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Adenosine Receptor Agonists: Synthesis and Biological Evaluation of the Diastereoisomers of 2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)NECA

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Abstract—Among the recently reported 2-(ar)alkynyl derivatives of 5′-*N*-ethylcarboxamidoadenosine (NECA), the (*R*,*S*)-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)NECA [(*R*,*S*)-PHPNECA or SCH 59761] was found to be a very potent agonist at A_1 and A_{2A} receptor subtypes, with a K_i of 2.5 nM and 0.9 nM, respectively. Furthermore, this compound showed an inhibitory activity on platelet aggregation 16-fold higher than NECA, being the most potent anti-aggregatory nucleoside reported so far. Since this compound bears a chiral carbon in the side chain, the diastereoisomer separation was undertaken both by chiral HPLC and by a stereospecific synthetic method. Binding assays have shown that the (*S*)-diastereomer is about fivefold more potent and selective than the (*R*)-diastereomer as agonist of the A_{2A} receptor subtype [(*S*)-PHPNECA, K_iA_{2A} = 0.5 nM; (*R*)-PHPNECA, K_iA_{2A} = 2.6 nM]. Functional studies indicated that (*S*)-PHPNECA possesses marked vasodilating activity and produces a relevant decrease in heart rate. Moreover, the (*S*)-diastereomer proved to be about ten times more potent than the (*R*)-diastereomer in inducing cardiovascular effects, in in vivo hemodynamic studies. However, the greatest difference between these two enantiomers resulted in the platelet aggregation test: in fact, the (*R*)-diastereomer displayed an inhibitory activity similar to that of NECA, whereas the (*S*)-diastereomer was 37-fold more active than NECA as an inhibitor of rabbit platelet aggregation, induced by ADP. These data suggest that (*S*)-PHPNECA could be a useful tool to investigate the mode of binding of agonists to the platelet adenosine receptor subtype. © 1997 Elsevier Science Ltd.

Introduction

A variety of studies have demonstrated, on the basis of biochemical and pharmacological experiments, that most adenosine actions are mediated by at least four extracellular receptors: A_1, A_{2A}, A_{2B} and A_3.[1,2]

Recently we reported that the introduction of (ar)alkynyl chains on the carbon in the 2 position of 5′-*N*-ethylcarboxamidoadenosine (NECA) greatly improved A_{2A} adenosine receptor affinity and selectivity.[3–5] One of the findings of our studies was that derivatives bearing an hydroxyl group α to the triple bond of the side chain showed the highest platelet anti-aggregatory activity in the series.[6] Among these α-hydroxyalkynyl derivatives, *N*-ethyl-1′-deoxy-1′-[6-amino-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide[4] [(*R*,*S*)-PHPNECA or SCH 59761, Fig. 1] was found to be a very potent agonist at A_1 and A_{2A} receptor subtypes, with a K_i of 2.5 nM and 0.9 nM, respectively. Furthermore, this compound showed an inhibitory activity on platelet aggregation, induced by ADP, approximately 16-fold higher than NECA, and is, therefore, the most potent inhibitor of platelet aggregation reported so far among nucleosides.[4]

Key words: adenosine receptors, nucleosides, platelet aggregation inhibitors, diastereoisomer separation.

Since (*R*, *S*)-PHPNECA contains a chiral carbon in the side chain, the enantiomeric resolution was attempted by synthetic methods, using chiral auxiliary derivatizating agents and enantioselective synthesis, and by instrumental methods, using chiral HPLC.

We already demonstrated that A_{2A} adenosine receptors strongly differentiate between geometric isomers of NECA derivatives in terms of affinity and selectivity. Specifically, in a series of 2-alkenyl derivatives of NECA, the *E*-diastereomers proved to be more potent and selective than the *Z*-isomers.[7,8] Hence, the present research was aimed both at finding more potent platelet

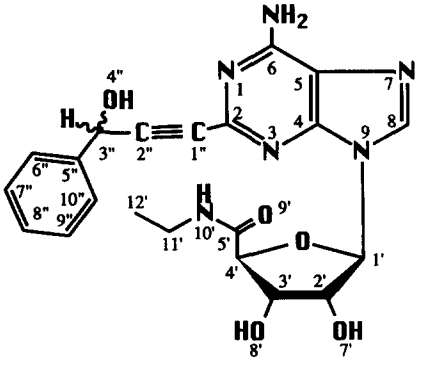


Figure 1. Structure of (*R*, *S*)-PHPNECA (1).

aggregation inhibitors and at better understanding the conformational requirement of the region of the A_{2A} and A_1 receptor area interacting with the substituents in the 2-position of NECA derivatives.

