

Design, synthesis and evaluation of racemic 1-(4-hydroxyphenyl)-2-[3-(substituted phenoxy)-2-hydroxy-1-propyl]amino-1-propanol hydrochlorides as novel uterine relaxants

C. L. Viswanathan,* M. M. Kodgule and A. S. Chaudhari

Department of Pharmaceutical Chemistry, Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai 400 098, India

Received 26 December 2004; revised 15 May 2005; accepted 19 May 2005

Available online 20 June 2005

Abstract—Novel 1-(4-hydroxyphenyl)-2-[3-(substituted phenoxy)-2-hydroxy-1-propyl]amino-1-propanol hydrochlorides were designed based on the pharmacophore for potent uterine relaxant activity and by utilizing the principles of structural hybridization. The designed molecules were synthesized as racemates by a novel route and were evaluated for uterine relaxant activity in vitro on isolated rat uterus and in vivo in pregnant rats. Their cAMP-releasing potential was studied using rat uterus tissue homogenates by the cAMP [³H] assay, and cardiac stimulant potential was evaluated on isolated guinea pig right atrium. All compounds exhibited potent uterine relaxant activity in vitro and produced a significant delay in the onset of labour in pregnant rats; their cAMP-releasing potential was slightly less, while their cardiac stimulant potential was insignificant as compared to isoxsuprine hydrochloride. © 2005 Elsevier Ltd. All rights reserved.

Premature labour is the leading cause of neonatal morbidity and perinatal mortality.¹ β_2 -adrenoceptor agonists are the drugs of choice for treating premature labour.¹ They relax the uterine smooth muscle by inhibiting the rate and force of myometrial contractions and this process involves the intracellular release of cAMP.² This leads to a gradual rise in plasma progesterone levels and maintenance of pregnancy. β_2 -Adrenoceptor agonists, such as isoxsuprine and ritodrine, when used in clinical practice for treating premature labour, also produce tachycardia³ due to cardiac β_1 -adrenoceptor stimulation. To reduce this side effect, co-administration of cardioselective β -blockers is recommended.⁴

The present work was aimed towards developing novel molecules with improved uterine relaxant property and with decreased cardiac stimulant activity. It is proposed to achieve this aim by generating a pharmacophore for potent β_2 -adrenoceptor stimulant activity,

designing novel molecules using the principle of structural hybridization, and synthesizing and evaluating them.

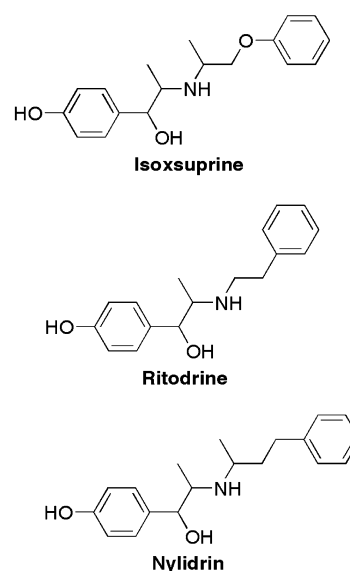


Figure 1. Structures of isoxsuprine, ritodrine, and nyldrin.

Keywords: Uterine relaxants; β_2 -Adrenoceptor stimulants; cAMP [³H] assay.

* Corresponding author. Tel.: +91 22 26670871; fax: +91 22 26670816; e-mail addresses: chelakara_viswanathan@yahoo.com; atulsc@rediffmail.com

A four-point pharmacophore was developed by using the structures of potent β_2 -adrenoceptor stimulants like isoxsuprine, ritodrine and nylidrin (Fig. 1). The study was carried out using the Chem-X⁵ software on a Pentium

