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# $N^6$-Ethyl-2-alkynyl NECAs, selective human $A_3$ adenosine receptor agonists

Ran Zhu,[a] Cynthia R. Frazier,[b] Joel Linden[b] and Timothy L. Macdonald[a,*]

[a]*Department of Chemistry, University of Virginia, Charlottesville, VA 22904-4319, USA*
[b]*Department of Internal Medicine, Cardiovascular Medicine, University of Virginia, Charlottesville, VA 22904-4319, USA*

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**Abstract**—A series of $N^6$-ethyl-2-alkynyl NECA (5′-*N*-ethylcarboxamidoadenosine) analogs were synthesized and their binding affinity with the four human adenosine receptors was evaluated. One of the compounds ZR1121 shows high affinity with $hA_3$ receptor and its selectivity over $hA_1$ receptor is 1–2 log orders greater than IB-MECA or Cl-IB-MECA, the currently employed selective $A_3$ agonists.


Adenosine is an endogenous nucleoside that modulates many physiological processes through four G-protein-coupled receptors: $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$.[1] Extensive study indicates that the $A_3$ receptor exhibits important physiologic functions in at least three different organ systems: the central nervous system,[2–4] the cardiovascular system,[5–7] and the immune system.[8–10] IB-MECA[11] and 2-Cl-IB-MECA are two widely used $A_3$ agonists in biological studies (Fig. 1). Although these compounds are selective against other adenosine receptor subtypes in rat, their selectivity is depressed when assessed at the human receptors due principally to their high affinity at human $A_1$ receptor. It is likely that the loss of selectivity is a consequence of the low homology of $A_3$ receptors between species.[12] In 2003, the first selective human adenosine $A_3$ agonist, CP-608039, an IB-MECA analog, was reported.[13]

In addition to IB-MECA analogs, 5′-*N*-ethylcarboxamidoadenosine (NECA) and its derivatives are also $hA_3$ agonists with potential selectivity over the other hA receptors. $N^6$-Alkylation and $C^2$ acetylation of NECA by Cristalli et al.[14] gave several $A_3$ agonists with moderate $hA_3/hA_1$ selectivity. The Cristalli studies on NECA derivatives have provided valuable SAR insight for the design of selective $A_3$ agonists. Their primary observations were that substitution at $N^6$ with small alkyl groups depresses potency at the $A_1$ receptor with little effect on the $A_3$ receptor thereby enhancing $A_3/A_1$ selectivity and that alkynyl substitution at $C^2$ modulates affinity with all four adenosine receptors. In light of these observations, we designed a series of $N^6$-ethyl-2-alkynyl NECA analogs as potential human $A_3$ receptor agonists.

The synthesis of the current series was adapted from well-established methods and modified according to individual compounds (Scheme 1). The starting material **5** was prepared from commercially available guanosine according to the literature method[15] and the ethyl amino group was conveniently installed to $N^6$-position at low temperature.[14] With the 3′- and 4′-hydroxyl groups protected, the 5′-primary alcohol was oxidized to the carboxylic acid **7** quantitatively by TEMPO and BAIB.[16] Introduction of the ethyl amido group provided the key intermediate **8**, which gave the final products (**9**–**35**) through palladium-catalyzed Sonogashira coupling of alkyne subunits.

Binding data at the human adenosine receptor subtypes for the series of analogs prepared are presented in Table 1. The binding affinity at $hA_3$ of the entire series is within the subnM to lower nM range. All of the compounds have low or no affinity at the $hA_{2A}$ or $hA_{2B}$ receptors. The $hA_3/hA_1$ selectivity varies significantly, which is a consequence primarily of the variation in the $hA_1$ affinity. Our lead compound, ZR1121 (**9**), has similar $hA_3$ binding affinity to IB-MECA or Cl-IB-MECA as shown

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* Corresponding author. Tel.: +1 434 924 7718; fax: +1 434 982 2302; e-mail: tlm@virginia.edu