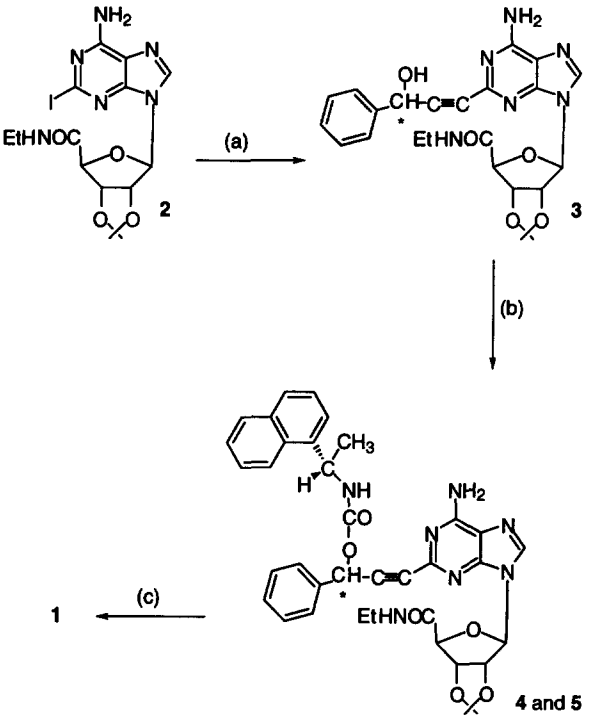
Chemistry

Synthetic method of separation of (*R*, *S*)-PHPNECA using chiral auxiliary

The synthesis of the diastereoisomer mixture *N*-ethyl-1′-deoxy-1′-[6-amino-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)-9*H*-purin-9-yl]-2′,3′-*O*-isopropyliden-β-D-ribofuranuronamide (**3**) was carried out starting from *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-2′,3′-*O*-isopropyliden-β-D-ribofuranuronamide[5] (**2**). Compound **2** was coupled at room temperature with (±)-1-phenyl-2-propyn-1-ol in the presence of cuprous iodide and triphenylphosphine palladium dichloride as catalysts to provide racemic **3**.

The diastereoisomer mixture **3** was derivatized by heating it to reflux with the chiral auxiliary (*R*)-1-(1-naphthyl)ethylisocyanate in dry chloroform in the presence of a catalytic amount of *N*,*N*-dimethylethanolamine for 48 h, as reported in Scheme 1. The diastereomeric mixture was separated by preparative TLC. The two carbamates **4** and **5** were obtained and their purity was checked by high performance TLC, ^{1}H NMR, and polarimetric analysis. The results are reported in Table 1. The absolute configurations were assigned by literature data for similar carbamate diastereomers:[9] in our case the compound that showed a higher R_f was the *R*–*R* diastereomer.

The cleavage of the auxiliary group, after many attempts, was performed using 50% formic acid at 60 °C, that also removed the 2′,3′-*O*-isopropylidene protecting group. The enantiomeric purity of the two compounds (a) PHPNECA and (b) PHPNECA was checked by chiral HPLC. Unfortunately, the result indicates that the hydrolysis of the carbamates, derived from propargylic alcohols, brought about racemization of compounds (probably due to high acidity or reactivity of the propargylic hydrogen).

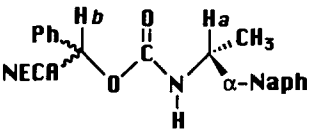


Scheme 1. (a) (±)-1-Phenyl-2-propyn-1-ol; (b) (*R*)-1-(1-naphthyl)-ethylisocyanate, *N*,*N*-dimethylethanolamine; (c) = 50% HCOOH.

Diastereoselective synthesis and molecular modeling of (*R*)- and (*S*)-PHPNECA (7 and 8)

The synthesis of pure derivatives was carried out by using a modification of the palladium-catalysed cross-coupling reaction. *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide[3] (2-iodoNECA, **6**) was in turn coupled with the recently commercially available (*R*)- and (*S*)-1-phenyl-2-propyn-1-ol, as reported in Scheme 2. Treatment of a solution of 2-iodoNECA (**6**) in a mixture of dry acetonitrile, DMF and triethylamine with cuprous iodide, triphenylphosphine palladium dichloride and the appropriate pure (*S* or *R*) alkyne, at room temperature for several hours in an atmosphere of nitrogen, gave the (*R*)-(**7**) and (*S*)-PHPNECA (**8**) isomers, respectively. It should be noted that the configuration changed, according to IUPAC

Table 1. Analytical data of diastereomers **4** and **5**



Compound	R_f[a]	pf	H[a]	H[b]	$[\alpha]^{20}_D$[b]
4 (*R*-*R*)	0.64	150–153 °C	5.52	6.28	−69.1
5 (*S*-*R*)	0.61	147–150 °C	5.53	6.32	−34.2

[a] R_f (chromatographic resolution factor) was calculated using high performance TLC (Whatman HP-KF), eluting with $CHCl_3$:cC_6H_{12}:CH_3CN:CH_3OH (80:10:5:5).
[b] Polarimetric analysis was performed in $CHCl_3$.

rules,[10] although no inversion of the chiral centre occurred (i.e., from the (*R*)-chain we get the (*S*)-nucleoside and vice versa, see Scheme 2).

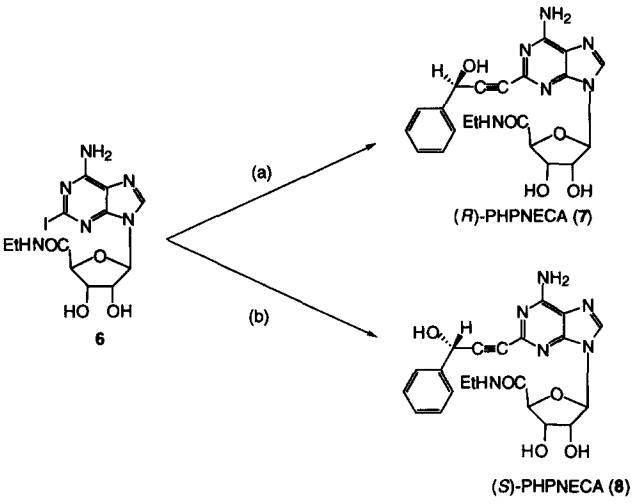
The cross coupling reaction involved only the ethynylic hydrogen of the triple bond since the enantiomeric excess of each compound was similar to that of the starting chain. The diastereoisomer purity was assessed by proton NMR spectral data. In fact, the proton NMR spectrum of the isomeric mixture (taken in DMSO-d_6 or CD_3OD) showed two triplets corresponding to the *N*-ethylcarboxamido methyl signal. The splitting of these methyl signals is reported in Table 2. Accordingly, the spectra of the two diastereoisomers [(*R*)- and (*S*)-PHPNECA] showed only one signal corresponding to the methyl, clearly excluding the presence of the other diastereomer up to the sensitivity of NMR apparatus.

