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A new chemical tool for exploring the physiological function of the PDE2 isozyme

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Abstract—Oxindole (**2**) is a potent and selective PDE2 inhibitor with a favorable ADME, physiochemical and pharmacokinetic profile to allow for use as a chemical tool in elucidating the physiological role of PDE2.

The phosphodiesterase (PDE) enzyme family controls intercellular levels of secondary messenger cAMP or cGMP through regulation of their hydrolysis. Phosphodiesterase Type II (PDE2) possesses a low affinity catalytic domain and an allosteric domain specific for cGMP. The low affinity catalytic site can hydrolyze both cAMP and cGMP with a lower apparent K_M for cGMP over cAMP. However, when cGMP binds to the allosteric site, the catalytic site undergoes a conformational change showing high affinity for cAMP. PDE2 is expressed throughout the body and therefore has a broad array of functions and potential therapeutic utility.[1] It has been shown that EHNA (erythro-9-(2-hydroxy-3-nonyl)adenine) (**1**) (Fig. 1), a potent adenosine deaminase inhibitor ($K_i = 10^{-9}$ M),[2] selectively inhibits PDE2.[3] However, the use of EHNA (**1**) as a chemical tool in determining the pharmacological role of PDE2 is limited due to low PDE2 potency and high potency in inhibiting adenosine deaminase. A recent advancement in the development of PDE2 inhibitors is marked by the discovery that analogs of phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002 inhibit PDE2 with potency approaching that of EHNA (**1**).[4] Another recent advancement in the area is the report that Bay 60-7550, a potent and selective PDE2 inhibitor structurally derived from EHNA (**1**), elevates intracellular cGMP in cultured neurons and is efficacious in animal models of memory and cognition.[5] These recent findings prompt us to report that oxindole (**2**) is a potent and selective PDE2 inhibitor with a favorable pharmacological profile to allow the use as a chemical tool in further defining the role of PDE2 across a broad range of disease states.

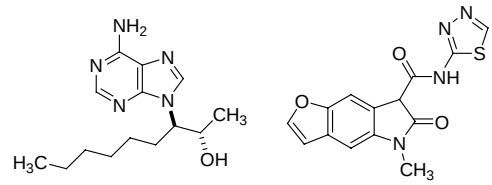

Figure 1. PDE2 inhibitors EHNA (**1**) and oxindole (**2**).

In a comparative evaluation of potency and selectivity across the phosphodiesterase isozyme family, oxindole (**2**) was found to be a potent inhibitor of PDE2 being over an order of magnitude more potent than EHNA (**1**) and both compounds lacked significant inhibition of other isozymes indicating a high degree of selectivity toward PDE2 (Table 1).[6,7] Also, **2** shows PDE2 potency higher than those reported for LY294002 analogs and PDE1 selectivity higher than that reported for Bay 60-7550.[4,5]

Keywords: Phosphodiesterase; PDE2.
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Table 1. Comparative PDE2 potency and selectivity of EHNA (**1**) and oxindole (**2**)

PDE isozyme	EHNA (**1**)	Oxindole (**2**)
PDE1 IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE2 IC_{50}, nM (*n*)[a]	635 ± 134 (2)	40 ± 18 (9)
PDE3 IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE4A IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE4B IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE4C IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE4D IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE5 IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE8A IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE8B IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE9 IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE10 IC_{50}, nM (*n*)	>16,000 (1)	>16,000 (1)
PDE11 IC_{50}, nM (*n*)	>16,000 (1)	11,600 (1)
PDE2 selectivity	>25X	290X

[a] Values are means of a number (*n*) experiments with standard deviation.

In considering that **2** might interact with other pharmacological targets that in turn could then compromise interpretation of results from further in vitro and in vivo functional profiling, **2** was assayed for interaction against a collection of 54 receptors and ion channels where no significant level of binding relevant to its PDE2 potency was found (Table 2). In addition, oxindole (**2**) showed no inhibition of 5-lipoxygenase (5-LO) or cyclooxygenase (COX-1), two enzymes which other oxindole derivatives inhibit and derive anti-inflammatory activity (Table 3).[8,9] The chemical structure of **2** displays proximal hydrogen bond donor and acceptor pairs which is associated with kinase inhibition which in turn would effect cell signaling.[10] Against a panel of thirty kinases, **2** showed no significant inhibition relative to the level of PDE2 inhibition (Table 3).[11]

