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Discovery of 7-hydroxyaporphines as conformationally restricted ligands for beta-1 and beta-2 adrenergic receptors†

Angela F. Ku and Gregory D. Cuny *

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A series of (–)-nornuciferidine derivatives was synthesized and the non-natural enantiomer of the aporphine alkaloid was discovered to be a potent β_1 - and β_2 -adrenergic receptor ligand that antagonized isoproterenol and procaterol induced cyclic AMP increases from adenylyl cyclase, respectively. Progressive deconstruction of the tetracyclic scaffold to less complex cyclic and acyclic analogues revealed that the conformationally restricted (6a-*R*,7-*R*)-7-hydroxyaporphine 2 (AK-2-202) was necessary for efficient receptor binding and antagonism.

G protein-coupled receptors (GPCRs), the largest protein superfamily in the human genome,¹ participate in a wide range of physiologic and pathophysiologic processes and have emerged as targets for chemotherapeutic development.² The β_2 -adrenergic receptor (β_2 -AR),^{2a,d} which is involved in pain signaling³ and the regulation of the respiratory,⁴ cardiovascular⁵ and neurological⁶ systems, is one of these important GPCRs. Many reported ligands for the β_2 -AR have a common pharmacophore consisting of an aromatic ring connected to a ethanolamine moiety (Fig. 1),^{7–10} including the antagonist (*R*)-pronethalol,⁸ the agonist (*R*)-salbutamol⁹ and the endogenous agonist epinephrine.^{7d,10}

7-Hydroxyaporphine alkaloids also possess the β_2 -AR pharmacophore raising the possibility that this class of natural products might possess β_2 -AR binding affinity. However, conformational restrictions imposed by the tetracyclic system could impede optimal spatial orientation of the functional groups necessary for efficient binding.¹¹ Herein we report the exploration of 7-hydroxyaporphine derivatives as β_2 -AR ligands. Furthermore, progressive dismantling of the tetracyclic scaffold to less conformationally restricted derivatives was conducted to evaluate the impact on receptor binding affinity.

(–)-Nornuciferidine is a 7-oxygenated aporphine alkaloid isolated from the bark of Bolivian *Duguetia spixiana* (Annonaceae) by Rasamizafy and coworkers¹² and was selected as the lead molecule for this study. We recently reported the synthesis of this natural product, as well as sev-

eral related compounds, employing acid catalysed reductive cyclization followed by Pd-mediated *ortho*-arylation as two key steps.¹³ As shown in Table 1, (–)-nornuciferidine has an absolute configuration of (*S,S*) at (C-a, C-b). However, several β_2 -AR ligands (*e.g.*, salbutamol) with a *R*-configuration at the corresponding C-b position were reported to be more active and/or selective than their racemates or stereoisomers.^{8,9} Therefore, nornuciferidine diastereomer 1, enantiomer 2, 6a-alkyl substituted derivative 3, two tricyclic derivatives 4 and 5, and the acyclic derivative 6 were selected for conducting the structure–activity relationship (SAR) analysis for β_2 -AR binding functional activity.

The preparation of test compounds 1–6 is summarized in Schemes 1 and 2. The synthesis was initiated with the conversion of (*R*)-(-)-2-chloromendelic acid (7) into ester 9. Then, coupling of alcohol 9 and isocyanate 10, derived from amine 8, furnished carbamate 11. Subsequent one-pot reductive cyclization with sequential addition of DIBAL-H, methanol and $\text{BF}_3 \cdot \text{OEt}_2$, followed by silyl deprotection with TBAF, gave oxazolidinone 12 as a mixture favouring the *anti*-isomer (*dr* 67:33). The diastereomers could be differentiated based on their ¹H NMR coupling constants. The assignment of absolute

Fig. 1 Examples of β_2 -AR ligands and the 7-hydroxyaporphine scaffold; β_2 -AR pharmacophore elements highlighted in red for ethanolamine and blue for the aromatic ring.

Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Science and Research Building 2, Houston, Texas 77204, USA.