The hypothesis that a possible interaction of the methyl group with some parts of the chiral center could account for this phenomenon has been supported using in vacuo simulation molecular modeling. The 3-D models were built following the crystal data of NECA as a template, in which the compound is reported in *syn* conformation.[11]

The local minima structures of (*S*)- and (*R*)-PHPNECA (Fig. 2, structures **a** and **b**), obtained in MM^+ force field,[12] showed that the distance between the methyl group and the phenyl ring is of about 4 Å, hence the methyl group is inside the inductive magnetic field of the phenyl ring (anisotropic effect).

A RHF/AM[13] semi-empiric single point method gave the static properties of diastereomers such as the atomic charges used for conformational analysis and the electrostatic map on the purine slab. Subsequently, the conformational analysis, using a 'random search', is applied at the three main torsion angles: (i) O6′-C1′-N9-C8, (ii) C5′-N10′-C11′-C12′, and (iii) C1″-C2″-C3″-C5″. In this analysis the set of *anti* and *syn* conformers are fairly isoenergetic: in fact they differ less than 1 kcal/mol from the predicted minimized structures, built following the literature data.[11,14,15]

The *syn* conformation has been selected for our study, since it has been previously reported that 2-alkynyl derivatives of NECA displayed in solution a *syn* conformation of the adenine base towards the sugar moiety and a possible hydrogen bond between the amidic NH and the endocyclic N-3.[14] The presence of this hydrogen bond has been confirmed by our model in which the distance between NH and N-3 is less than 2.2 Å (Fig. 2, a and b). Furthermore, the superimposition



Scheme 2. Synthesis of (*R*) and (*S*) PHPNECA. (a) (*S*)-Phenyl-2-propyn-1-ol; (b) (*R*)-1-phenyl-2-propyn-1-ol.

of the minimized structures of the two diastereomers on the flat purine plane (Fig. 2, frontal view c and lateral view d) showed the main difference in the phenyl moiety.

Some differences were also found in the electrostatic map of the two compounds such as different electrostatic field of hydroxyl group in the area near the methyl core (Fig. 3).

Separation of (*R,S*)-PHPNECA using chiral HPLC

The separation of the two diastereomers was also performed using chiral HPLC and some attempts, together with their operating conditions, are reported in Table 3.

The column Chiralcel OD-J was chosen for its best resolution. The absolute configuration of the compounds was assigned according to the above reported NMR method: the first peak (retention time: 15.57 min) corresponded to (*R*)-PHPNECA whereas the second (retention time: 22.19 min) corresponded to (*S*)-PHPNECA.

Results and Discussion

Adenosine receptor binding affinity

The interaction of the new 2-alkynyl diastereoisomer derivatives (*R*)- and (*S*)-PHPNECA (**7** and **8**) with A_1 and A_{2A} adenosine receptors was evaluated using

Table 2. Chemical shift of methyl groups in 5′ position of compounds **7** and **8**

	(*R*)-PHPNECA (7)	(*S*)-PHPNECA (8)	*J* (Hz)	Δδ
Chemical shift in DMSO-d_6 (ppm)	0.954	0.937	7.2	0.017
Chemical shift in CD_3OD (ppm)	1.035	1.022	7.2	0.013

