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Synthesis and biological studies of yohimbine derivatives on human α_{2C}-adrenergic receptors

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Abstract—A series of yohimbine derivatives was synthesized and evaluated for binding affinity at the human α_{2C}-adrenergic receptors expressed in Chinese hamster ovary cells. It has been found that compound **5** shows a higher affinity for α_{2C}-AR than the parent compound yohimbine **1**, thereby illustrating that the nature of the linkers affect binding potencies on these receptors.

Adrenoreceptors (ARs) are membrane proteins belonging to the superfamily of G-protein-coupled receptors.[1] With the aid of pharmacological and molecular biological techniques, the α-adrenoceptor subtypes α_1 and α_2 were determined. Detailed studies have shown that these initial subtypes were further divided into α_{1A}, α_{1B}, and α_{1D}; and α_{2A}, α_{2B}, α_{2C}, and α_{2D} subtypes, depending on species and tissues.[2] This knowledge has led to a search for selective agonist and antagonists for each subtype. Although there are a number of α_2-AR antagonists,[3] only a small set of compounds have shown a degree of selectivity among the three subtypes of α_2-AR. However, these compounds suffer from either low subtype selectivity or binding to receptor sites outside the α_2-AR subfamily.[3a,4] The importance of α_{2C}-AR antagonists in treating Raynaud's disease was illustrated by Flavahan et al.[5] The most common probes used in these studies are agonists such as clonidine, and antagonists such as yohimbine and yohimbine like compounds viz rauwolscine, corynanthine.

Yohimbine **1** is known to be a potent and selective α_2-AR antagonist, and has been used extensively as a pharmacological probe for studying the α_2-AR.[6] In order to improve selectivity, the bivalent ligand approach was introduced recently based on the concept that a bivalent ligand should first undergo univalent binding, followed by the binding of the second pharmacophore to a recognition site on a neighboring receptor.[7] Using this approach we prepared several yohimbine dimers with methylene and methylene–diglycine spacer linkages and it has been found that such compounds with spacers of $n = 3$ (**2**) and $n = 24$ (**3**) showed the highest potency and selectivity for the α_{2C}-AR in receptor binding and in functional studies measuring cAMP changes using a cell based luciferase reporter gene assay.[8] Since the functional roles for the α_2-adrenoceptor subtypes need to be explored; yohimbine monomeric analogs were synthesized and evaluated for their binding affinity on human α_{2C}-AR with a goal of finding suitable pharmacological probes and potential therapeutic agents. The significance of functional groups in compounds affecting selectivity and affinity in α_2-AR system were detailed recently by Pigini et al.[9] The objective of our investigation was to find whether a second pharmacophore is essential for binding and how the nature of the linkers affect binding potencies on these receptors.

The monomeric analogs of yohimbine were synthesized by coupling yohimbinic acid with reagents having free amino groups under standard peptide coupling conditions. 1,3-Dicyclohexylcarbodiimide (DCC) was used as the coupling agent and *N*-hydroxybenzotriazole (HOBT) was used as an additive to catalyze the reaction

