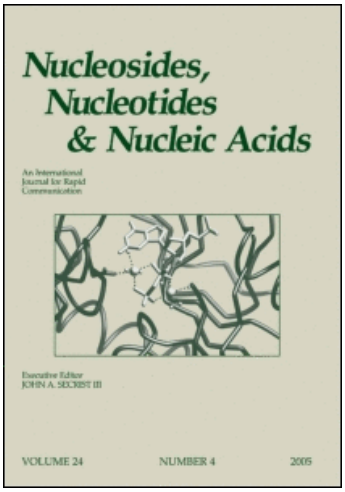

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PLATELET AGGREGATION INHIBITORY ACTIVITY OF SELECTIVE A2 ADENOSINE RECEPTOR AGONISTS.

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Abstract. A series of new 2-alkynyl, 2-cycloalkynyl, and 2-aralkynyl derivatives of adenosine-5'-ethyluronamide (NECA) were synthesized and evaluated in binding studies and functional assays to assess their potency and selectivity at A_2 vs A_1 receptors. The new derivatives were also tested as inhibitors of rabbit platelet aggregation induced by ADP. While the presence of an aromatic or heteroaromatic ring conjugated to the triple bond decreased antiplatelet activity, the introduction of a hydroxyl group or a heterocyclic ring on the alkynyl side chain increased the antiaggregatory activity in comparison with NECA, resulting in the most potent inhibitors of platelet aggregation so far known in the nucleoside series. However, the presence of an α-quaternary carbon markedly reduced the antiaggregatory potency without affecting the A_2 binding affinity, suggesting that the platelet receptor is not a typical A_{2a} site.

Adenosine is known to modulate a number of physiological functions, and a variety of studies have demonstrated that most adenosine actions are mediated by

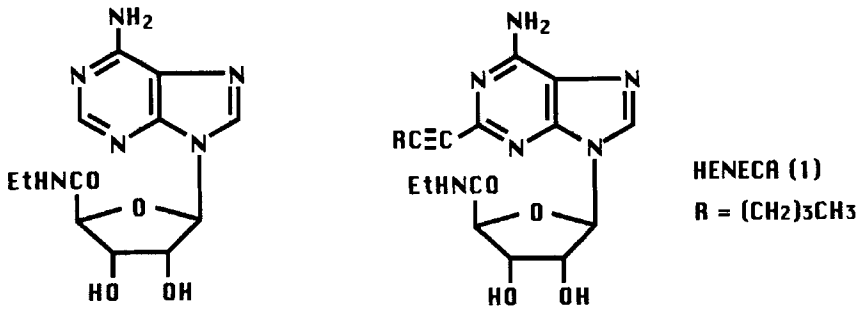


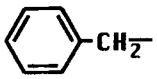
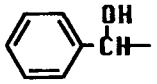
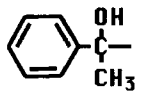
FIGURE 1

TABLE I. *In vitro* Pharmacological Activity of 2-Alkynyl Derivatives of NECA.

Compd	R[a]	Binding assay[b] K_i (nM) Rat brain A_1	Rat striatum A_2	Select. A_1/A_2	Anti-aggr.[c] Potency ratio vs NECA Rabbit Platelet A_2
NECA		10.4 (9.4-11.6)	7.8 (6.6-9.1)	1.3	1.00
1	$(CH_2)_3CH_3$	130 (116-145)	2.2 (1.9-2.6)	59	3.00
2	CH_2OH	14.1 (7.8-25.6)	9.1 (6.0-13.7)	1.5	2.30
3	$(CH_2)_2OH$	47.3 (42.8-52.4)	10.8 (9.8-12.0)	4.4	1.10
4	$(CH_2)_3OH$	99.9 (89.6-111)	11.3 (10.1-12.5)	8.8	2.20
5	$(CH_2)_4OH$	42.1 (39.9-44.5)	6.8 (6.0-7.7)	6.2	4.80
6	$CH(OH)CH_3$	11.1 (10.1-12.2)	7.6 (6.6-8.7)	1.5	4.70
7	$CH(OH)CH_2CH_3$	20.4 (18.5-22.6)	12.4 (10.8-14.2)	1.6	14.10
8	$CH_2CH(OH)CH_3$	69.6 (64.7-74.9)	56.4 (52.3-60.8)	1.2	3.20
9	1-Hydroxycyclopentyl	4.0 (3.5-4.5)	0.6 (0.5-0.7)	6.7	5.30
10	CH(OH)Ph	2.5 (2.2-2.9)	0.9 (0.7-1.3)	2.8	15.70
11	$CH_2N(CH_3)_2$	27.9 (25.0-31.1)	2.3 (2.2-2.4)	12	2.30
12	$(CH_2)_3Cl$	37.9 (34.1-42.0)	1.0 (0.8-1.2)	38	2.30
13	$(CH_2)_3CN$	184 (167-204)	4.7 (4.1-5.5)	39	2.10
14	CH_2-N-imidazolyl	178 (166-191)	17 (9.1-30.0)	10.4	3.5
15	CH_2-N-piperidyl	28 (22.0-34.0)	4.3 (3.2-5.8)	6.5	4.7
16	CH_2-N-piperazyl-4-methyl	36 (32.0-40.0)	19 (15.0-24.0)	2.3	nd
17	CH_2-N-morpholyl	91 (78.0-105)	27 (16.0-48.0)	3.4	2.6
18	CH_2-N-thiomorpholyl	53 (44.0-63.0)	5.9 (4.4-7.9)	8.9	2.9

