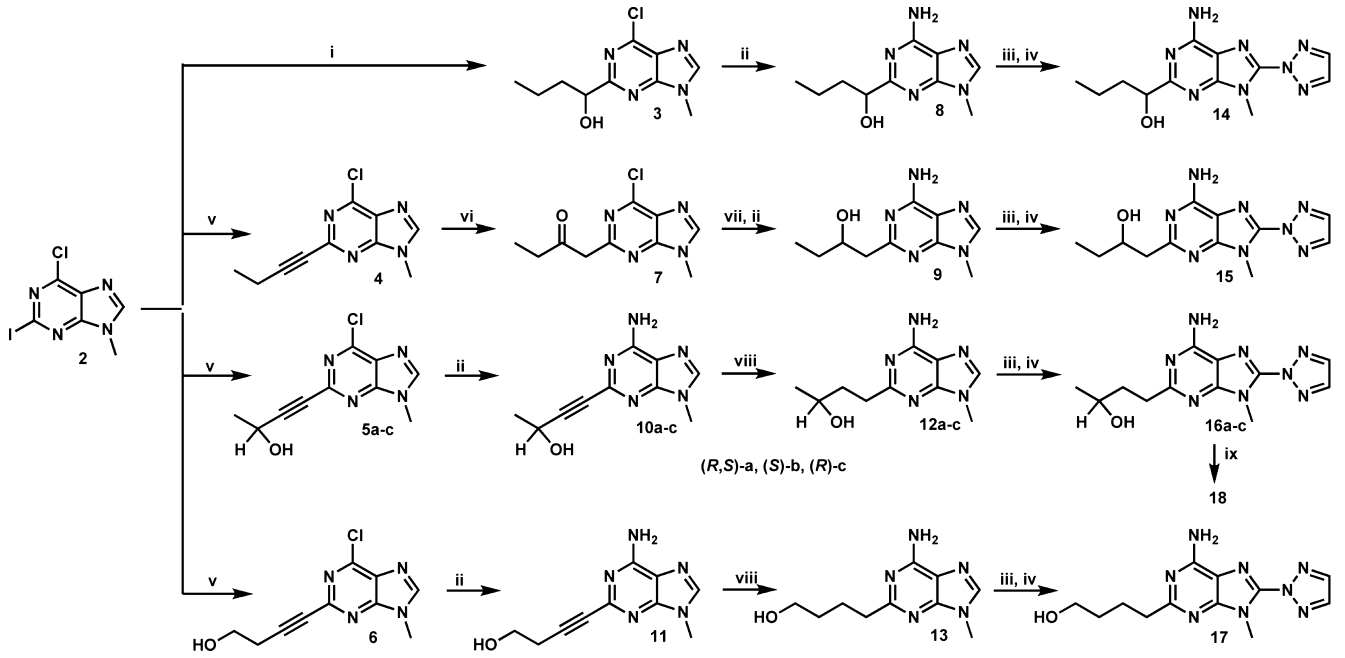


Figure 1. Structure of five metabolites of **1**.

Scheme 1. Synthesis of Metabolites 14−18[a]



[a]Reagents and conditions: (i) (1) THF, *i*PrMgCl, −78 °C, (2) $CH_3(CH_2)_2CHO$, −78 °C to rt, 20 h; (ii) NH_3(aq)/dioxane, 70 °C, 16 h; (iii) MeOH, THF, acetate buffer pH 4, Br_2, −14 °C to rt, 20 min; (iv) DMF, Cs_2CO_3, 1*H*-1,2,3-triazole, 90 °C, 16 h; (v) dioxane, alkyne, TEA, $Pd(PPh_3)_2Cl_2$, CuI, rt, 1 h; (vi) (1) $TiCl_4$, *t*-$BuNH_2$, toluene, 1,3,5-$Me_3C_6H_2NH_2$, 120 °C, 14 h, (2) HCl 2N, rt, 1 h; (vii) MeOH, $NaBH_4$, rt, 1 h; (viii) EtOH, H_2 (4 atm), Pd/C, 50 °C, 40 h; (ix) DMSO, THF, IBX, rt, 20 h.

We began by investigating the halogen-magnesium exchange reaction with 6-chloro-2-iodo-9-methyl-9*H*-purine (**2**). Reaction of 2 with *i*-PrMgCl at −78 °C, followed by addition of butanaldehyde at the same temperature, afforded the expected 6-chloro-2-(1-hydroxybutyl)purine derivative **3** in 59% yield. However, when the I−Mg exchange reaction was performed at 0 °C and addition of butanaldehyde, purine derivatives with the secondary alcohol moiety at the 8 position was obtained in 41% yield, similarly to that found by Dvořák.[7]

As evidence of the regioselectivity, ^{13}C NMR spectroscopy revealed the disappearance of the signal of the C2−I of **2** (δ 116.0 ppm) and appearance of a new signal for C2-CHOH (δ 166.3 ppm). Furthermore, the structure of compound **3** was confirmed by its mass spectrum (ESI+: 241−243), which showed the presence of the two isotopes of the chlorine atom in a 3/1 ratio. This regioselectivity is similar to that observed in Sonogashira or Suzuki coupling when applied to the same dihalopurine (**2**). Regioselective amination of the 6 position of **3** with aqueous ammonia in dioxane in a sealed tube at 60 °C yielded the 6-amino compound (**8**). Selective nucleophilic bromination at 8-position using bromine in buffered solution (pH 4), followed by reaction with 1*H*-1,2,3-triazole, gave **14** in

moderate yield from **8**, as reported with the unfunctionalized alkyl derivatives (Scheme 1).[9]