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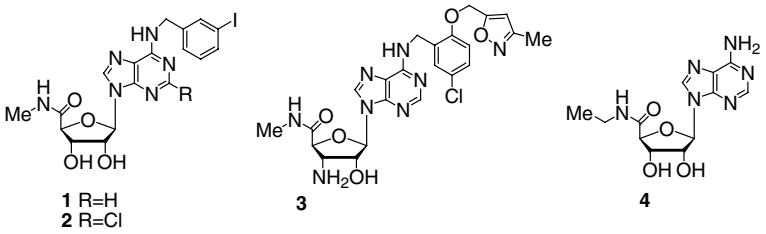
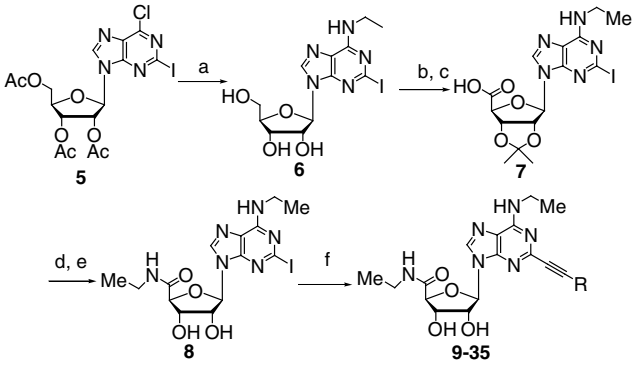



**Figure 1.** Structures of some $A_3$ agonists. **1**, IB-MECA; **2**, 2-Cl-IB-MECA; **3**, CP-608039; **4**, NECA.




**Scheme 1.** Reagents and conditions: (a) $EtNH_2$; (b) *p*-TsOH hydrate, 2,2-dimethoxylpropane, $CH_2Cl_2$; (c) BAIB, TEMPO, $CH_3CN/H_2O$ 1:1; (d) HCOOH; (e) $SOCl_2$, MeOH, then $EtNH_2$; (f) alkyne, $Pd(PPh_3)_2Cl_2$ or $Pd(PPh_3)_4$, CuI, triethylamine, DMF or acetonitrile.

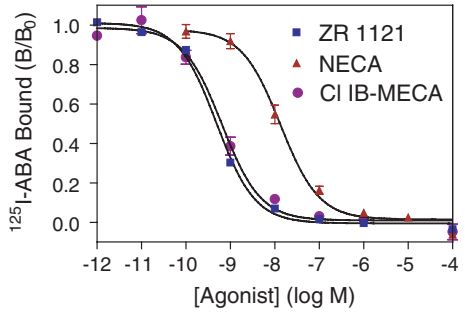



**Figure 2.** Competition for radioligand binding to hA3 receptors. Comparison of **9** with Cl-IB-MECA and NECA in binding assay.

in Figure 2. A functional assay in transfected cell lines (Fig. 3) proves **9** to be a full agonist of $hA_3$ receptor. The $hA_3/hA_1$ selectivity of ZR1121, 746-fold, is approximately 100 times higher than that of Cl-IB-MECA.

The α-position to acetylene seems to be a critical interaction site for the $hA_1$ receptor. Most of the compounds with hydroxyl groups at this position have high affinity at $hA_1$ receptor ranging from 1.24 to 11.3 nM (except for **34**). Absence of this hydroxyl group (**9**–**13**, **18**, and **19**) depresses binding affinity at $hA_1$ ($K_i > 50$ nM). Compounds **14**, **15**, **20**, and **21** have intermediate selectivities, due to their moderate $hA_1$ affinity. Compared with similar analogues that have

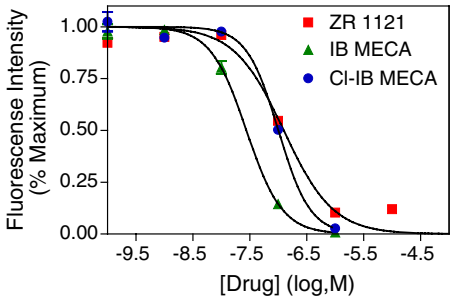



**Figure 3.** Inhibition of isoprotenol-stimulated cAMP gated channel activity by **9**, **1**, and **2** in HEK 293 cells expressing $hA_3$ receptors and cyclic nucleotide gated channels. $ED_{50}$ values (nM) are: ZR 1121, 125; IB MECA, 28; and Cl-IB MECA, 100.

been reported,[17] the acetylene substituent appears to be the critical functionality within this scaffold that modulates $hA_1$ affinity and therefore profoundly impacts the $hA_3/hA_1$ selectivity.