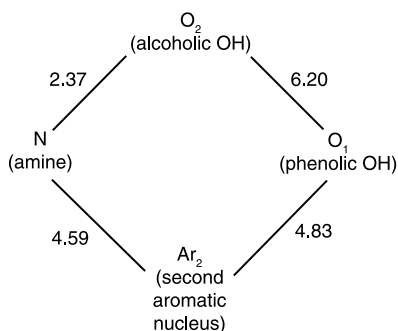
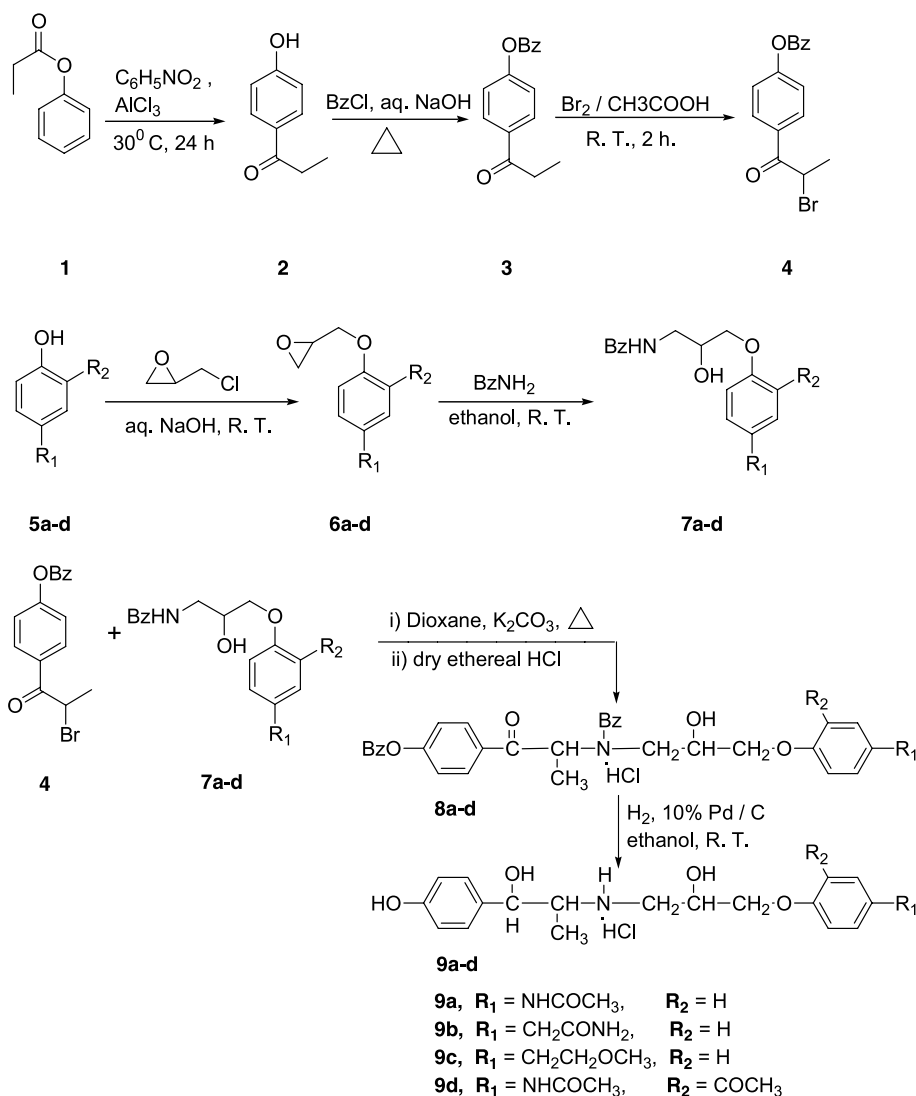


Figure 2. Pharmacophoric points (distances in Angstrom units).

