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Synthesis and evaluation of uterine relaxant activity for a novel series of substituted *p*-hydroxyphenylethanolamines

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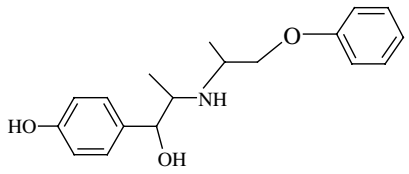
Abstract—Novel racemic 1-(4-hydroxyphenyl)-2-[3-(substituted phenoxy)-2-hydroxy-1-propyl]aminopropan-1-ol hydrochlorides (**9a**–**h**) were synthesized by condensing racemic 1-(*p*-hydroxyphenyl)-2-aminopropan-1-ol hydrochloride (**6**) with substituted aryloxymethyloxiranes (**8a**–**h**) in DMF in presence of anhydrous potassium carbonate and then reacting with dry hydrogen chloride gas. They 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 cAMP [^{3}H] assay and cardiac stimulant potential was evaluated in dog. 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 higher than isoxsuprine hydrochloride except for **9b** and **9c**. Finally insignificant cardiac stimulant potential was noted for these compounds when compared to isoxsuprine hydrochloride.

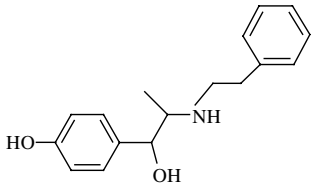
1. Introduction

Adrenergic agonists with selectivity for β_2-adrenoceptors are used in the management of premature labour, which is the leading cause of neonatal morbidity and perinatal mortality.[1] They act by releasing cAMP intracellularly which leads to inhibiting myometrial contractions.[2] It also leads to increase in plasma progesterone levels and maintenance of pregnancy. However, many of the β-adrenergic agonists like isoxsuprine, ritodrin and nylidrin (Fig. 1) used in treating premature labour also produce tachycardia[3] by stimulation of cardiac β-adrenergic receptors. This was a serious side effect and resulted in withdrawal of treatment in some cases. In order to reduce this side effect, co-administration of cardioselective β-adrenergic blockers was recommended.[4]

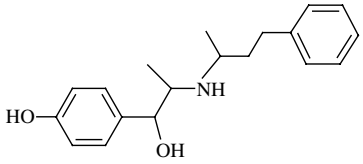
To overcome this side effect, we developed a novel class of β-adrenergic receptor agonists by pharmacophore generation and by incorporation of essential structures to reduce tachycardia. The design, synthesis and pharmacological evaluation of four novel compounds from this series was reported earlier.[5] The successful development of novel uterine relaxants devoid of serious side effects prompted us to extend this work and generate



Isoxsuprine



Ritodrine



Nylidrin

Figure 1. Structures of isoxsuprine, ritodrine and nylidrin.

Keywords: Uterine relaxants; β_2-Adrenoceptor stimulants; cAMP [^{3}H] assay; Phenylethanolamines.
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more potent analogues. In the present work, we report the synthesis and the pharmacological evaluation of a series of eight compounds.The synthetic process followed is quite novel and has advantages over the method reported earlier.[5] The cardiac stimulant activity was also determined by an alternate method.

2. Results and discussion

2.1. Chemistry

A novel series of racemic 1-(4-hydroxyphenyl)-2-[3-(substituted phenoxy)-2-hydroxy-1-propyl]aminopropan-1-ol hydrochlorides (**9a**–**h**) were synthesized by condensing substituted aryloxymethyloxiranes (**8a**–**h**) with racemic 1-(*p*-hydroxyphenyl)-2-aminopropan-1-ol hydrochloride (**6**) in DMF in presence of anhydrous potassium carbonate and then reacting with dry hydrogen chloride (Scheme 1). The substituted aryloxymethyloxiranes were prepared from substituted phenols (**7a**–**h**) by reacting with epichlorohydrin in aqueous NaOH. Compound **6** was synthesized from phenyl propionate by Fries rearrangement to get *p*-hydroxypropiophenone. The hydroxyl group was protected by benzylation and then the compound was brominated at the alpha position using Br_2 in $CHCl_3$. The bromo compound was then condensed with benzylamine in ethanol to get an amine (**5**) which was then catalytically reduced using 10% Pd–C and H_2 at 40 psi and finally converted into hydrochloride salt.

All the steps involved in the synthetic process were optimized with respect to reaction conditions and yields. For example, during the synthesis of **6** catalytic hydrogenation was used to achieve keto reduction to alcohol and both N- and O-debenzylations. The synthetic scheme followed has advantages over the method reported earlier.[5] The hydrogenation step is used only once to synthesize 6 in bulk, while in the previous method[5] this step is involved in the synthesis of each title compound.

2.2. Pharmacology

The title compounds (**9a**–**h**) were evaluated in vitro for uterine relaxant activity in isolated rat uterus preparation[6,7] using isoxsuprine hydrochloride as the standard and the IC_{50} for the inhibition of oxytocin induced sustained contractions were recorded (Table 1). The study showed potent inhibition of oxytocin induced sustained contractions.