An additional important structural determinant is the substitution status of the $N^6$ nitrogen. The $N^6$-ethyl-NECA series reported here can be contrasted with $N^6$ mono-ethylated adenosine analogs reported previously that demonstrated good $A_3$ affinity, but lacked high $hA_3/hA_1$ selectivity.[15]

One observation worth being pointed out is that, **9** is not very active at rodent $A_3$ receptors. In preliminary rat $A_3$ receptor binding assay, **9** is 1–2 log orders less active than Cl-IB-MECA. In the $rA_1$ binding assay, compound **9** shows a similar degree of decrease in affinity, compared with Cl-IB-MECA.

In conclusion, a series of $N^6$-ethyl-2-alkynyl NECA derivatives were synthesized. One compound, ZR1121 (**9**), exhibits similar $hA_3$ affinity to IB-MECA or Cl-IB-MECA, but with significantly improved $hA_3/hA_1$ selectivity. This is the first non-IB-MECA-based selective human $A_3$ receptor agonist. SAR studies indicate that the α-hydroxyl substitution of acetylene ligand is critical for binding to the $A_1$ receptor and its removal can consequently lead to $A_3/A_1$ selectivity. Although only one para-substituted phenyl acetylene ligand was examined in this series, we expect further modification on the phenyl ring to provide an additional highly selective $hA_3$ agonist.