Purine-derived Grignard reagents react with aldehydes to produce substituted purine derivatives bearing α-hydroxyalkyl groups in the 2-position, which are not easily accessible by other methods. We sought to extend this procedure to the synthesis of β-hydroxyalkyl derivative, such as compound **15**, by treating the 6-chloro-2-magnesiated purines with the appropriate epoxide (1,2-epoxybutane). Using different conditions and catalyst systems, the epoxide-opening reaction failed to give the desired product as did the use of purine copper intermediates prepared by oxidative addition of highly reactive zerovalent copper.[11]

Although a wide variety of C6 substituted purine nucleosides bearing β-functionalized alkyl groups are known, the same cannot be said for the C2 position.[12] After several unsuccessful attempts,[13] we chose to synthesize the β-hydroxyalkyl derivative via a Sonogashira type coupling with butyne, followed by regioselective hydration of the substituted alkyne and reduction of the resulting ketone. We then focused on the preparation of purine derivatives having an acetylenic side chain and on the regioselective (β) hydration of unsymmetrical carbon−carbon triple bond in order to provide direct access to β ketones. Preparation of 2-alkynylpurines from 2-halogenated purine is effectively catalyzed by palladium in the presence of copper and a base.[9a] Thus, when butyne was bubbled through a homogeneous reddish solution containing **2**, $Pd(PPh_3)_2Cl_2$, and CuI in TEA until the reaction mixture turned black, the regioselective formation of **4** was achieved in high yield. We investigated the conversion of alkynyl purine into ketone **7** by hydration. Traditionally, mercury(II) compounds or expensive transition-metal catalysts have been employed to give the corresponding Markovnikov product,[14] whereas a general method for selective anti-Markovnikov hydration reaction of internal alkynes has proven unsuccessful.[15] Therefore, we explored the methodology developed by Ackermann et al. for the efficient regioselective indirect anti-Markovnikov hydration of unsymmetrically substituted internal alkynes, consisting of $TiCl_4$-catalyzed hydroamination and subsequent hydrolysis of the generated imine/enamine to the desired ketone.[15d] On that basis, treatment of **4** with the sterically hindered *t*-butylamine and mesitylamine in toluene in the presence of catalytic amount of $TiCl_4$, followed by treatment with acidic water, afforded the regioselective ketone **7** in moderate yield. Standard $NaBH_4$ reduction gave chemoselectively[16] the β secondary alcohol **9**, which yielded compound **15** via subsequent substitution of the chlorine atom in 6-position with an amino group, bromination, and triazolation at C8.

This palladium−copper-catalyzed cross-coupling reaction can be extended further to include other hydroxyl functionalized terminal alkynes.[17] For example, racemic propargyl alcohol (3-butyn-2-ol) and its enantiomers are commercially available. Reaction of these agents with **2** under the above cross-coupling conditions gave the alkynes **5a−c** in excellent yields. No base-promoted isomerization from intermediates was detected.[18] The hydroxyl-bearing terminal alkyne, 3-butyn-1-ol, was used with **2** under standard Sonogashira alkynylation condition to afford the primary alcohol **6** in good yield.

Unfortunately, attempted hydrogenation of **5a−c**, and **6** resulted in both alkyne reduction and dehalogenation. The dehalogenation result was circumvented by selective C2 amination of **5a−c** and **6**, followed by hydrogenation of the resulting adenines **10a−c** and **11** to furnish **12a−c** and **13**, respectively, in almost quantitative yields, even though higher temperature and longer times were required. Finally, the standard procedure of C-8 bromination and subsequent reaction with triazole gave compounds **16a−c** and **17**. Ketone **18** was obtained from **16a** by oxidation with 2-iodoxybenzoic acid.

RESULTS AND DISCUSSION

The synthesized compounds were evaluated for their binding affinity for cloned hA_1 and hA_{2A} receptors and for their intrinsic activity on the hA_{2A} receptor (Table 1).[19]

Table 1. Binding Affinity and Inhibition Activity on Agonist-Mediated cAMP Accumulation in Human A_{2A} Receptor Overexpressing Cells and Binding Affinity to Human A_1 Receptor

compds	A_{2A} binding K_i [nM][a,b]	cAMP inhibition IC_{50} [nM][a]	A_1 binding K_i [nM][a,b]
1	11 (5.8−20)	430 (190−970)	100 (59−180)
14	22 (8.0−57)	2300 (290−18000)	160 (130−210)
15	64 (37−110)		
16a	8.4 (4.5−16)	450 (130−1600)	34 (28−40)
16b	19 (9.1−38)	260 (55−1200)	53 (43−66)
16c	7.5 (4.2−13)	420 (130−1300)	34 (26−43)
17	53 (25−110)	2100 (360−12000)	150 (120−190)
18	12 (6.7−23)	990 (160−6300)	200 (150−260)

[a]95% confidence intervals. [b]K_i values were calculated from IC_{50} values, obtained from competition curves by the method of Cheng and Prusoff, and are the mean of four determinations performed in triplicate.