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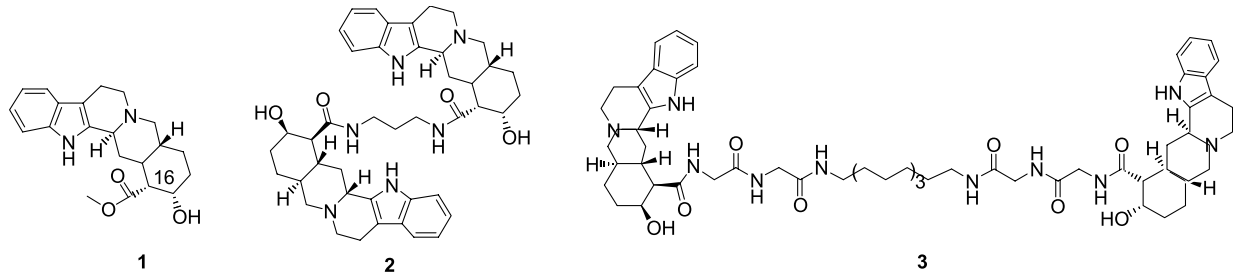
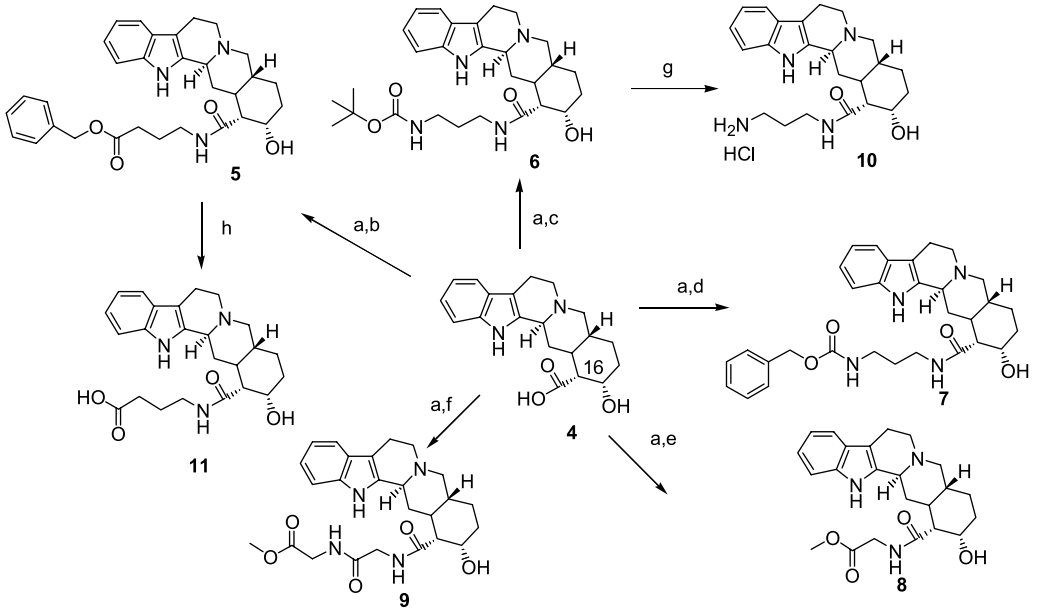
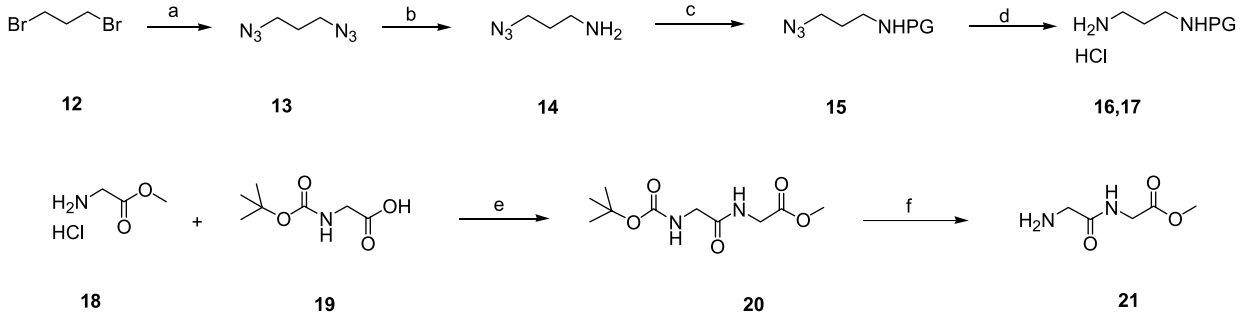


and to suppress the epimerization at C-16. Accordingly the yohimbine monomeric analogs were prepared as shown in Scheme 1. The yohimbine monomeric analogs **10** and **11** were prepared by the deprotection of **6** using HCl in ether and by the catalytic hydrogenation of **5** using 10% Pd/C in ethyl acetate, respectively. Attempts to prepare compound **11** by ester hydrolysis was successful although isolation was not and this prompted us to prepare its benzyl derivative followed by reduction. The structures of all the yohimbine monomeric analogs synthesized were characterized by ^{1}H NMR, MS, and CHN analyses. The mono-*N*-protected-1,3-diaminopropanes **16** and **17** were prepared as illustrated in Scheme 2[10] whereas **21** was prepared by the deprotection of the coupled product **20** of glycine methyl ester hydrochloride **18** and *N*-*t*-Boc glycine **19** with TFA at 0 °C in methylene chloride medium.