Title compounds (**9a**–**h**) were then evaluated in vivo for uterine relaxant activity using pregnant Sprague–Dawley rats by a method developed by us.[8] They were evaluated in two doses (10 and 15 mg/kg). Each dose was administered orally to a group of six rats from day 13 to 21 of gestation. The delay in onset of labour was calculated for each rat by comparing with control. The average values are given in Table 2 along with the concentrations in micromoles in parentheses. Title compounds produced significant delay in the onset of labour (25–36 h) in in vivo study. Title compounds were more potent than isoxsuprine hydrochloride when activity is compared on a mole to mole basis. There was significant increase in the average weight of the pups (Table 2) in the drug treated groups compared to control and this can be attributed to the increase in gestation periods. There was no mortality recorded in pregnant rats or in the pups delivered.

Title compounds (**9a**–**h**) and isoxsuprine hydrochloride were evaluated for their ability to release cAMP using cAMP [^{3}H] assay system (TRK 432) from Amersham International plc, UK[9], and by following the procedure reported earlier.[5] The cAMP (pmol) released at a dose of 10 mg/kg body weight are given in Table 1. Results indicate that except for title compounds **9b** and **9c** all others were more potent than isoxsuprine hydrochloride when activity was compared on a mole to mole basis.

Title compounds (**9a**–**h**) were evaluated in vivo in dog for cardiac stimulant activity[10] at doses 1.0 and 1.5 mg/kg body weight. The heart rate in beats per minute (bpm) was recorded and is given in Table 2. Insignificant increase in heart rate was recorded for title compounds and one compound **9h** showed slight cardiac depression. In contrast isoxsuprine hydrochloride exhibited clinically significant tachycardia (heart rate > 75 bpm) at a much lower dose (0.05 mg/kg body weight).

3. Conclusions

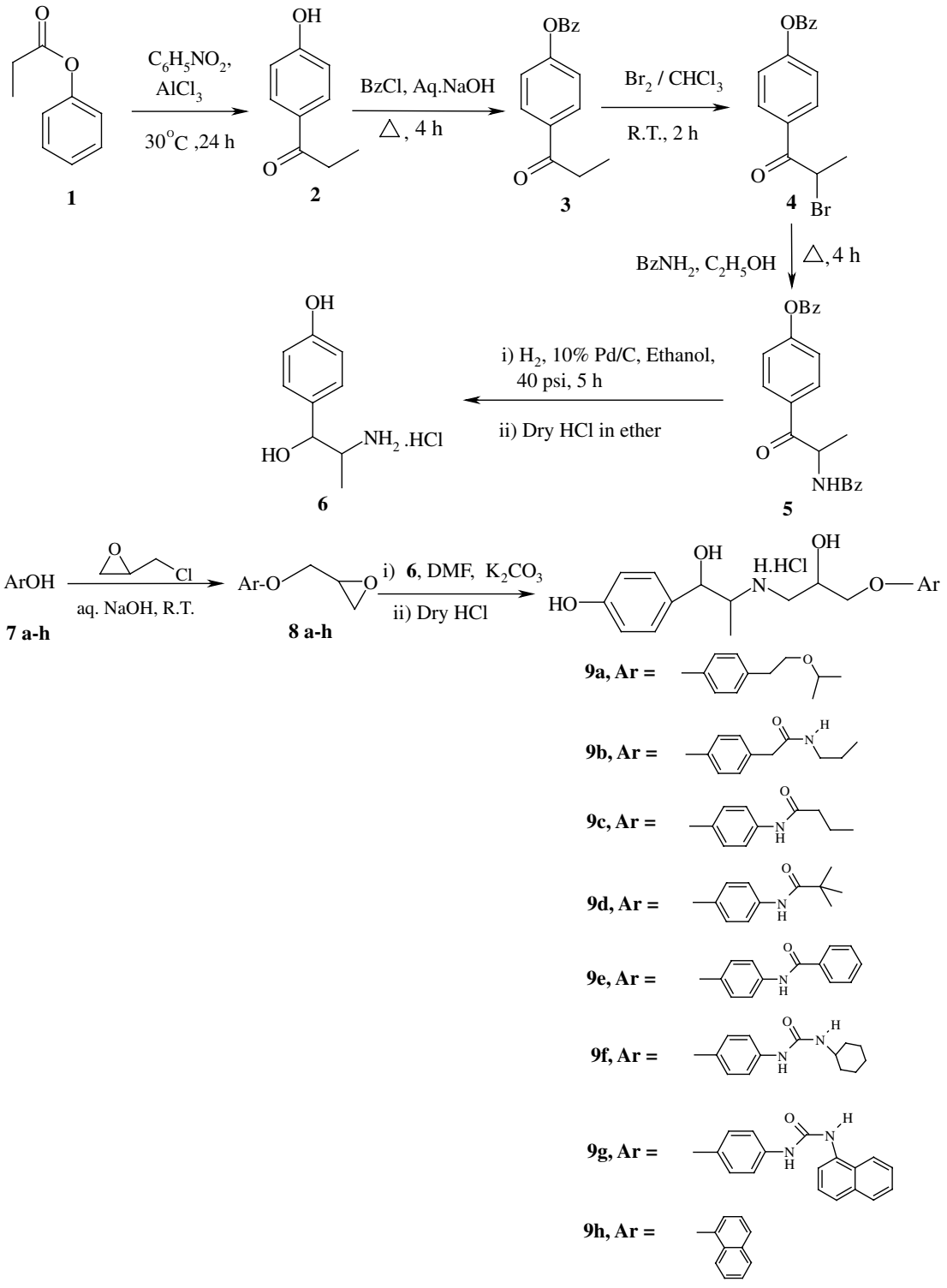
A novel series of substituted *p*-hydroxyphenylethanolamines were synthesized by novel synthetic process, characterized by spectral and elemental analysis, and were found to exhibit potent uterine relaxant activity when evaluated both in in vitro and in vivo models. cAMP release assay indicated that their activity were higher than that of the standard drug isoxsuprine hydrochloride except for **9b** and **9c**. Finally title compounds were devoid of cardiac stimulant side effects.