The main objective was to investigate the activities of the metabolites but in the process limited insight into the structure−activity relationships of the adenine structural class was obtained. Table 1 shows that all metabolites behave as adenosine receptor antagonists at the human A_{2A} receptor with affinities and intrinsic activities comparable to that of **1** (0.16−1.40-fold) and (0.20−1.40-fold), respectively. This indicates that metabolic hydroxylation of the butyl chain retains **1**-like activity. Compared to compound **1**, the hydroxylated metabolites **14**, **15**, and **17** showed slightly decreased affinity toward the hA_{2A} receptor, whereas hydroxylation on the butyl side chain in γ position leads to metabolite **16a** that is slightly more active than the parent compound, confirming that highly flexible substituents can be accepted in this area by the A_{2A} receptor, as reported.[3,20]

Assuming that **1** and its metabolites dock into the A_{2A} receptor similarly to ZM 241385,[20a] the alkyl and hydroxyalkyl side chain would fit into the lipophilic cavity defined by transmembrane helices VI and VII, in which one ordered water molecule is present. Presumably, metabolite **16a**, in contrast to **1**, will form a hydrogen-bonding water-mediated interaction with His264 or Asn253. Although it was not the intention of this work, in order to further refine this hypothesis, it would be

useful to screen a larger group of compounds and perform more rigorous modeling to probe the potential interactions of water within the binding pocket. Despite of enantiomers of chiral compounds are generally recognized to behave differently in biochemical processes, the enatioselectivity of the A_{2A} receptors was poor, as evidenced by the fact that the enantiomers of **16a**, i.e. (*S*)-**16b** and (*R*)-**16c**, showed similar affinity and activity. The lack of stereoselectivity in the recognition of the two enantiomers as well as the substituent freedom in this area of the A_{2A} receptor was confirmed by the comparable affinity and intrinsic activity values obtained with the ketone **18**.

When examined at the human A_1 receptor, compounds **16a**–**c** bind with higher affinity than **1**. In the case of the two enantiomers **16b** and **16c**, the (*R*)-isomer binds with higher affinity than the (*S*)- form at both the hA_{2A} and hA_1 receptor. Whether this is true also in the case of the enantiomers of compounds **14** (Figure 1) has yet to be verified.

The metabolites with higher affinity on the hA_{2A} receptor in vivo **14**, **16a**, and **18** were evaluated by Irwin test. As expected, three metabolites tested induced reactivity and stereotyped movements, either limbic (sniffing) or striatal (biting and licking) at all doses. For **16a** and **18** these behaviors were still present at long times (240 min) after dosing, showing that they are retained in the CNS for a time sufficiently to contribute to the activity of ST1535.

■ CONCLUSIONS

Five metabolites of the antiparkison agent **1**, which are formed in vivo by enzymatic oxidation of the 2-butyl chain, were evaluated as adenosine hA_{2A} and hA_1 receptor antagonists. The metabolites were synthesized from 6-chloro-2-iodo-9-methyl-9*H*-purine in four steps by reaction with butanal or the appropriate butyne derivative.

The affinity data for the hA_{2A} and hA_1 adenosine receptor indicate that enzymatic hydroxylation (oxidation) of the 2-butyl side chain can modulate potency and intrinsic activity and that the long lasting pharmacological activity of **1** is due to these metabolites.

■ EXPERIMENTAL SECTION

Chemistry. *General Methods.* All reactions were run in air unless otherwise noted. Column chromatography purifications were performed in flash chromatography conditions using Merck 230–400 mesh silica gel. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates (Silica Gel 60 F254) that were visualized by exposure to ultraviolet light. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 200 spectrometer, using $CDCl_3$, CD_3OD, acetone-d_6, or DMSO-d_6 as solvent. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. Coupling constants (J values) are given in hertz (Hz). ESI-MS spectra was taken on a Waters Micromass ZQ instrument, and only molecular ions (M + 1) are given. Enantiomeric purity were determined on an HPLC instrument (Chiracel AD-H column; mobile phase hexane/*i*-PrOH 9:1, flux 1.0 mL min^{-1}, λ = 287 nm). Optical rotation analysis was performed using a Perkin-Elmer 241 polarimeter using a sodium lamp (λ 589 nm, D-line); $[\alpha]_D^{20}$ values are reported in 10^{-1} deg cm^2 g^{-1}; concentration (c) is in g per 100 mL. IR spectra were obtained on a Nicolet Avatar 360 FT-IR spectrometer, absorbances are reported in cm^{-1}. Melting points were determined on a Buchi SMP-510 capillary melting point apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba analyzer, and the results are within ±0.4 of the theoretical values (C, H, N). Purity of tested compounds was greater than 95%.

Starting Materials. Butyraldehyde, but-1-yne, but-3-yn-2-ol, (*R*)-but-3-yn-2-ol, (*S*)-but-3-yn-2-ol, but-3-yn-1-ol, and 1*H*-1,2,3-triazole are commercially available. 6-Chloro-2-iodo-9-methyl-9*H*-purine (**6**) and 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX) were prepared from 2-amino-6-chloro-9*H*-purine[10] and *o*-iodobenzoic acid,[21] respectively, as reported.

*1-(6-Chloro-9-methyl-9H-purin-2-yl)butan-1-ol (**3**).* To a solution of 6-chloro-2-iodo-9-methyl-9*H*-purine (**2**) (3.7 g, 12.6 mmol) in anhydrous THF (48 mL) at −78 °C under an atmosphere of nitrogen was added isopropylmagnesium chloride (2 M solution in THF, 7.6 mL, 15.1 mmol). After 30 min, butyraldehyde (1.7 mL, 16.4 mmol) was added at −78 °C dropwise. The reaction mixture was stirred at −78 °C for 8 h and then allowed to warm to room temperature overnight. The reaction was quenched with a saturated solution of NH_4Cl (20 mL). The organic solvent was evaporated under reduced pressure, and the aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure. The residue obtained was purified by flash chromatography (gradient from CH_2Cl_2/MeOH 98:2 to CH_2Cl_2/MeOH 96:4) to give **3** (1.8 g, 59%). MS (ESI) (m/z): 241–243 [M + H]$^+$. ^{1}H NMR (CD_3OD) δ 0.96 (t, J = 7.0 Hz, 3H), 1.38–1.53 (m, 2H), 1.81–1.90 (m, 2H), 3.95 (s, 3H), 4.83 (t, J = 6.5 Hz, 1H), 8.47 (s, 1H). ^{13}C NMR (CD_3OD) δ 12.8, 18.4, 29.2, 38.9, 73.8, 129.1, 147.5, 149.6, 152.5, 166.3. FTIR (nujol, cm^{-1}): 3405, 2959, 2873; mp 165–166 °C (acetone/petroleumeum ether). Anal. Calcd for $C_{10}H_{13}ClN_4O$ (240.08): C, H, N.