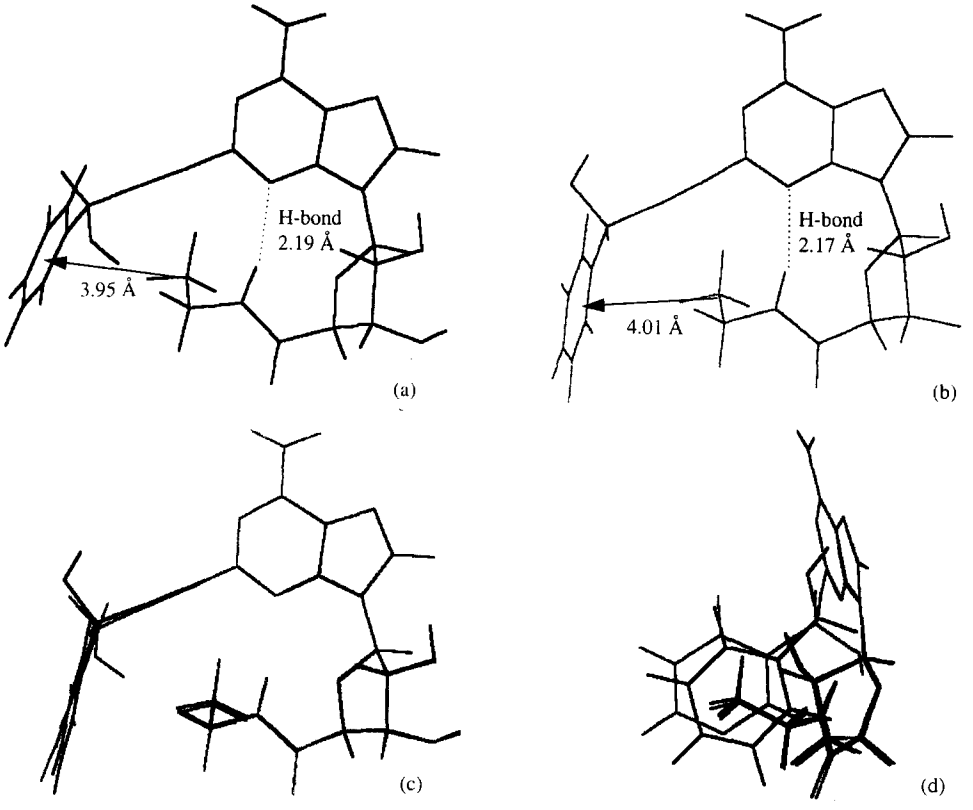


Figure 2. Molecular modeling studies. Figures **a** and **b** correspond to the minimized 3-D structures of (*R*)- and (*S*)-PHPNECA (black and gray, respectively). Superimposition of minimized structures are reported in **c** (frontal view) and in **d** (lateral view).

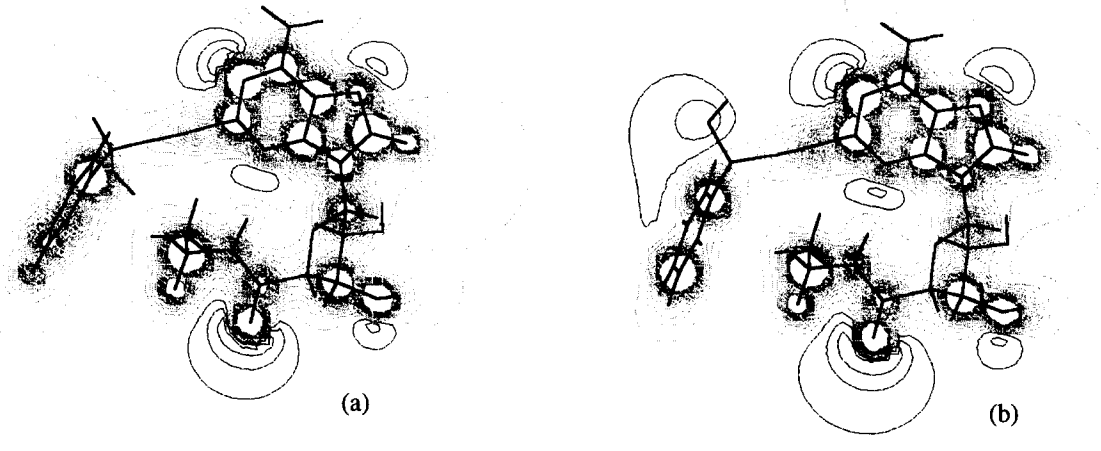


Figure 3. Electrostatic isopotential maps for (*R*)- and *S*)-PHPNECA (**a** and **b**, respectively). The potential surface has been calculated in the plane of purine moiety. Regions of negative potential have been drawn in dark and the area of positive potential has been drawn in gray. The value of external lines is of 0.18 Kcal/mol.

radioligand binding techniques. Affinity for A_{2A} receptors was determined in competition assays in rat striatum using [^{3}H]CGS 21680 as radioligand.[16] Affinity for A_1 receptors was determined in competition assays in rat brain using [^{3}H]CHA.[17]

HENECA[3] (A_{2A} selective agonist), CCPA[18] (A_1 selective agonist), and (*R*, *S*)-PHPNECA[4] were included as reference compounds. The results are reported in Table 4 and showed that the (*S*)-diastereomer **8** is about fivefold more potent at A_{2A} receptor subtype than the (*R*)-diastereomer **7** [**8**, K_iA_{2A} = 0.5 nM; **7**, K_iA_{2A} = 2.6

Table 3. Separation conditions of the diastereomer mixture using HPLC

Column	Mobile phase	Flow rate	Separation results
Chiralcel OD-H (Dialcel Chemical Industries Ltd)	20% EtOH:Hexane	0.3	Good
Chiralcel OJ (Dialcel Chemical Industries Ltd)	20% EtOH:0.1% Et_2NH:Hexane	0.8	Excellent[a]
Chiralcel OD-*R* (Dialcel Chemical Industries Ltd)	100% EtOH	0.3–0.5	One peak
Whelk-01(Regis Tecn.)	100% EtOH	0.2	Sticks to column

[a]Retention time (min): 15.571 (*R*-diastereomer, 7), 22.186 (*S*-diastereomer, 8).

nM]. (*S*)-PHPNECA showed also to be slightly more selective at A_{2A} subtype than the (*R*)-diastereomer and than the mixture itself. The A_{2A} binding assay curves of the two diastereomers, compared to the *R,S*-mixture, are reported in Figure 4.

Effect in isolated tissues

Negative chronotropic activity (A_1) was assessed in spontaneously beating rat atria and vasodilatation (A_{2A}) in rat aorta, according to methods described elsewhere.[4] Results are summarized in Table 4.

These functional studies indicated that (*S*)-PHPNECA is slightly more potent than the (*R*)-diastereomer and than the diastereomer mixture itself. Therefore, both these derivatives possess a marked vasodilating activity associated with relevant decrease in heart rate (Table 4).

Platelet aggregation studies

The anti-aggregatory effect of the new alkynyl derivatives of NECA, **7** and **8**, on rabbit platelet aggregation induced by ADP is reported in Figure 5, as a potency ratio calculated versus NECA (in our experimental conditions the IC_{50} value of NECA is 0.2 μM).