In comparing the solubility and ADME properties of EHNA (**1**) and oxindole (**2**), both compounds showed an appreciable level of solubility and cell permeability however, oxindole (**2**) shows over a twofold increase in metabolic stability over EHNA (**1**) as measured by half-life in rat liver microsomes (Table 4). [12] In parental Madin–Darby canine kidney (MDCK) and transfectant multidrug resistant (MDR) overexpressing human p-glycoprotein (P-gp) cell lines, oxindole (**2**) showed a moderate rate of absorption with no apparent efflux.[13]

Having found a favorable solubility and ADME profile for oxindole (**2**), the in vivo pharmacokinetic profile was determined in rats, which showed a moderate rate of clearance and a low volume of distribution leading to a moderate half-life and level of oral bioavailability (Table 5).[14] Furthermore, unbound plasma concentrations of oxindole (**2**) were achieved which exceeded its IC_{50} value for over 2 h.

The synthesis of oxindole **2** proceeds via an eight-step linear sequence in a 33% overall yield starting from Metol **3**, an inexpensive and readily available starting material (Scheme 1). Acylation of **3** with chloroacetyl chloride occurs chemoselectively at the aniline nitrogen to give amide **4**.[15] Cyclization of **4** by way of a intramolecular Friedel–Crafts alkylation occurs regioselectively to yield **5**.[16] Acylation of **5** with chloroacetyl chloride followed by Fries rearrangement of ester **6** affords ketone **7**.[17] Reduction of **7** and subsequent acid-catalyzed dehydration of alcohol **8** gives furan **9**. Acylation of **9** with diethylcarbonate followed by aminolysis of ester **10** with 2-aminothiadiazole affords **2** as a crystalline solid.

Table 2. Broad ligand binding profile of oxindole (**2**)

Target	% binding at 10 μM[a]
Adenosine A_1	16
Adenosine A_{2a}	24
Adenosine A_3	35
Adrenergic α_1	0
Adrenergic α_2	16
Adrenergic β_1	15
Adrenergic β_2	12
Norepinephine Uptake	12
Angiotensin-I	11
Angiotensin-II	4
Benzodiazepine	11
Bradykinin B_1	0
Bradykinin B_2	5
Dopamine D_1	5
Dopamine D_2	12
Dopamine D_3	0
Dopamine D_4	0
Dopamine Uptake	11
GABA	3
GABA Uptake	8
AMPA	20
Kainate	0
NMDA	7
Histamine H_1	7
Histamine H_2	0
Histamine H_3	0
MCR4	12
Muscarinic M_1	9
Muscarinic M_2	0
Muscarinic M_3	0
Muscarinic M_4	1
Choline Uptake	0
Neurokinin K_1	0
Nicotinic (neuronal)	7
Nicotinic (muscle)	0
Opiate δ	3
Opiate κ	0
Opiate μ	3
PAF	21
Serotonin 5-HT_{1A}	23
Serotonin 5-HT_{2A}	15
Serotonin 5-HT_{2C}	0
Serotonin 5-HT_3	0
Serotonin 5-HT_4	0
Serotonin 5-HT_7	0
Serotonin Uptake	0
Glucocorticord	0
Thyroid hormone	0
Vasopressin V_1	5
Vasopressin V_2	13
Ca^{2+} channel D (dihydro pyridine)	0
Ca^{2+} channel D (dilitiazem)	0
Ca^{2+} channel L (verapamil)	0
Ca^{2+} channel N	0

[a] Values are from a single experiment with duplicate determinations.

Table 3. Off target inhibition profile of oxindole (**2**)

Target	% inhibition at 10 μM[a]
5-Lipoxygenase	35
Cyclooxygenase	0
MKK1	0
MAPK2	12
JNK1a1	0
SAPK2a	0
SAPK2b	5
SAPK3	7
SAPK4	13
RSK1	1
MAPKAP-K2	8
MSK1	6
PRAK	28
PKA	16
PKCa	13
PDK1	0
PKBa	14
SGK	0
p70S6K	23
GSK3β	16
ROCK-II	20
AMPK	8
CHK1	0
CK2	32
PHK	0
LCK	5
CSK	5
CDK2	2
CK1	10
DYRK1A	53
NEK6	7
NEK2	0

[a] Values are from a single experiment with duplicate determinations.