General Procedure for Sonogashira Reaction. To a solution of 6-chloro-2-iodo-9-methyl-9*H*-purine (**2**) (6.8 g, 23.3 mmol), CuI (454 mg, 2.23 mmol), and bis-triphenylphosphine palladium dichloride (811 mg, 1.15 mmol) in dioxane (93 mL) were added triethylamine (4.9 mL, 34.5 mmol) and the appropriate alkyne (25.6 mmol) (butyne was bubbled via the dispersion tube until the reaction mixture turns black, about 30 min). The reaction mixture was stirred at room temperature for 1 h. The volatiles were removed under reduced pressure, and water (100 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (4 × 150 mL). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure.

*2-(But-1-ynyl)-6-chloro-9-methyl-9H-purine (**4**).* The residue was purified by flash chromatography (gradient from CH_2Cl_2/MeOH 98:2 to CH_2Cl_2/MeOH 95:5) to obtained a brown oil (4.31 g, 84%). MS (ESI) (m/z): 221–223 [M + H]$^+$. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.5 Hz, 3H), 2.50 (q, J = 7.5 Hz, 2H), 3.92 (s, 3H), 8.10 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 13.0, 13.1, 30.5, 78.7, 91.9, 130.5, 146.0, 146.2, 150.8, 152.1. FTIR (nujol, cm^{-1}): 2935, 2218. Anal. Calcd for $C_{10}H_9ClN_4$ (220.05): C, H, N.

*4-(6-Chloro-9-methyl-9H-purin-2-yl)but-3-yn-2-ol (**5a**).* The residue was purified by flash chromatography (CH_2Cl_2/MeOH 94:6) to obtained a white solid (4.18 g, 76%). MS (ESI) (m/z): 237–239 [M + H]$^+$. ^{1}H NMR (acetone-d_6) δ 1.50 (d, J = 6.0 Hz, 3H), 3.95 (s, 3H), 4.77 (m, 2H), 8.51 (s, H). ^{13}C NMR (acetone-d_6) δ 23.5, 57.31, 57.39, 81.4, 89.9, 130.6, 144.7, 148.2, 149.2, 152.7. FTIR (nujol, cm^{-1}): 3375, 3061, 2230; mp 121–122 °C (acetone/petroleumeum ether). Anal. Calcd for $C_{10}H_9ClN_4O$ (236.05): C, H, N.

*(S)-4-(6-Chloro-9-methyl-9H-purin-2-yl)but-3-yn-2-ol (**5b**).* The residue was purified by flash chromatography (CH_2Cl_2/MeOH 94:6) to obtained a white solid (4.12 g, 75%). The chemical–physical data were the same as those of the racemate; mp 130–131 °C (acetone–petroleum ether); $[\alpha]_D^{20}$ −26.4 (c 0.189 in MeOH). Anal. Calcd for $C_{10}H_9ClN_4O$ (236.05): C, H, N.

*(R)-4-(6-Chloro-9-methyl-9H-purin-2-yl)but-3-yn-2-ol (**5c**).* The residue was purified by flash chromatography (CH_2Cl_2/MeOH 94:6) to obtained a white solid (4.07 g, 74%). The chemical-physical data were the same as those of the racemate; mp 130–131 °C (acetone–petroleum ether). $[\alpha]_D^{20}$ +26.8 (c 0.201 in MeOH). Anal. Calcd for $C_{10}H_9ClN_4O$ (236.05): C, H, N.

*4-(6-Chloro-9-methyl-9H-purin-2-yl)but-3-yn-1-ol (**6**).* The residue was purified by flash chromatography (CH_2Cl_2/MeOH 94:6) to obtain an off-white solid (4.12 g, 75%). MS (ESI) (m/z): 237–239 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 2.62 (t, J = 6.5 Hz, 2H), 3.61 (br s, 2H), 3.82 (s, 3H), 4.97 (br s, 1H), 8.64 (s, 1H). ^{13}C NMR (DMSO-

d_6) δ 23.3, 30.6, 59.6, 80.6, 88.1, 130.4, 144.7, 148.9, 149.3, 152.8. FTIR (nujol, cm^{-1}): 3416, 2934, 2360, 2342, 2238; mp 151−152 °C (EtOH). Anal. Calcd for $C_{10}H_9ClN_4O$ (236.05): C, H, N.

1-(6-Chloro-9-methyl-9H-purin-2-yl)butan-2-one (7). *t*-$BuNH_2$ (365 μL, 3.48 mmol) was added to a solution of $TiCl_4$ 1 M in CH_2Cl_2 (580 μL, 0.58 mmol) in toluene (7.5 mL) under N_2. Mesitylamine (613 μL, 4.4 mmol) and **4** (640 mg, 2.9 mmol) were added. The solution was stirred at 120 °C for 14 h. The mixture was cooled at room temperature, then aqueous HCl (2 N, 20 mL) was addedand stirred at 20 °C for 1 h. The solution was basified with aqueous solution of Na_2CO_3 2N and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and the solvent evaporated under reduce pressure. The residue obtained was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to give **7** as an oil (330 mg, 48%). MS (ESI) (*m*/*z*): 239−241 [M + H]$^+$. ^{1}H NMR ($CDCl_3$) δ 1.09 (t, *J* = 7.5 Hz, 3H), 2.62 (q, *J* = 7.5 Hz, 2H), 3.91 (s, 3H), 4.16 (s, 2H), 8.07 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 7.7, 30.3, 36.2, 52.6, 132.0, 145.6, 150.6, 152.7, 159.0, 206.6. FTIR (nujol, cm^{-1}): 2923, 1716. Anal. Calcd for $C_{10}H_{11}ClN_4O$ (238.06): C, H, N.