A major finding emerged from this study: both the diastereomers are very potent inhibitors of platelet aggregation. However, the (*R*)-diastereomer proved to be as active as NECA, whereas the (*S*)-diastereomer was 37-fold more active.

In vivo studies

The diastereomers **7** and **8** were also tested in in vivo haemodynamic studies in spontaneously hypertensive rats (SHRs), employing telemetry instrumentation.

Baseline haemodynamic values, recorded during two hours before compound administration, were 180±7 and 125±9 mm Hg on systolic and diastolic blood pressure (BP), respectively, and 270±21 beats/min on heart rate (HR).

Based on previous experiments[19] the (*R,S*)-PHPNECA (**1**) was administered ip at a dose expected to induce a decrease of about 50 mm Hg on diastolic blood pressure

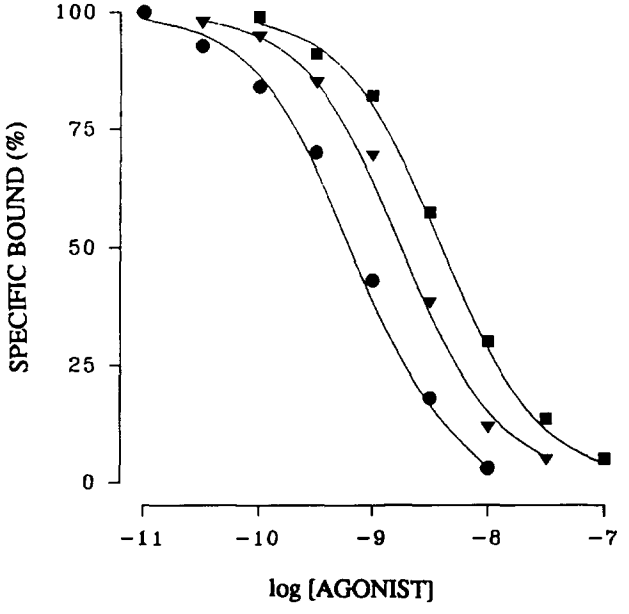


Figure 4. Mean dose–response curves for binding affinity at A_{2A} rat striatum adenosine receptor replacing [^{3}H]CGS 21680 as radioligand (● (*S*)-PHPNECA, ▼ (*R,S*)-PHPNECA, ■ (*R*)-PHPNECA).

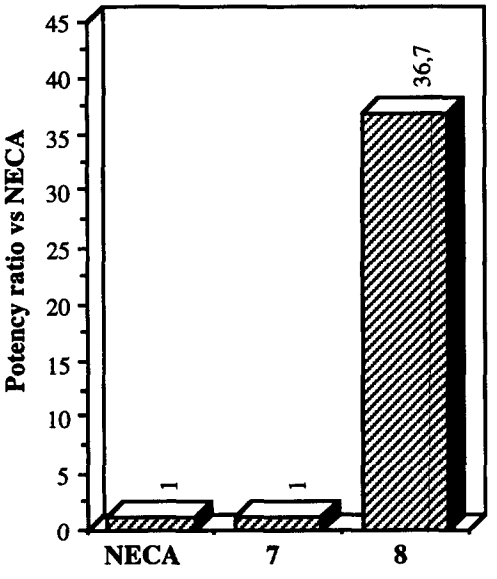


Figure 5. Anti-aggregatory activity on rabbit platelets of diastereomers (*R*)-PHPNECA (**7**) and (*S*)-PHPNECA (**8**), in comparison to NECA (IC_{50} Å, 0.2 μM). Data are means of at least three separate experiments.

Table 4. In vitro pharmacological activity of the new 2-phenylhydroxypropynyl derivatives of NECA **7** and **8**

Compounds	Binding assay[a] K_i (nM)			Functional activity[b] EC_{50} (nM)	
	Rat brain A_1	Rat striatum A_{2A}	Selectivity A_1/A_{2A}	Rat atria A_1	Rat aorta A_{2A}
HENECA[c]	130 (116–145)	2.2 (1.9–2.6)	59	>10 μM	596 (244–1460)
CCPA[c]	1.3 (1.1–1.4)	650 (555–762)	0.002	8.2 (4.4–15.3)	>10 μM
(*R,S*)-PHPNECA (**1**)[c]	2.5 (2.2–2.9)	0.9 (0.7–1.3)	2.8	110 (35.4–345)	123 (50.4–301)
(*R*)-PHPNECA (**7**)	5.9 (5.4–6.4)	2.6 (2.3–2.9)	2.3	85.6 (69.0–106)	132 (70.7–247)
(*S*)-PHPNECA (**8**)	4.0 (3.7–4.3)	0.5 (0.4–0.6)	8	52.0 (32.1–84.2)	29.9 (16.7–53.8)

[a]Receptor binding affinity at A_1 and A_{2A} receptors was determined using [^{3}H]CHA and [^{3}H]CGS21680 as radioligands, respectively. Data are geometrical means from at least three separate experiments; 95% confidence limits in parenthesis.
[b]Data are means from at least three separate experiments; 95% confidence limits in parenthesis.
[c]See ref 4.

(i.e., 0.001 mg/kg), accompanied by a reflex increase in HR. These effects reach a peak 15–30 min after injection, and last about 2 h.

These actions were confirmed in the present study (Table 5), and both PHPNECA diastereomers were tested at the same dose level. (*S*)-PHPNECA was even more effective than the diastereomeric mixture on diastolic BP, with similar increase on HR. Conversely, (*R*)-PHPNECA did not affect either BP or HR at the dose of 0.001 mg/kg, whereas at 0.01 mg/kg it produced apparent hemodynamic effects (Table 5). Hence, (*R*)-PHPNECA is about ten times less potent than the (*S*)-diastereomer in inducing cardiovascular effects.