IV 1.6 GHz computer. The conformers of each molecule were generated by using dynamics simulations at a temperature of 310 °C and the sampling time was 5×10^{-4} s. Lowest energy conformations were identified for each molecule and were then used for arriving at the pharmacophore (Fig. 2). Based on the pharmacophore, four novel molecules with the general structure 1-(4-hydroxyphenyl)-2-[3-(substituted phenoxy)-2-hydroxy-1-propyl]amino-1-propanol were designed.

Reacting phenylpropionate **1** with anhydrous AlCl_3 in nitrobenzene at 30 °C yielded 4-hydroxypropiophenone **2** (mp 147 °C). This was condensed with benzyl chloride in aqueous NaOH to yield 4-benzyloxypropiophenone **3** (mp 102–103 °C), which, on bromination with Br_2 in glacial acetic acid at room temperature, gave 2-bromo-4'-benzyloxypropiophenone **4** (mp 75–76 °C). The reaction of substituted phenols (**5a–d**) with epichlorohydrin in aqueous NaOH at room temperature yielded the corresponding substituted phenoxymethyloxiranes (**6a–d**), which were then reacted with benzylamine in ethanol at room temperature yielded the corresponding substituted phenoxymethyloxiranes (**7a–d**), which were then reacted with benzylamine in ethanol



Scheme 1. Synthesis of compounds **9a–d**.

at room temperature to yield substituted phenoxypropa-nolamines (7a–d).

Finally, heating compound 4, in turn, with compounds 7a–d in dioxane in the presence of K_2CO_3 , followed by treatment with dry HCl, gave compounds 8a–d, which were then reduced catalytically using H_2 and 10% Pd/C in ethanol at room temperature to get compounds 9a–d as racemates (Scheme 1).

All compounds were purified by silica gel column chromatography. The physical constants and spectral characteristics of the final compounds are given in Table 1. Fries rearrangement of compound 1 in nitrobenzene at 30 °C gave a higher yield (60%) of para isomer than the reaction in CS_2 and reactions at higher temperatures. In the final step, the reduction of keto function to secondary alcohol, and simultaneous N- and O-debenzylations were achieved in a single step at a hydrogen pressure of 2 bar.

Final compounds 9a–d were evaluated for uterine relaxant activity in isolated rat uterus preparation. The point where the inhibition of oxytocin induced sustained

contractions was recorded at different concentrations (Table 2).^{6,7}

Compounds 9a–d were then evaluated in pregnant Sprague–Dawley rats by a method developed by us.⁸ Each dose was evaluated in a group of six rats. The dose of standard drug isoxsuprine hydrochloride used was 10 mg/kg body weight/day and was administered orally from day 13 to 21 of gestation. Similarly compounds 9a–d were evaluated at doses 10 and 15 mg/kg/day. The rats were weighed daily until parturition and on delivery, the delay in onset of labour was calculated for each rat by comparing with control (see Table 2). Litter size and average weight of pup were also noted.

Compounds 9a–d and isoxsuprine hydrochloride were administered p.o. to Sprague–Dawley female virgin rats in an oestrus phase. Three hours later, the rats were sacrificed and the uterine strips were isolated, weighed, placed in 2 ml Tris–EDTA buffer and homogenized using Kinematica AG, Polytron. The homogenates were centrifuged at 12,000g using Hettich Zentrifugan, Universal 16 R. The supernatants were deproteinised using