General Procedure for Amination. To a solution of the appropriate 6-chloro-2-(hydroxyalkynyl)-9-methyl-9*H*-purine derivatives (**3**, **7**, **5a**−**c**, or **6**) (20 mmol) in dioxane (30 mL) was added 30% w/w water solution of ammonia (60 mL). The reaction mixture was stirred in an autoclave at 70 °C for 16 h. The solution was evaporated at atmospheric pressure at 50 °C and then under reduced pressure.

1-(6-Amino-9-methyl-9H-purin-2-yl)butan-1-ol (8). The residue was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to obtain an off-white solid (3.5 g, 79%). MS (ESI) (*m*/*z*): 222 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 0.87 (t, *J* = 7.0, 3H), 1.25−1.43 (m, 2H), 1.51−1.86 (m, 2H), 3.71 (s, 3H), 4.37−4.46 (m, 1H), 4.77 (d, *J* = 5.5 Hz, 1H), 7.24 (br s, 2H), 8.06 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 14.9, 19.4, 30.3, 40.0, 73.9, 118.3, 142.3, 151.2, 156.5, 166.4. FTIR (nujol, cm^{-1}): 3405, 2959, 2873; mp 185−186 °C (EtOH). Anal. Calcd for $C_{10}H_{15}N_5O$ (221.13): C, H, N.

1-(6-Amino-9-methyl-9H-purin-2-yl)butan-2-ol (9). To a solution of 1-(6-chloro-9-methyl-9*H*-purin-2-yl)butan-2-one (**10**) (180 mg, 0.75 mmol) in MeOH (9 mL) was added $NaBH_4$ (84 mg, 2.25 mmol). The mixture was stirred at room temperature for 1 h, and water (30 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to yield a white solid which was treated immediately with concentrated aqueous ammonia (see general procedure for 0.75 mmol). The residue obtained was purified by flash chromatography (gradient from CH_2Cl_2/MeOH 98:2 to CH_2Cl_2/MeOH 94:6) to obtain an yellowish oil (139 mg, 91%). MS (ESI) (*m*/*z*): 222 [M + H]$^+$. ^{1}H NMR ($CDCl_3$) δ 1.00 (t, *J* = 7.5 Hz, 3H), 1.54−1.63 (m, 2H), 2.78−3.01 (m, 2H), 3.77 (s, 3H), 3.99 (br d, *J* = 6.0 Hz, 1H), 6.14 (br s, 2H), 7.70 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 10.0, 29.8, 29.8, 44.1, 71.5, 117.8, 140.7, 150.5, 154.8, 164.0. FTIR (nujol, cm^{-1}): 3320, 3115, 2970; mp not determinable. Anal. Calcd for $C_{10}H_{15}N_5O$ (221.13): C, H, N.

4-(6-Amino-9-methyl-9H-purin-2-yl)but-3-yn-2-ol (10a). The residue was purified by flash chromatography (CH_2Cl_2/MeOH 93:7) to obtain a yellowish solid (3.39 g, 78%). MS (ESI) (*m*/*z*): 218 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 1.42 (d, *J* = 6.5 Hz, 3H), 3.67 (s, 3H), 4.54 (br s, 1H), 5.59 (br s, 1H), 7.35 (br s, 2H), 8.13 (s,1H). ^{13}C NMR (DMSO-d_6) δ 24.7, 29.9, 56.8, 83.1, 87.6, 130.0, 142.7, 145.6, 150.3, 156.1. FTIR (nujol, cm^{-1}): 3355, 2239; mp 138−140 °C (EtOH). Anal. Calcd for $C_{10}H_{11}N_5O$ (217.10): C, H, N.

(S)-4-(6-Amino-9-methyl-9H-purin-2-yl)but-3-yn-2-ol (10b). The residue was purified by flash chromatography (CH_2Cl_2/MeOH 93:7) to obtained an yellowish solid (3.34 g, 77%). The chemical−physical data were the same as those of the racemate; mp 146−148 °C (EtOH); $[\alpha]_D^{20}$ −24.9 (*c* 0.202 in MeOH). Anal. Calcd for $C_{10}H_{11}N_5O$ (217.10): C, H, N.

(R)-4-(6-Amino-9-methyl-9H-purin-2-yl)but-3-yn-2-ol (10c). The residue was purified by flash chromatography (CH_2Cl_2/MeOH 93:7) to obtain a yellowish solid (3.33 g, 77%). The chemical−physical data were the same as those of the racemate; mp 146−148 °C (EtOH); $[\alpha]_D^{20}$ +24.7 (*c* 0.198 in MeOH). Anal. Calcd for $C_{10}H_{11}N_5O$ (217.10): C, H, N.

4-(6-Amino-9-methyl-9H-purin-2-yl)but-3-yn-1-ol (11). The residue was crystallized from dioxane to obtained an off-white solid (3.08 g, 71%). MS (ESI) (*m*/*z*): 218 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 2.52 (t, *J* = 7.0 Hz, 2H), 3.53−3.63 (m, 2H), 3.68 (s, 3H), 4.91 (t, *J* = 5.5 Hz, 1H), 7.25 (br s, 2H), 8.09 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 23.2, 29.8, 60.0, 82.2, 83.5, 118.5, 142.6, 146.0, 150.4, 156.1. FTIR (nujol, cm^{-1}): 3368, 2241; mp 158−159 °C (EtOH). Anal. Calcd for $C_{10}H_{11}N_5O$ (217.10): C, H, N.