Conclusions

The two diastereomers of PHPNECA proved to be very potent (low nM range) in rat brain A_1 and A_{2a} receptor binding and functional assays, the (*S*)-diastereomer being slightly more active and selective than the (*R*)-diastereomer.

Also in haemodynamic studies the (*S*)-diastereomer was found to be tenfold more potent than the (*R*)-diastereomer. Furthermore, these compounds proved to be extremely active in vivo as shown by the cardiovascular effects induced by a dose of 0.001 mg/kg given ip on the SHRs (Table 5).

However, a greater difference in potency was observed in the inhibition of rabbit platelet aggregation. While (*R*)-PHPNECA is equipotent with NECA, the (*S*)-diastereomer **8** showed a potency about 37-fold higher than that of the reference compound NECA.

Hence, (*S*)-PHPNECA proved to be the most potent inhibitor of platelet aggregation induced by ADP so far known among adenosine derivatives, with a IC_{50} in the low nM range. These data suggest that (*S*)-PHPNECA could be a useful tool to investigate the mode of binding of agonists to the platelet adenosine receptor subtype.

Experimental

Chemistry

Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian VX 300 MHz spectrometer. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck) and high performance TLC (Whatman HP-KF). For column chromatography, silica gel 60 (Merck) was used. Polarimetric analysis was performed with Perkin–Elmer Model 241 & 241MC apparatus.

HPLC separation was performed with the following parameters:

- Column: Chiralcel OD-J (Daicel Chemical Industries Ltd.)
- Flow rate: 0.8 mL/min (pressure: 290 ψ)
- Mobile phase: 20% EtOH:0.1% Et_3N:Hexane

Elemental analysis results are within±0.4% of theoretical values.

Table 5. Peak changes from baseline value induced by the diastereomer mixture **1** and the diastereomers **7** and **8** in conscious SHRs

Compound/dose	Diastolic blood pressure (mm/Hg)	Heart rate (Beats/min)
(*R,S*)-PHPNECA/0.001 mg/kg	−45±6	+145±23
(*R*)-PHPNECA/0.001 mg/kg	Not effective	Not effective
(*R*)-PHPNECA/0.01 mg/kg	−51±6	+129±21
(*S*)-PHPNECA/0.001 mg/kg	−53±8	+151±18

Data are means ± SE (n = 5).

The alkyne derivatives were purchased from Fluka with the following characteristics:

(*R*)-1-phenyl-2-propyn-1-ol $[\alpha]^{20}_D$ −28±2°; er [*R*]:[*S*] > 96:4, ee > 92% (GC).

(*S*)-1-phenyl-2-propyn-1-ol $[\alpha]^{20}_D$ +28±2°; er [*S*]:[*R*] > 98:2, ee > 96% (GC).

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)-9*H*-purin-9-yl]-2′,3′-*O*-isopropyliden-β-D-ribofuranuronamide (3).** To a solution of 260 mg (0.55 mmol) of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-2′,3′-*O*-isopropyliden-β-D-ribofuranuronamide[5] (**2**) in 20 mL of dry acetonitrile, 10 mL of DMF and 3 mL of triethylamine under an atmosphere of N_2 were added 10 mg (0.015 mmol) of bis(triphenylphosphine)palladium dichloride and 1 mg (0.005 mmol) of cuprous iodide. To the mixture was added (±)-1-phenyl-2-propyn-1-ol (3.2 mmol) and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for 20 h; flash chromatography on silica gel column, eluting with chloroform:cyclohexane:methanol (88:10:2) gave 182 mg (69%) of **3** as vitreous solid: ^{1}H NMR (Me_2SO-d_6) δ 0.70 (t, 3H, NCH_2CH_3), 1.36 and 1.56 (s 3H each, $C(CH_3)_2$), 2.90 (m, 2H, NCH_2CH_3), 4.56 (s, 1H, H-4′), 5.36 (s, 2H, H-2′ and H-3′), 5.62 (d, 1H, J = 5.9 Hz, *CH*OH), 6.26 (d, 1H, J = 1.1 Hz, CH*OH*), 6.29 (s, 1H, H-1′), 7.34–7.61 (m, 5H, H-Ph), 7.35 (s, 2H, NH_2), 7.67 (t, 1H, NH), 8.33 (s, 1H, H-8). Anal. calcd for $C_{24}H_{25}N_6O_5 \cdot H_2O$: C, 60.37, H, 5.28, N, 17.60; found: C, 60.55, H, 5.39, N, 17.89.

***N*-Ethyl-1′-deoxy-1′-(6-amino-2-{3-[*N*-1-(1*R*)-(1-naphtyl)-ethyl]carbamyl-(3*R*)-3-phenyl-1-propyn-1-yl}-9*H*-purin-9-yl)-2′,3′-*O*-isopropyliden-β-D-ribofuranuronamide (4) and *N*-ethyl-1′-deoxy-1′-(6-amino-2-{3-[*N*-1-(1*R*)-(1-naphtyl)ethyl]carbamyl-(3*S*)-3-phenyl-1-propyn-1-yl}-9*H*-purin-9-yl)-2′,3′-*O*-isopropyliden-β-D-ribofuranuronamide (5).** To a solution of 220 mg (0.46 mmol) of racemic **3** in dry $CHCl_3$ was added 0.1 mL (0.57 mmol) of (*R*)-1-(1-naphthyl)ethylisocyanate in the presence of catalytic amount of *N*,*N*-dimethylethanolamine and heated at 65 °C for 48 h. The purification of the two diastereomers was performed on preparative TLC eluting with chloroform: cyclohexane:methanol:acetonitrile (80:10:5:5) which gave 83 mg of **4** (27%), and 90 mg of **5** (29%) as vitreous solids.