Table 1. Physical constants and spectral data of the final compounds

Compound	R ₁	R ₂	Melting point (free base)	% Yield	¹ H NMR (δ in ppm)
9a	–NHCOCH ₃	–H	57–58 °C	62%	δ 1.0 (d, 3H, –CH ₃), δ 2.2 (2s, 2H, 2 –OH), δ 2.9 (m, 1H, –CH–), δ 3.1 (s, 3H, –COCH ₃), δ 3.3 (m, 4H, OCH ₂ , HO–C–CH ₂), δ 3.9 (m, 2H, 2 CHOH), δ 4.2 (s, 1H, –NH), δ 4.6 (2s, 2H, Ar–OH, NHCO), δ 6.8–7.3 (m, 8H, Ar–H)
9b	–CH ₂ CONH ₂	–H	47–49 °C	60%	δ 1.0 (d, 3H, –CH ₃), δ 2.1 (2s, 2H, 2 –OH), δ 2.5 (m, 1H, –CH–), δ 3.4 (s, 2H, –CH ₂ CO), δ 3.7 (m, 4H, OCH ₂ , –COHCH ₂), δ 3.9 (m, 2H, 2 –CHOH), δ 4.2 (s, 1H, –NH), δ 4.6 (s, 1H, Ar–OH), δ 5.0 (s, 2H, –NH ₂), δ 6.8–7.3 (m, 8H, Ar–H)
9c	–CH ₂ CH ₂ OCH ₃	–H	85–87 °C	58%	δ 1.2 (d, 3H, –CH ₃), δ 2.0 (2s, 2H, 2 –OH), δ 2.5 (m, 1H, –CH–), δ 3.2 (m, 5H, CH ₂ COCH ₃), δ 3.6 (t, 2H, ArCH ₂ –), δ 3.8 (m, 4H, OCH ₂ , HO–C–CH ₂), δ 3.9–4.2 (m, 2H, 2 –CHOH), δ 5.1 (m, 2H, ArOH, N–H), δ 6.8–8.0 (m, 8H, Ar–H)
9d	–NHCOCH ₃	–COCH ₃	78–79 °C	60%	δ 1.2 (d, 3H, –CH ₃), δ 2.0 (2s, 2H, 2 –OH), δ 3.3 (2s, 6H, 2 –COCH ₃), δ 3.7 (m, 4H, –OCH ₂ , HO–C–CH ₂), δ 4.2 (m, 2H, 2 –CHOH), δ 4.7 (s, 1H, –NHCO), δ 5.1 (m, 2H, –N–H, ArOH), δ 7–8 (m, 7H, Ar–H)

Table 2. In vitro, in vivo uterine relaxant activity and cAMP assay

Compound	Inhibition of oxytocin induced contraction IC ₅₀ (μmol)	Delayed onset of labour (h)		cAMP released (pmol) at 10 mg (μmol)
		10 mg (μmol)	15 mg (μmol)	
9a	6.07	25.0 (24.3)	31.78 (36.5)	3.48 (24.3)
9b	6.32	24.02 (24.3)	31.70 (36.5)	3.56 (24.3)
9c	6.02	27.05 (24.3)	33.67 (36.4)	3.66 (24.3)
9d	6.58	23.70 (22.0)	30.07 (33.1)	3.53 (22.0)
Isoxsuprine HCl	9.15	31.63 (29.6)	—	5.03 (29.6)

Table 3. In vitro cardiac activity

Compound	Heart rate at different doses (beats per minute)				
	Control	10 μmol	25 μmol	50 μmol	100 μmol
9a	62	60	58	55	55
9b	65	64	64	60	57
9c	62	60	56	53	50
9d	65	63	59	58	55
	Control	0.5 μmol	0.75 μmol	1.0 μmol	1.25 μmol
Isoxsuprine HCl	62	70	74	79	84

trichloroacetic acid (TCA) and the tubes were centrifuged again. The supernatants were freed from TCA by repeated extractions with ether and then the pH was adjusted to 7.5. The cAMP (^3H) assay system (TRK 432) from Amersham International plc, UK, was used as per the procedure listed in their booklet.⁹ To assay tubes placed in an ice bath was added standard cAMP in concentrations of 0, 1, 2, 4, 8 and 16 pmol/50 μl Tris–EDTA buffer, 50 μl (0.9 pmol) cAMP [^3H] and 100 μl of binding protein. Tubes were vortex-mixed for 5 s and allowed to stand in a refrigerator at 2–8 $^{\circ}\text{C}$ for 2 h. Charcoal suspension (100 μl) was then added to each tube, vortex-mixed and centrifuged. Two hundred microlitres of supernatant was removed from each tube and was placed in scintillation vials for counting.