E-mail: gdcuny@central.uh.edu

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Table 1 Human β_2 -AR binding inhibition

Compound	Type	R ¹	R ²	(C-a, C-b)	IC ₅₀ (μM)
(-)-Nornuciferidine	A	H	—	(S,S)	1.6 ± 0.5
1	A	H	—	(S,R)	0.21 ± 0.035
2	A	H	—	(R,R)	0.0014 ± 0.0005
3	A	Me	—	rel-(R,R)	0.020 ± 0.0025
4	B	—	Cl	(R,R)	>10
5	B	—	H	(R,R)	>10
6	C	—	—	(-,R)	8.4 ± 0.5

configurations was previously discussed.¹³ Oxazolidinone **12** was then carried forward directly to the *ortho*-phenol arylation step. After heating at 110 °C for 40 minutes in the presence of 10 mol% XPhos precatalyst and Cs₂CO₃, arylated products **13-anti** and **13-syn** were obtained in 70% and 24% yields, respectively. Subsequent methylation and removal of the carbamate converted **13-anti** and **13-syn** into the desired products **2** and **1**, respectively.

Compound **3** (Table 1, type A) has the same structure as (±)-nornuciferidine except for a methyl group at C-a. This compound was generated by methylation of phenol **14**,¹⁴ an

intermediate previously reported by our group, followed by hydrolysis of the carbamate.

Compounds **4** and **5** (Table 1, type B) possess the same absolute configuration as compound **2**, but the linkage between the biaryl ring systems was disconnected, imparting increased freedom of rotation. These two compounds differ from one another in the *ortho*-substituent (R²). Compound **4** (R² = Cl) was synthesized by methylation and hydrolysis of intermediate **12-anti**. Compound **5** (R² = H) was derived from two commercially available starting materials, mandelic ester **16** and amine **17**. After formation of carbamate **19**, one-pot reductive cyclization provided oxazolidinone **20** exclusively as the *anti*-isomer. Subsequent hydrolysis furnished compound **5**.

Compound **6** (Table 1, type C) is an acyclic *R*-enantiomer with a fully rotatable ethanolamine scaffold. As shown in Scheme 2, hydrolysis of mandelic ester **16**, followed by EDC coupling with amine **17**, generated amide **21** in 69% yield. Then, reduction of **21** with borane, produced *in situ* by addition of NaBH₄ and BF₃·OEt₂, generated compound **6** in 82% yield.

The specific binding affinities of (-)-nornuciferidine and compounds **1–6** for human β_2 -AR were evaluated in a radioligand binding assay using [³H]-CGP-12177.¹⁵ The half maximal inhibitory concentration (IC₅₀) values are summarized in Table 1. The natural product (-)-nornuciferidine was found to be a moderately potent ligand for β_2 -AR (IC₅₀ = 1.6 ± 0.5 μM). When the chirality at C-b was switched from *S* to *R* (e.g., compound **1**), the binding affinity increased by 7.6-fold

Scheme 1 Summarized synthetic routes for compounds **1–5**.

Scheme 2 Synthetic route for compound 6.

($IC_{50} = 0.21 \pm 0.035 \mu M$). Surprisingly, when the absolute configurations at C-a and C-b were switched from (*S,S*) in (–)-nornuciferidine to (*R,R*) in compound 2, the binding affinity dramatically increased by almost a thousand fold ($IC_{50} = 0.0014 \pm 0.0005 \mu M$). However, addition of a substituent at C-a as in compound 3 reduced potency by 14.3-fold ($IC_{50} = 0.020 \pm 0.0025 \mu M$) compared to compound 2. Interestingly, releasing part of the conformational restriction of the aporphine ring system by disconnecting the biaryl linkage resulted in complete loss of activity with or without an *ortho*-substituent ($IC_{50} > 10 \mu M$ for 4 and 5) likely due to rotation of the C-a–C-b bond changing the relative orientation of the amino alcohol. Further dismantling of the tetrahydroisoquinoline (*e.g.*, the bond between C-b and the phenyl residue) restored weak binding activity ($IC_{50} = 8.4 \pm 0.5 \mu M$ for 6).

Based on the β_2 -AR binding assay results, compound 2 was selected for several additional studies. First, the compound was evaluated for selectivity among human β -AR subtypes.¹⁶ As displayed in Table 2, compound 2 demonstrated significant binding activity at the β_1 -AR ($IC_{50} = 0.0017 \pm 0.0003 \mu M$). However, it was about 150-fold less potent for β_3 -AR ($IC_{50} = 0.24 \pm 0.064 \mu M$). Next, the function of 2 was examined in cellular assays.^{17,18} The compound failed to agonize either β_1 - or β_2 -AR at $10 \mu M$, whereas, it blocked isoproterenol¹⁷ and procaterol¹⁸ induced cyclic AMP increases from adenylyl cyclase in human CHO cells (Eurofins Panlabs Discovery Services Taiwan Ltd.) at β_1 ($IC_{50} = 0.017 \pm 0.014 \mu M$) and β_2 -AR ($IC_{50} = 0.0069 \pm 0.0027 \mu M$), respectively.