General Procedure for Hydrogenation. The appropriate 6-amino-2-(hydroxylalkynyl)-9-methyl-9*H*-purine derivatives (**10a**−**c**, **11**) (6.5 mmol) was placed in an autoclave with EtOH (30 mL), and palladium 10% on graphite (20% in weight) was added. The mixture was stirred under 4 atm of hydrogen at 50 °C for 40 h (After 15 h was added further hydrogen). The hot mixture was filtered through Celite, washed with hot EtOH (5 × 20 mL), and the solution obtained was evaporated under reduced pressure to give a residue that was used for the following reaction without further purification.

4-(6-Amino-9-methyl-9H-purin-2-yl)butan-2-ol (12a). Off-white solid (1.19 g, 83%). An analytical sample was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) for the characterization. MS (ESI) (*m*/*z*): 222 [M + H]$^+$. ^{1}H NMR (CD_3OD) δ 1.21 (d, *J* = 6.5 Hz, 3H), 1.90 (m, 2H), 2.85 (m, 2H), 3.35 (br s, 2H), 3.84 (s, 3H), 3.89 (m, 1H), 8.00 (s, 1H). ^{13}C NMR (CD_3OD) δ 22.0, 28.7, 35.0, 37.7, 66.9, 116.6, 141.5, 150.4, 155.6, 165.4. FTIR (nujol, cm^{-1}): 3405, 2959, 2873; mp 145−146 °C (EtOH). Anal. Calcd for $C_{10}H_{15}N_5O$ (221.13): C, H, N.

(S)-4-(6-Amino-9-methyl-9H-purin-2-yl)butan-2-ol (12b). Off-white solid (1.15 g, 80%). An analytical sample was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) for the characterization. The chemical−physical data were the same as those of the racemate; mp 152−153 °C (EtOH); $[\alpha]_D^{20}$ +6.2 (*c* 1.835 in MeOH). Anal. Calcd for $C_{10}H_{15}N_5O$ (221.13): C, H, N.

(R)-4-(6-Amino-9-methyl-9H-purin-2-yl)butan-2-ol (12c). Off-white solid (1.16 g, 81%). An analytical sample was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) for the characterization. The chemical−physical data were the same as those of the racemate; mp 152−153 °C (EtOH). $[\alpha]_D^{20}$ −5.8 (*c* 1.646 in MeOH). Anal. Calcd for $C_{10}H_{15}N_5O$ (221.13): C, H, N.

4-(6-Amino-9-methyl-9H-purin-2-yl)butan-1-ol (13). Off-white solid (1.0 g, 70%). An analytical sample was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) for the characterization. MS (ESI) (*m*/*z*): 222 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 1.38−1.52 (m, 2H), 1.65−1.80 (m, 2H), 2.64 (t, *J* = 7.5 Hz, 2H), 3.37−3.43 (m, 2H), 3.67 (s, 3H), 4.34 (br s, 1H), 7.01 (br s, 2H), 7.97 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 25.5, 29.7, 32.8, 39.0, 61.1, 117.4, 141.3, 151.0, 156.1, 164.9. FTIR (nujol, cm^{-1}): 3368, 2241; mp 167−168 °C (EtOH). Anal. Calcd for $C_{10}H_{15}N_5O$ (221.13): C, H, N.

General Procedure for Bromination and Displacement with Triazole. Bromine (2.1 mL, 41 mmol) was added dropwise, at −14 °C, to the appropriate 6-amino-2-(hydroxyalkyl)- 9-methyl-9*H*-purine derivatives (**8**, **9**, **12a**−**c**, **13**) (3.6 mmol) dissolved in a mixture of MeOH (10 mL), THF (10 mL), and acetate buffer pH = 4 (10 mL) (obtained by dissolving 4 g of NaOAc in 100 mL of water and adjusting with HOAc). The reaction was stirred at this temperature for 10 min and then at room temperature for 10 min. Excess of bromine was eliminated with $Na_2S_2O_5$ and the reaction brought to pH 8−9 by addition of saturated solution of $NaHCO_3$. The organic solvents were evaporated under reduced pressure, and the aqueous phase was extracted with CH_2Cl_2 (6 × 50 mL). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 6-amino-8-bromo-2-(hydroxyalkyl)-9-methyl-9*H*-purine derivatives that was used for the substitution with 1*H*-1,2,3-triazole without further purification. To a solution of the appropriate 6-amino-8-bromo-2-(hydroxyalkyl)-9-methyl-9*H*-purine derivatives (3.6 mmol) in anhydrous DMF (15 mL) were added Cs_2CO_3 (4.69 g, 14.4 mmol) and then 1*H*-1,2,3-triazole (0.83 mL, 14.4 mmol). The mixture was stirred at 90 °C for 16 h. The solvents were evaporated under reduced pressure, and the residue obtained was suspended in water and

extracted with CH_2Cl_2 (4 × 80 mL). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure.

*1-(6-Amino-9-methyl-8-(1H-1,2,3-triazol-2-yl)-9H-purin-2-yl)-butan-1-ol (**14**).* The residue was purified by flash chromatography (CH_2Cl_2/MeOH 97:3) and ($CHCl_3$/MeOH 95:5) to obtained a white solid (311 mg, 30%). MS (ESI) (*m/z*): 289 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 0.87 (t, *J* = 7.0, 3H), 1.27−1.45 (m, 2H), 1.55−1.88 (m, 2H), 3.82 (s, 3H), 4.41−4.50 (m, 1H), 4.84 (d, *J* = 5.0 Hz, 1H), 7.53 (br s, 2H), 8.33 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 14.0, 18.4, 30.4, 39.0, 73.1, 115.0, 137.7, 141.3, 150.5, 155.7, 166.8. FTIR (nujol, cm^{-1}): 3405, 2959, 2873; mp 186−188 °C (EtOH). Anal. Calcd for $C_{12}H_{16}N_8O$ (288.14): C, H, N.