Table 4. Comparative ADME and solubility profile of EHNA (**1**) and oxindole (**2**)

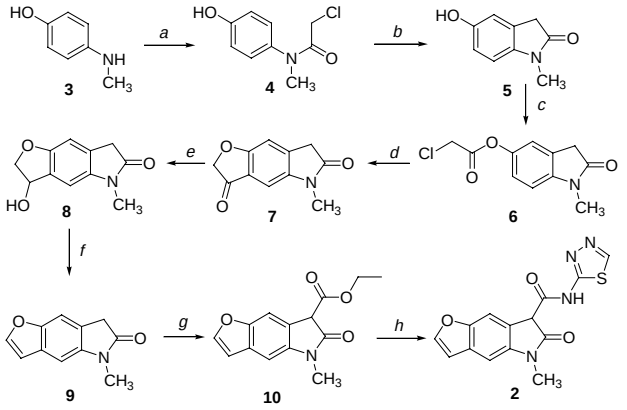
	EHNA (**1**)	Oxindole (**2**)
Rat liver microsome $t_{1/2}$ (min)	31	83
CACO-2 apical P_{app} ($\times 10^{-6}$ cm/s)	34	5
CACO-2 basolateral P_{app} ($\times 10^{-6}$ cm/s)	18	32
MDCK apical P_{app} ($\times 10^{-6}$ cm/s)	NT	3.0
MDR basolateral P_{app} ($\times 10^{-6}$ cm/s)	NT	4.6
Turbidimetric solubility (pH 7, μg/ml)	>65	55

NT, not tested.

Table 5. Rat pharmacokinetics of oxindole (**2**)

Clearance Cl (ml/min/kg)	8.8
Volume of distribution V_{dss} (L/kg)	0.1
Half life $t_{1/2}$ (h)	1.6
T_{max} (h)	0.8
C_{max} (ng/ml)	8303
Oral bioavailability % F	41

Oxindole (**2**) is a potent and selective PDE2 inhibitor that possesses a suitable solubility and in vitro ADME profile leading to a moderate level of in vivo oral bioavailability and half-life in rats. Against a panel of 54 receptors and ion channels, oxindole (**2**) shows no significant interaction nor is effective in inhibiting 5-lipoxygenase (5-LO) or cyclooxygenase (COX-1), two enzymes which other oxindole derivatives inhibit and derive anti-inflammatory activity. In regard to mediating off target cell signaling pathways, oxindole (**2**) showed no significant inhibition against a panel of 30 kinases. Thus, oxindole (**2**) is positioned to be a useful chemical tool in defining the role of PDE2 in a broad range of disease states.



Scheme 1. Reagents and conditions: (a) $ClCOCH_2Cl$, TEA, DMAP, DMF, 0 °C, 83%; (b) $AlCl_3$, 180 °C, 89%; (c) $ClCOCH_2Cl$, Py CH_2Cl_2, 0 °C; 94% (d) $AlCl_3$, 180 °C, 76%; (e) $NaBH_4$, MeOH, 0 °C, 92%; (f) TFA, MeCN, 20 °C, 88%; (g) $(EtO)_2CO$, Na, EtOH, reflux, 97%; (h) 2-amino-1,3,4-thiadiazole, 4 Å Sieves, PhH, reflux, 80%.

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yttrium silicate beads (Amersham Biosciences®) bind preferentially to the linear nucleotide compared to the cyclic nucleotide. In the case of PDE2, ^{3}H-cGMP and unlabeled cGMP are added to the reaction and when the product, ^{3}H-GMP, is in close proximity to the beads, the scintillant within the bead is excited, which is detected using a Packard scintillation counter. The enzyme concentration used is in the linear range and the K_M of the enzyme was determined (15 μM). The final substrate concentration is <1/3 of K_M (1 μM) so that IC_{50} values would approximate the K_i values (a) Bardelle, C.; Smales, C.; Ito, M.; Nomoto, K.; Wong, E. Y. M.; Kato, H.; Saeki, T.; Staddon, J. M. *Anal. Biochem.* **1999**, *275*, 148; (b) Laliberte, F.; Han, Y.; Govindarajan, A.; Giroux, A.; Liu, S.; Bobechko, B.; Lario, P.; Bartlett, A.; Gorseth, E.; Gresser, M.; Huang, Z. *Biochemistry* **1997**, *36*, 2968; (c) Card, G. L.; England, B. P.; Suzuki, Y.; Fong, D.; Powell, B.; Lee, B.; Luu, C.; Tabizizad, M.; Gillette, S.; Ibrahim, P. N.; Artis, D. R.; Bollag, G.; Milburn, M.; Kim, S.-H.; Schlessinger, J.; Zhang, K. Y. J. *Structure* **2004**, *12*, 2233.

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