4: ^{1}H NMR (Me_2SO-d_6) δ 0.65 (t, 3H, NCH_2CH_3), 1.34 and 1.55 (s, 3H each, $C(CH_3)_2$), 1.48 (d, 3H, J = 6.7 Hz, NaphCHCH_3), 2.85 (m, 2H, NCH_2CH_3), 4.55 (s, 1H, H-4′), 5.35 (s, 2H, H-2′ and H-3′), 5.52 (m, 1H, NH*CH*CH_3), 6.28 (s, 1H, C≡C*CH*), 6.56 (s, 1H, H-1′), 7.38–8.36 (m, 16H, H-Ph, H-Naph, 2NH, NH_2), 8.33 (s, 1H, H-8). $[\alpha]^{20}_D$ −69.1°. Anal. calcd for $C_{37}H_{37}N_7O_6 \cdot H_2O$: C, 65.77, H, 5.52, N, 14.51; found: C, 66.02, H, 5.77, N, 14.20.

5: ^{1}H NMR (Me_2SO-d_6) δ 0.71 (t, 3H, NCH_2CH_3), 1.37 and 1.57 (s, 3H each, $C(CH_3)_2$), 1.53 (d, 3H, J = 6.9 Hz, NaphCHCH_3), 2.90 (m, 2H, NCH_2CH_3), 4.57 (s, 1H, H-4′), 5.39 (s, 2H, H-2′ and H-3′), 5.53 (m, 1H, NH*CH*CH_3), 6.32 (s, 1H, C≡C*CH*), 6.56 (s, 1H, H-1′), 7.39–8.36 (m, 16H, H-Ph, H-Naph, 2NH, NH_2), 8.35 (s, 1H, H-8). $[\alpha]^{20}_D$ −34.2°. Anal. calcd for $C_{37}H_{37}N_7O_6 \cdot H_2O$: C, 65.77, H, 5.52, N, 14.51; found: C, 66.06, H, 5.71, N, 14.18.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-((3*R*)-3-hydroxy-3-phenyl-1-propyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (7).** To a solution of 170 mg (0.39 mmol) of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide[3] (**6**) in 10 mL of dry acetonitrile, 5 mL of DMF and 2.5 mL of triethylamine under an atmosphere of N_2 were added 6 mg (0.009 mmol) of bis(triphenylphosphine)-palladium dichloride and 1 mg (0.005 mmol) of cuprous iodide. To the mixture was added (*S*)-1-phenyl-2-propyn-1-ol (1.95 mmol) and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for 20 h. Chromatography on silica gel column eluting with chloroform:methanol (85:15) gave 100 mg of compound **7** (59%) as chromatographically pure solid; a sample was crystallized from a mixture of acetonitrile:methanol (9:1); mp 150–152 °C (dec); $[\alpha]^{20}_D$ −10.8; ^{1}H NMR (Me_2SO-d_6) δ 0.94 (t, 3H, CH_2CH_3), 3.18 (m, 2H, CH_2CH_3), 4.10 (m, 1H, H-3′), 4.28 (s, 1H, H-4′), 4.55 (m, 1H, H-2′), 5.58 (d, 1H, J = 8.1 Hz, C*H*OH), 5.92 (d, 1H, J = 7.5 Hz, H-1′), 6.26 (d, 1H, J = 8.1 Hz, CHO*H*), 7.36 (m, 3H, H-Ph), 7.50 (d, J = 6.9 Hz, 2H, H-Ph), 7.58 (s, 2H, NH_2), 8.45 (s, 1H, H-8), 8.58 (t, 1H, NH). Anal. calcd for $C_{20}H_{20}N_6O_5 \cdot H_2O$: C, 54.30, H, 5.01, N, 19.00; found: C, 54.48, H, 5.23, N, 18.84.

***N*-Ethyl-1′-deoxy-1-[6-amino-2-((3*S*)-3-hydroxy-3-phenyl-1-propyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (8).** To a solution of 170 mg (0.39 mmol) of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide[3] (**5**) in 10 mL of dry acetonitrile, 5 mL of DMF and 2.5 mL of triethylamine under an atmosphere of N_2 were added 6 mg (0.009 mmol) of bis(triphenylphosphine)palladium dichloride and 1 mg (0.005 mmol) of cuprous iodide. To the mixture was added (*R*)-1-phenyl-2-propyn-1-ol (1.95 mmol) and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for 20 h; chromatography on silica gel column eluting with chloroform:methanol (85:15) gave 125 mg of compound **8** (73%) as chromatographically pure solid; a sample was crystallized from a mixture of acetonitrile:methanol (9:1); mp 150–152 °C (dec); $[\alpha]^{20}_D$ −21.7°; ^{1}H NMR (Me_2SO-d_6) δ 0.95 (t, 3H, CH_2CH_3), 3.18 (m, 2H, CH_2CH_3), 4.10 (m, 1H, H-3′), 4.28 (s, 1H, H-4′), 4.55 (m, 1H, H-2′), 5.58 (d, 1H, J = 8.1 Hz, C*H*OH), 5.92 (d, 1H, J = 7.5 Hz, H-1′), 6.26 (d, 1H, J = 8.1 Hz, CHO*H*), 7.36 (m, 3H, H-Ph), 7.50 (d, J = 6.9 Hz, 2H, H-Ph), 7.58 (s, 2H, NH_2), 8.45 (s, 1H, H-8), 8.58 (t, 1H, NH). Anal. calcd for $C_{20}H_{20}N_6O_5 \cdot H_2O$: C, 54.30, H, 5.01, N, 19.00; found: C, 54.46, H, 5.20, N, 18.81.