In order to examine the broader selectivity of 2, it was screened against a panel of 45 GPCRs and monoamine transporters,¹⁹ including serotonin (*e.g.*, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT₃, 5-HT_{5A}, 5-HT₆ and 5-HT₇), adrenergic (*e.g.*, α_{1A} , α_{1B} , α_{1D} , α_{2A} , α_{2B} , α_{2C} , β_1 , β_2 and β_3), dopamine (*e.g.*, D₁, D₂, D₃, D₄ and D₅), histamine (*e.g.*, H₁, H₂, H₃ and H₄), muscarinic (*e.g.*, M₁, M₂, M₃, M₄ and M₅), γ -aminobutyric acid type A (GABA_A), sigma (*e.g.*, σ_1

Table 2 Human β -AR subtype binding inhibition and adenylyl cyclase antagonism in CHO cells for compound 2

β -AR subtype	Binding inhibition (IC_{50} , μM)	Cellular adenylyl cyclase antagonism (IC_{50} , μM)
β_1	0.0017 ± 0.0003	0.017 ± 0.014
β_2	0.0014 ± 0.0005	0.0069 ± 0.0027
β_3	0.24 ± 0.064	ND ^a

^a ND = not determined.

and σ_2), δ -opioid (DOR), κ -opioid (KOR), μ -opioid (MOR), peripheral-type benzodiazepine (PBR) receptors, as well as dopamine (DAT), norepinephrine (NET), serotonin (SERT) transporters and benzodiazepine (BZP) rat brain site, which is an allosteric benzodiazepine binding site of the GABA_A receptor.

The compound was initially accessed at $10 \mu M$ and for those that demonstrated >50% inhibition determinations of inhibition constant (K_i) values were conducted.²⁰ The profiling results are shown in Table 3. Overall, compound 2 was found to be quite selective, demonstrating moderate to weak activities (K_i values 0.12 – $1.2 \mu M$) against seven of the molecular targets and potent activity ($K_i < 0.10 \mu M$) against only one other GPCR (*e.g.*, 5-HT_{1A}) besides β_1 - and β_2 -ARs.

In conclusion, modification of the absolute stereochemistry in (–)-nornuciferidine resulted in the discovery of 2 with the configuration of (*R,R*) at (C-a, C-b) as a potent ligand for human β_1 - and β_2 -ARs. The compound was selective (>10-fold) versus β_3 -AR and 40 other molecular targets and blocked isoproterenol and procaterol induced cyclic AMP increases from adenylyl cyclase in human CHO cells at β_1 - and β_2 -AR, respectively. Due to the conformationally restricted structure, compound 2 (AK-2-202) may help reveal the preferred binding conformations for β_1 - and β_2 -AR ligands. Additional structure–activity relationship studies using related aporphine derivatives could further refine the pharmacophore for β_1 - and β_2 -AR antagonism, as well as for generating biased agonists.²¹ Finally, compound 2 defines very specific chemical space that may be used for *in silico* screening or design to achieve structurally distinct compounds that elicit similar pharmacology.

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Table 3 Profiling results of 2 against 45 GPCRs and monoamine transporters

Target	K_i^a (μM)	Target	K_i^a (μM)	Target	K_i^a (μM)
5-HT _{1A}	0.071	α_{2B}	0.571	M ₂	—
5-HT _{1B}	1.209	α_{2C}	—	M ₃	—
5-HT _{1D}	0.857	β_1	0.019	M ₄	—
5-HT _{1E}	—	β_2	0.013	M ₅	—
5-HT _{2A}	—	β_3	0.308	GABA _A	—
5-HT _{2B}	0.246	D ₁	—	σ_1	—
5-HT _{2C}	—	D ₂	—	σ_2	—
5-HT ₃	—	D ₃	—	DOR	—
5-HT _{5A}	—	D ₄	—	KOR	—
5-HT ₆	0.862	D ₅	—	MOR	—
5-HT ₇	0.120	H ₁	—	PBR	—
α_{1A}	—	H ₂	—	DAT	—
α_{1B}	—	H ₃	—	NET	—
α_{1D}	—	H ₄	—	SERT	—
α_{2A}	1.147	M ₁	—	BZP	—

^a Means test compound shows <50% inhibition at $10 \mu M$ in the primary radioligand competition binding assay.

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Conflicts of interest

The authors declare no competing interest.

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