[a]The structure of compounds is reported in Figure 1. [b]Receptor binding affinity at A_1 and A_2 receptors was determined using [^{3}H]CHA and [^{3}H]CGS21680 as radioligands, respectively. Data are geometrical means from at least three separate experiments. [c]The potency ratio was calculated using the concentration of the test compound close to the IC_{50} value. In our experimental conditions the IC_{50} value for NECA was 0.2 μM.

TABLE 2.

Cpd	R[a]	Binding assay[b] K_i (nM) A_1	A_2	Anti-aggreg. activity IC_{50} (nM)[c]
NECA		10.4	7.8	200
19		27.4	1.6	1100
10		2.5	0.9	13
20		32.7	1.7	810

[a]The structure of compounds is reported in Figure 1. [b]See note b in TABLE 1. [c]The IC_{50} values are calculated from the potency ratios: the IC_{50} value for NECA is 200 nM.

at least four extracellular receptors designated as A_1, A_{2a}, A_{2b} and A_3 on the basis of biochemical experiments and receptor cloning.[1] At A_2 receptors the most active compounds are C-2 substituted adenosine analogues, and recently we have reported the synthesis of N-ethyl-1'-deoxy-1'-(6-amino-2-hexynyl-9H-purin-9-yl)-β-D-ribofuranuronamide (HENECA, **1**), which possesses high affinity at A_2 receptors combined with a good A_2 vs A_1 selectivity (FIG. 1).[2]

In addition, HENECA was found to be the most potent inhibitor of platelet aggregation so far known in the nucleoside series.[2-4] Moreover, Dionisotti and coworkers demostrated that HENECA exhibits effective in vivo inhibitory activity on platelet function in the rabbit, whereas the selective adenosine A_1 agonist 2-chloro-N^6-cyclopentyladenosine (CCPA)[5] is ineffective.[6] The therapeutic potential of HENECA for the treatment of cardiovascular diseases prompted us to synthesize a number of new 2-(ar)alkynyl and cycloalkynyl derivatives of NECA bearing hydroxyl, amino, chloro, and cyano groups or substituted aromatic or heteroaromatic rings in the side chain. The synthesis was accomplished by three general methods, starting from the common intermediate 1'-deoxy-1'-(6-amino-2-iodo-9H-purin-9-yl)-2',3'-O-isopropylidene-ß-D-ribofuranuronic acid.[7,8]

The new derivatives were studied in binding and functional assays to assess their potency for the A_2 compared to A_1 adenosine receptors. The results of

binding assays and functional activity have been reported elsewhere.[7,8] Some compounds show subnanomolar activity in the rat striatal binding experiments and some compounds are very potent in inducing vasorelaxation without an appreciable effect on the heart rate. The compounds were also tested as inhibitors of rabbit platelet aggregation induced by ADP, and the results are reported as potency ratio vs the parent compound NECA.[7,8]

In the case of platelet receptor, the presence of aromatic rings, conjugated or not to the triple bond, is detrimental for the anti-aggregatory activity.

However, the introduction of polar groups in α to the triple bond markedly increases the potency, as shown in TABLE 1, where are listed all the compounds that proved more potent than NECA itself. Moreover, the anti-aggregatory activity is potentiated by polar groups only when steric hindrance is avoided, as shown in TABLE 2, indicating that this polar subregion in platelets is not able to accommodate even additional methyl groups.

These findings corroborates the conclusion, reached in previous studies, that the platelet A_2 receptor is not a typical A_{2a} site.[4,7]

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