The effects produced by the introduction of functional groups in the side chain at C-16 of yohimbine were



Scheme 1. Reagents and conditions: (a) DCC, HOBT, THF, Et_3N, rt; (b) $NH_2(CH_2)_3COOBz$; (c) $NH_2(CH_2)_3NHBoc$ **16**; (d) $NH_2(CH_2)_3NHCbz$ **17**; (e) NH_2CH_2COOMe **18**; (f) $NH_2CH_2CONHCH_2COOMe$ **21**; (g) HCl in ether, 30 min; (h) H_2, 10% Pd/C, EtOAc. [Compound **13**, the diazidopropane is a very dangerous explosion hazard and it should be handled carefully.]



Scheme 2. Reagents and conditions: (a) NaN_3, DMF/H_2O, 80 °C, 20 h; (b) Ph_3P, Et_2O/EtOAc–5% HCl, rt, 24 h; (c) $(Boc)_2O$/CbzCl, NaOH, THF–H_2O, rt; (d) Ph_3P, THF, rt, 24 h; PG = Boc, Cbz; (e) EDC, HOBT, Et_3N, DCM; (f) TFA, THF, 0 °C.

Table 1. Binding data (K_i) of compounds **1**–**11** on human α_{2C}-AR

Compound	$K_i \pm$ SEM (nM)
1	**0.88 ± 0.03**
2	8.50 ± 1.00
4	19.00 ± 2.30
5	**0.65 ± 0.03**
6	2.80 ± 0.63
7	1.1 ± 0.24
8	8.00 ± 0.70
9	5.20 ± 0.70
10	32.00 ± 3.40
11	3.00 ± 0.70

The radioligand binding studies of yohimbine monomeric analogs were carried out using CHO cells expressing homogeneous population of α_{2C}-ARs. The competition binding assays were performed with [^{3}H]rauwolscine (0.1 μCi, 0.7 nM) and nonspecific binding was determined in the presence of 10 μM phentolamine. K_i values were calculated from the equation of Cheng and Prusoff[11] and the data represent the mean of four to nine experiments. The IC_{50} values were determined by nonlinear regression analysis of competition data using the GRAPHPAD PRISM computer program.

noticeable as seen in Table 1. In general, a comparison of K_i values clearly points out that yohimbine dimer **2** has weak affinity for human α_{2C}-ARs as compared to monomeric analogs. Results show that among the monomeric derivatives, compound **5** exceeds the binding affinity of the parent compound yohimbine **1** toward α_{2C}-ARs. Compounds **6**, **7**, and **11** possessed comparable affinities to the parent compound while **8** and **9** displayed relatively lower binding potency than yohimbine. The introduction of a carboxyl group at the side chain led to derivative **11** a structure similar to yohimbinic acid **4** on the other hand surpasses the affinity of **4** for the human α_{2C}-ARs whereas the amino analog **10** exhibited weaker affinity.

The order of binding affinities exhibited by yohimbine **1** on human α_2-AR subtypes was α_{2C} (0.88 nM) $\geqslant \alpha_{2A}$ (1.4 nM) > α_{2B} (7.1 nM), while the order of the most potent compound **5** was found to be α_{2C} (0.65 nM) > α_{2A} (29 nM) > α_{2B} (1300 nM).

In conclusion we describe herein the synthesis and radioligand binding studies of a series of yohimbine derivatives which led to the conclusion that a second pharmacophore is not essential and, binding affinity depends on the nature of the substituent in the side chain found in the bivalent ligand approach.[12] Our future efforts will be oriented in optimizing the potent compound **5** in order to understand the mechanism of binding on these receptors.

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