*1-(6-Amino-9-methyl-8-(1H-1,2,3-triazol-2-yl)-9H-purin-2-yl)-butan-2-ol (**15**).* The residue was purified by flash chromatography (CH_2Cl_2/MeOH 96:4) and ($CHCl_3$/MeOH 95:5) to obtained a white solid (338 mg, 26%). MS (ESI) (*m/z*): 289 [M + H]$^+$. ^{1}H NMR ($CDCl_3$) δ 1.05 (t, *J* = 7.5 Hz, 3H), 1.56−1.69 (m, 2H), 2.92 (dd, *J* = 15.5 and 9.0 Hz, 1H), 3.06 (dd, *J* = 15.5 and 3.0 Hz, 1H), 4.01−4.09 (m, 1H), 4.09 (s, 3H), 6.16 (br s, 2H), 7.78 (br s, 1H), 8.04 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 10.1, 29.8, 31.4, 44.3, 71.5, 115.3, 137.3, 142.1, 151.0, 154.7, 164.8. FTIR (nujol, cm^{-1}): 3346, 3125, 2958, 1664; mp 144−146 °C (EtOH). Anal. Calcd for $C_{12}H_{16}N_8O$ (288.14): C, H, N.

*4-(6-Amino-9-methyl-8-(1H-1,2,3-triazol-2-yl)-9H-purin-2-yl)-butan-2-ol (**16a**).* The residue obtained was purified by two flash chromatography (CH_2Cl_2/MeOH 92:8 and then $CHCl_3$/MeOH 95:5) to obtained an off-white solid (279 mg, 27%). MS (ESI) (*m/z*): 289 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 1.09 (d, *J* = 6.0 Hz, 3H), 1.78 (m, 2H), 2.72 (m, 2H), 3.66 (m, 1H), 3.77 (s, 3H), 4.45 (br s, 1H), 7.32 (br s, 2H), 8.29 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 24.0, 30.6, 35.9, 38.5, 66.2, 114.9, 138.0, 141.4, 151.2, 156.2, 166.4. FTIR (nujol, cm^{-1}): 3346, 3125, 2958, 1664; mp 153−155 °C (acetone/petroleum ether). Anal. Calcd for $C_{12}H_{16}N_8O$ (288.14): C, H, N.

*(S)-6-Amino-9-methyl-8-(1H-1,2,3-triazol-2-yl)-9H-purin-2-yl)-butan-2-ol (**16b**).* The residue obtained was purified by two flash chromatography (CH_2Cl_2/MeOH 92:8 and then $CHCl_3$/MeOH 95:5) to obtained an off-white solid (285 mg, 27%). The chemical−physical data were the same as those of the racemate; $[\alpha]_D^{20}$ +1.01 (*c* 1.29 in MeOH); mp 145−147 °C (acetone/petroleum ether). Anal. Calcd for $C_{12}H_{16}N_8O$ (288.14): C, H, N.

*(R)-6-Amino-9-methyl-8-(1H-1,2,3-triazol-1-yl)-9H-purin-2-yl)-butan-2-ol (**16c**).* The residue obtained was purified by two flash chromatography (CH_2Cl_2/MeOH 92:8 and then $CHCl_3$/MeOH 95:5) to obtained an off-white solid (290 mg, 28%). The chemical−physical data were the same as those of the racemate. $[\alpha]_D^{20}$ −1.3 (*c* 1.23 in MeOH); mp 145−147 °C (acetone/petroleum ether). Anal. Calcd for $C_{12}H_{16}N_8O$ (288.14): C, H, N.

*4-(6-Amino-9-methyl-8-(1H-1,2,3-triazol-2-yl)-9H-purin-2-yl)-butan-1-ol (**17**).* The residue was purified by flash chromatography (CH_2Cl_2/MeOH 93:7) to obtained a white solid (311 mg, 30%). MS (ESI) (*m/z*): 289 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 1.39−1.53 (m, 2H), 1.68−1.82 (m, 2H), 2.67 (t, *J* = 7.5 Hz, 2H), 3.27−3.42 (m, 2H), 3.77 (s, 3H), 4.39 (br s, 1H), 7.38 (br s, 2H), 8.32 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 25.4, 30.6, 32.8, 39.1, 61.0, 114.9, 138.1, 141.4, 151.1, 156.2, 166.2. FTIR (nujol, cm^{-1}): 3318, 3101, 1664, 1608, 1587; mp 191−193 °C (EtOH). Anal. Calcd for $C_{12}H_{16}N_8O$ (288.14): C, H, N.

*4-(6-Amino-9-methyl-8-(2H-1,2,3-triazol-2-yl)-9H-purin-2-yl)-butan-2-one (**18**).* IBX (1.5 g, 5.3 mmol), freshly prepared, was added to a solution of **16a** (520 mg 1.8 mmol) in THF (1 mL) and DMSO (8.5 mL). The mixture was stirred at room temperature for 20 h. Water was added and the solution was basified by addition of sature solution of $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure. The residue obtained was purified by flash chromatography (CH_2Cl_2/MeOH 97:3) to give **18** (400 mg, 78%) as a white solid. MS (ESI) (*m/z*): 287 [M + H]$^+$. ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H), 3.07 (m, 4H), 4.04 (s, 3H), 5.71 (bs, 1H), 8.00 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 30.2, 31.2, 32.9, 41.2, 115.4, 137.1, 142.1, 151.4, 154.8, 164.7, 208.2. FTIR (nujol, cm^{-1}): 3293, 3101, 1713; mp 214−216 °C (EtOH). Anal. Calcd for $C_{12}H_{14}N_8O$ (286.13) C, H, N.