Fifty microliters of supernatants obtained after tissue extractions (for samples) was similarly analyzed. The counts were recorded as counts per minute (cpm) using β -scintillation counter. From a plot of C_0/C_x vs c , where C_0 and C_x are the cpm in the absence and presence of standard cAMP, respectively, and c is the concentration of standard cAMP, the amount of cAMP present in samples was determined (see Table 2).

Compounds **9a–d** and isoxsuprine hydrochloride were evaluated in an isolated guinea pig right atrium.¹⁰

Test compounds **9a–d** exhibited potent inhibition of oxytocin induced sustained contractions of rat uterus and produced a significant delay in the onset of labour (24–34 h) in pregnant rats and their activity is comparable to that of isoxsuprine hydrochloride. Increase in gestation period led to a significant increase in the average weight of the pups, but no effect was observed on the mean litter size. No mortality was recorded in pregnant rats or in the pups delivered. The cAMP release study showed that the test compounds were slightly less potent. Test compounds inhibited an ISP induced increase in the heart rate and exhibited an insignificant cardiac depression at higher doses studied, while isoxsuprine hydrochloride, even at much lower doses, exhibited significant cardiac stimulation. The present work thus led

to the development of novel uterine relaxants free from cardiac stimulation side effects.

Acknowledgments

The authors thank the Indian Council of Medical Research (ICMR), New Delhi, for the financial support and Dr. S. B. Moodbidri, Deputy Director, Institute for Research in Reproduction (IRR), Parel, Mumbai 400 012, for assistance in pharmacological evaluation.

References and notes

1. Caritis, S. N. *Drugs* **1983**, 26, 243.
2. Gabbe, S. G.; Niebyl, J. R.; Simpson, J. L. In *Obstetrics: Normal and Problem Pregnancies*; Churchill Livingstone: New York, 1991, p 829.
3. Schenken, R. S.; Hayashi, R. H.; Valenzuela, G. V.; Castillo, M. S. *Am. J. Obstet. Gynecol.* **1980**, 137, 773.
4. Mahon, W. A.; Reid, D. W. *J. J. Pharmacol. Exp. Ther.* **1967**, 156, 178.
5. Chem-X, Chemical Design Ltd, Oxon, England, Jan. 1997.
6. Gaddum, J. H.; Peart, W. S.; Vost, M. *J. Physiol. (London)* **1949**, 108, 467.
7. *Selected Topics in Experimental Pharmacology*; Sheth, U. K., Dadkar, N. K., Kamat, U. G., Eds.; Shri Mohanlal B. Kothari Publishers: Bombay, 1972, p 160.
8. Kodgule, M. M.; Viswanathan, C. L.; Moodbidri, S. B.; Vanage, G. *Indian J. Pharm. Sci* **1997**, 59, 42.
9. Cyclic AMP [^3H] assay system (code TRK 432), Amersham International plc, Amersham, UK.
10. Black J. W.; Duncan W. A. M.; Shanks R. G. *Br. J. Pharmacol.* **1965**, 25, 577. The atrium was mounted in a jacketed organ bath filled with Ringer–Locke solution under a resting tension of 1 g. Carbogen gas was bubbled and the temperature was maintained at 37 $^{\circ}\text{C}$ and the tissue was allowed to equilibrate for 45 min. Ten micromole of isoproterenol sulfate (ISP, Ref. Std) was added to the organ bath and the heart rate was recorded using a force transducer on a polygraph. Compounds **9a–d** at doses of 10, 25, 50 and 100 μmol were added to the bath and incubated for 3 min, then 1 μmol ISP was added and the heart rate was recorded (Table 3). In a similar manner isoxsuprine hydrochloride was evaluated at 0.5, 0.75, 1.0 and 1.25 μmol doses.