Molecular modeling

Molecular modeling in vacuo simulation was performed using HyperChem 4.5 (HyperCube Inc.) and Chem3D Plus 3.1.1. (Cambridge Scientific Computing) software. The models were built using Chem3D Plus software. The files corresponding at two diasteromers were transferred in HyperChem package and the geometry was optimized in MM^+ force field,[12] using a RMS gradient of 0.02 Kcal/mol Å.

RHF/AMI single point calculation gave the static properties of compounds such as the atomic charges and electrostatic potential.[13]

Low energy conformers were found using 'random search' HyperChem command applied at the following torsion angles: (i) O6′-C1′-N9-C8, (ii) C5′-N10′-C11′-C12′, and (iii) C1″-C2″-C3″-C5″. They were then minimized with a RMS gradient of 0.1 Kcal/mol Å. Subsequently the lowest energy conformers were optimized until convergence of 0.02 Kcal/mol Å.

Superimposition of the two minimized diasteromers was carried out with the 'RMS overlay' HyperChem command using the N3-C4-C5-N7 torsion angles.

Biological studies

Receptor binding assays. Cerebral membranes were obtained from male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 150–200 g. Adenosine A_1 and A_{2A} receptor binding assays were performed according to Bruns et al.[17] and Jarvis et al.[16] using [^{3}H]N^6-cyclohexyladenosine ([^{3}H]CHA) and [^{3}H]2-[*p*-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H]CGS 21680), respectively. The IC_{50} values were estimated by probit models.[20] K_i values were calculated from the Cheng–Prusoff equation[21] using 1 nM as the K_d for [^{3}H]CHA and 18.5 nM for [^{3}H]CGS 21680 in A_1 and A_{2A} binding studies, respectively.

Isolated tissues. Rats were sacrificed by decapitation and both heart and thoracic aorta were removed and placed in Krebs Henseleit's solution according to a method previously described.[22] Briefly, isolated spontaneously beating rat atria were used to measure drug interaction with A_1 receptors. The decrease in heart rate evoked by cumulative addition of the agonist was measured. Vascular tissue is used specifically to measure the interaction of adenosine analogues with A_{2A} receptors. Specimens of rat aorta were cleaned of connective tissue, cut into rings and allowed to equilibrate in an organ bath. Sub-maximal contractions of vascular rings were obtained by PGF_{2a} (3 μM). The compounds were then added cumulatively and relaxation was measured isometrically. The relationship between the contractile response (y) and the log dose was modeled with a straight line after arcsin transformation of dependent variable in order to obtain least square estimates of EC_{50} values for each preparation.[23] The average dose–response function was computed as a mean constant curve (i.e., a curve whose constants are the mean of those estimated from each preparation). The effective dose of each compound was expressed as mean EC_{50} with 95% confidence limits. The analysis was carried out by SAS PROC GLM.[24]

Platelet aggregation assay. The platelet aggregation assay was performed according to the Born turbidimetric technique,[25] as previously described.[26] Compounds were dissolved in saline containing 10% of dimethyl sulphoxide (DMSO), which was present in the platelet rich plasma at a final concentration of 0.3%. The maximal amplitude of aggregation, recorded 5 min after the addition of ADP 5 μM, was used for quantitative evaluation of the aggregation process. Percentage of inhibition was calculated in relation to control values. The potency ratio was calculated versus NECA, the reference adenosine analogue, after logit-log transformation, and fitted by the weighted least square method.[20] The anti-aggregatory activity was evaluated using a concentration of test compound close to the IC_{50} value.

In vivo studies

Animals. Male spontaneously hypertensive rats (SHRs) (20 weeks old) were supplied by Charles River, Calco, Italy. They were acclimatized to standard conditions and housed in individual cages for one week before the surgical operation, with free access to food and water.

Telemetry instrumentation. The rats were anaesthetized with pentobarbital (30 mg/kg ip). A midline laparatomy was performed and a tract of the abdominal aorta (about 1 cm below the renal arteries) was carefully isolated. The catheter tip was inserted in the descending aorta above the iliac bifurcation and the sensor was affixed to the muscle. After recovery from anaesthesia, rats were housed individually in cages placed on the radio-frequency receivers. Gentamicin (5 mg/kg ip twice a day) was administered for 2 days following surgery to prevent infection.

Haemodynamic measurements. Blood pressure and heart rate were recorded and analysed by a Dataquest IV system (Data Sciences, St. Paul, MN, U.S.A.). The system consists of blood pressure sensors (model TA11PAC40), receivers (model RA1010), a consolidation matrix (BCM100), a personal computer (Everex 486/33) and Dataquest IV software. Haemodynamic recordings were taken every 5 min, starting 2 h before administration of drugs and continuing up to 24 h thereafter. Each recording lasted for 10 s, and the heamodynamic value of all cardiac cycles within this period (about 50 at baseline) were averaged.

Drugs. All substances were dissolved in dimethylsulphoxide 2% in water, and were administered intraperitoneally in a volume of 2 mL/kg body weight.

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