Procedure for O-Acetylation. To a solution of 6-amino-2-(hydroxyalkyl)-9-methyl-8-(1*H*-1,2,3-triazol-2-yl)-9*H*-purine derivatives (**16a−c**) (20 mg, 0.07 mmol) in pyridine (0.7 mL) was added acetic anhydride (9 mg, 0.084 mmol). The solution was stirred at room temperature for 1 h. The homogeneous solution was loaded directly onto a silica gel column, and the product was purified by flash chromatography (CH_2Cl_2/MeOH 95:5) yielding the title compound (18 mg, 78%) as a white solid. The enantiomeric excess was determined to be >96% for **16b** and >98% for **16c** by chiral HPLC analysis (chiracel AD-H column; hexane/*i*-PrOH 9:1, flux 1.0 mL min^{-1}, λ = 287 nm).

Pharmacology. *In Vitro Studies. Adenosine hA$_1$ Receptor Binding Assay.* Binding affinity to the adenosine hA$_1$ receptor (Perkin-Elmer) was evaluated using membranes from human recombinant CHO-K1 cells stably transfected with the human adenosine A$_1$ receptor (10 μg of protein/sample) with a single concentration of [^{3}H]DPCPX (1.7 nM) in the presence of various concentrations (0.01−10000 nM) of test compounds for 60 min at 25 °C in a total volume of 200 μL/tube of 25 mM Hepes, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 100 mM NaCl, pH = 7.4. Nonspecific binding was determined in the presence of 10 μM cold DPCPX (8-cyclopentyl-1,3-dipropylxanthine, Sigma Aldrich).

Adenosine hA$_{2A}$ Receptor Binding Assay. Binding affinity to the adenosine hA$_{2A}$ receptor (Perkin-Elmer) was evaluated using membranes from human recombinant HEK 293 cells (human embryo kidney cells) expressing the human A$_{2A}$ receptor subtype. To remove endogenous adenosine, the membrane suspension was incubated with 2 IU/mL adenosine deaminase (ADA, Sigma−Aldrich) for 30 min at 37 °C. Competition binding experiments was performed by incubating membranes (5−10 μg of protein/sample) with a single concentration of the A$_{2A}$ antagonist [^{3}H]ZM241385 (Biotrend), corresponding to its K_d (2 nM) in the presence of various concentrations (0.01−10000 nM) of test and reference compounds in 96-well filter plate for 1 h at 4 °C in a total volume of 200 μL/well of appropriate buffer (50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$). Nonspecific binding was determined in the presence of 10 μM of unlabeled ZM241385. Four experiments were performed in triplicate by Janus automated workstation.

Measurement of cAMP Level. cAMP quantitative determination was performed with an enzyme immunoassay system (catalogue RPN2255 Amersham Biosciences) according to manufacturer's instructions. In brief, HEK-293 cells, stably expressing the human adenosine A$_{2A}$ receptor gene (Perkin-Elmer), were plated on 96-well dishes at a concentration of 10^3 cells/well 48 h before test compound exposure. Before cell stimulation with the compounds, cells were treated for 10 min at 37 °C with 0.5 mM of the phosphodiesterase inhibitor Ro20-1724 (Sigma−Aldrich) and with 2 IU/mL of ADA (ADA, Sigma−Aldrich). Medium was changed to medium containing scalar concentrations of test compounds (from 10^{-4} to 10^{-10} M) at 37 °C and, after 10 min, 100 nM NECA, an adenosine A$_{2A}$ agonist, was added for an additional 20 min. At the end of treatment, cAMP was extracted and quantitated.

In Vivo Study. Irwin Test. General behavioral observations in CD-1 mice were recorded by camera (Charles River, Milan), 5−6 weeks old (4 animals/group), 60 and 240 min after oral 20, 100, and 200 mg/kg administration by Irwin test. Stereotypies such as sniffing, licking, and biting were scored. The compounds were suspended in a solution containing 0.1% Tween 80 in 0.5% CMC (medium viscosity, Sigma-Aldrich Milan) and administered in a volume of 10 mL/kg to each animal.

Data Evaluation. Data were analyzed using nonlinear regression with GraphPad PRISM commercial software. Test compound concentrations causing a half-maximal inhibition of control values (IC_{50} calculated by GraphPad Prism software) were calculated from values of four independent experiments. For binding experiments only, inhibitory binding constants (K_i) were calculated from 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 its dissociation constant.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H NMR and ^{13}C NMR spectra for compounds **3**−**18**. Copies of HPLC chromatogram for compounds **16a**−**c**. Irwin test of compounds **14**, **16a**, and **18**. Binding profile for compound **14**, **16a**, **17**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*For G.P.: phone, +390722303320; fax, +390722303313; E-mail, giovanni.piersanti@uniurb.it. For P.M.: E-mail, patrizia.minetti@virgilio.it.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

PD, Parkinson's disease; DMF, dimethylformamide; THF, tetrahydrofuran; IC_{50}, half-maximal inhibitory concentration; IBX, 2-iodoxybenzoic acid; DMSO, dimethyl sulfoxide; TEA, triethylamine; CHO, Chinese hamster ovary; NECA, *N*-ethylcarboxamidoadenosine

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(5) LC/MS/MS, relative abundance, species distribution, and in vivo pharmacological evaluation of the metabolites of **1** (compounds **14**−**18**) will be reported elsewhere.

(6) Possible chemical structure consistent with MW_{ST1535} + 14 would result from hydroxylation of the 2-*n*-butyl side chain followed either by oxidation of the resulting alcohols to ketones, then confirmed to be **18**, or by dehydration and allylic oxidation to β,γ unsaturated alcohols.

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