**Table 1.** Binding affinity at human adenosine receptors[a]

| Compound | R | $K_i$ (nM) | | | | |
|---|---|---|---|---|---|---|
| | | $A_1$[b] | $A_{2A}$[c] | $A_{2B}$[d] | $A_3$[b] | $A_1/A_3$ |
| **9** | *p*-$PhOCH_3$ | 558 ± 124 | 4963 ± 2534 | NI[e] | 0.748 ± 0.215 | 746 |
| **10** | *n*-$C_4H_9$ | 87.0 ± 5.3 | 543 ± 154 | NI | 1.02 ± 0.25 | 85.3 |
| **11** | *n*-$C_6H_{13}$ | 176 ± 92 | 571 ± 75 | NI | 6.05 ± 2.27 | 29.1 |
| **12** | *n*-$C_8H_{17}$ | 69.6 ± 11.3 | NI | NI | 6.20 ± 0.96 | 11.2 |
| **13** | 1-Cyclohexenyl | 280 ± 65 | 6707 ± 1423 | NI | 3.77 ± 1.41 | 74.2 |
| **14** | 1-Hydroxycyclopentyl | 34.4 ± 12.0 | 935 ± 223 | 26,466 ± 5043 | 2.03 ± 0.39 | 16.9 |
| **15** | 1-Hydroxycyclohexyl | 44.2 ± 16.1 | 344 ± 107 | 5533 ± 2240 | 1.91 ± 0.41 | 23.1 |
| **16** | 1-Aminocyclohexyl | 716 ± 112 | 7350 ± 3804 | NI | 27.2 ± 7.9 | 26.3 |
| **17** | $CH_2Ph$ | 15.5 ± 2.4 | 645 ± 52 | 21,465 ± 14557 | 1.35 ± 0.33 | 11.5 |
| **18** | $(CH_2)_3Ph$ | 65.3 ± 7.7 | 321 ± 68 | 24,057 ± 13736 | 2.42 ± 0.73 | 27.0 |
| **19** | $(CH_2)_4OH$ | 109 ± 21 | 1107 ± 284 | NI | 1.28 ± 0.17 | 85.2 |
| **20** | $(CH_2)_2OH$ | 19.9 ± 2.3 | 642 ± 118 | NI | 2.40 ± 0.26 | 8.29 |
| **21** | (*R*,*S*)-$CH_2CH(OH)CH_3$ | 51.6 ± 1.3 | 2433 ± 388 | NI | 4.58 ± 0.72 | 11.3 |
| **22** | (*R*)-$CH(OH)CH_3$ | 4.24 ± 1.32 | 2130 ± 514 | NI | 1.60 ± 0.16 | 2.65 |
| **23** | (*S*)-$CH(OH)CH_3$ | 7.98 ± 0.28 | 785 ± 346 | 23,800 ± 2835 | 2.22 ± 0.39 | 3.59 |
| **24** | (*R*)-$CH(OH)(CH_2)_4CH_3$ | 2.80 ± 0.85 | 680 ± 141 | NI | 0.709 ± 0.296 | 3.95 |
| **25** | (*S*)-$CH(OH)(CH_2)_4CH_3$ | 3.12 ± 1.05 | 467 ± 177 | 8473 ± 2754 | 0.542 ± 0.074 | 5.76 |
| **26** | (*R*,*S*)-CH(OH)-*c*-pentyl | 6.81 ± 0.47 | 152 ± 24 | 7920 ± 1489 | 1.84 ± 0.94 | 3.70 |
| **27** | (*R*,*S*)-CH(OH)-*c*-hexyl | 5.59 ± 0.54 | 59.3 ± 9.0 | 8970 ± 3504 | 1.31 ± 0.40 | 4.27 |
| **28** | (*R*,*S*)-CH(OH)Ph | 1.63 ± 0.29 | 129 ± 28 | 1913 ± 870 | 0.763 ± 0.260 | 2.14 |
| **29** | (*R*,*S*)-CH(OH)-*o*-$PhOCH_3$ | 4.27 ± 0.64 | 497 ± 161 | 3862 ± 1688 | 1.98 ± 0.88 | 2.16 |
| **30** | (*R*,*S*)-CH(OH)-*m*-$PhOCH_3$ | 1.24 ± 0.26 | 207 ± 38 | 1268 ± 550 | 1.17 ± 0.33 | 1.06 |
| **31** | (*R*,*S*)-CH(OH)-*p*-$PhOCH_3$ | 1.52 ± 0.21 | 711 ± 221 | 2481 ± 1489 | 0.754 ± 0.383 | 2.02 |
| **32** | (*R*,*S*)-CH(OH)-*o*-$PhNO_2$ | 3.47 ± 1.15 | 371 ± 81 | 7620 ± 2654 | 1.29 ± 0.40 | 2.69 |
| **33** | (*R*,*S*)-CH(OH)-*m*-$PhNO_2$ | 11.3 ± 3.1 | 401 ± 68 | 1188 ± 152 | 3.45 ± 1.15 | 3.28 |
| **34** | (*R*,*S*)-CH(OH)-*m*-COOH | 131 ± 14 | 3783 ± 714 | NI | 20.1 ± 3.1 | 6.51 |
| **35** | (*R*,*S*)-CH(OH)-*m*-$PhCOOCH_3$ | 6.11 ± 1.11 | 143 ± 22 | NI | 2.13 ± 0.51 | 2.87 |
| **8** | | 20.6 ± 2.9 | 1955 ± 1543 | NI | 4.03 ± 1.05 | 5.11 |
| **4** | | 1.04 ± 0.10 | 124 ± 39 | 884 ± 129 | 10.2 ± 3.4 | 0.102 |
| **1** | | 3.98 ± 0.16 | 510 ± 160 | 2040 | 0.215[f] | 18.5 |
| **2** | | 5.26 ± 0.48 | NI | NI | 0.637 ± 0.080 | 8.26 |

[a] Values represent the average of at least three experiments unless noted, each run in triplicate.
[b] Displacement of $^{125}$I-ABA binding to HEK cells.
[c] Displacement of $^{125}$I-ZM241385 at low affinity $A_{2A}$ binding to HEK cells.
[d] Displacement of $^{125}$I-ABOPX at low affinity $A_{2B}$ binding to HEK cells.
[e] NI Inhibition at 100 μM is less than 50% or no inhibition observed.
[f] $n = 1$.

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