4. Experimental

Reactions were monitored by TLC using silica gel G coated plates. Compounds were purified by column chromatography. Carbon and hydrogen were determined by combustion, while nitrogen was determined by Kjeldahl's method. Calculated values are given in parentheses. Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded on Jasco FT-IR 5300 by KBr pellet method. Proton NMR spectra were recorded on Gemini-200 FT-NMR.

4.1. General procedure for synthesis of racemic 1-(4-hydroxyphenyl)-2-[3-(substituted phenoxy)-2-hydroxy-1-propyl]aminopropan-1-ol hydrochlorides (9a–h)

A solution of 0.18 M of substituted phenols (**7a**–**h**) in 100 ml of 10% aq NaOH was placed in a round-bottomed flask fitted with a mechanical stirrer. Then



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

33.3 g (0.36 M) of epichlorohydrin was slowly added with stirring and the mixture was stirred for 8–10 h at temperatures 25–60 °C. The solid oxirane formed was filtered and washed with little methanol to remove entrapped epichlorohydrin and then with water to remove alkali. In the case of liquid oxiranes, the oily layer was separated and washed with water in a separating funnel and then dried. The dried liquid was then distilled under reduced pressure to remove excess epichlorohydrin. The oxiranes (**8a**–**h**) formed were then used for further condensation.

In a round-bottomed flask fitted with a reflux condenser and a mechanical stirrer was placed 40.5 g (0.2 M) of **6** in 100 ml DMF and stirred with 40 g of anhydrous K_2CO_3 for 15 min. Then oxiranes, (**8a**–**h**) were added

Table 1. In vitro uterine relaxant activity and cAMP assay

Compound	Inhibition of oxytocin induced contraction IC_{50} (μM)	cAMP released (pmol) at 10 mg (μM)
9a	5.81	4.37 (23.4)
9b	6.08	3.61 (22.1)
9c	5.96	3.81 (22.8)
9d	6.01	3.93 (22.1)
9e	5.88	3.99 (21.16)
9f	5.75	4.45 (20.26)
9g	5.69	4.79 (21.66)
9h	5.93	4.25 (24.78)
Isoxsuprine HCl	8.57	5.03 (29.6)

in turn and stirred for 10–15 h. DMF was then removed under vaccum and the residue was extracted with 100–150 ml solvent ether. The extract was washed with 2 N HCl for removal of unreacted **6** and then with water and then dried over anhyd $MgSO_4$. Dry HCl was passed through the ether extract when hydrochloride salt separated out. The salt was filtered and recrystallized from ethanol.

4.1.1. Compound 9a. Yield: 85%; mp 158–159 °C. IR (KBr, cm^{-1}): 3374–3325 O–H, N–H, stretch; 2980–2892 C–H stretch; 1178 C–O–C stretch; ^1H NMR δ ppm: 1.4 (m, 9H, 3 $-CH_3$), 2.2 (s, 2H, 2 –OH), 2.7 (t, 2H, $ArCH_2$–), 3.3–3.4 (m, 6H, 2 $-OCH_2$, –N–CH–, –OCH–), 3.8 (d, 2H, Ar–OCH_2–), 4.5 (m, 1H, –C*H*(OH)), 5.4 (d, 1H, Ar–CH–), 6.7–7.2 (m, 8H, Ar–H), 8.6 (s, 2H, $-^+NH_2$–), 9.0 (s, 1H, Ar–OH); Elemental analysis (%): C 61.96 (62.18), H 7.83 (7.78), N 3.24 (3.18).

4.1.2. Compound 9b. Yield: 72%; mp 195–196 °C. IR (KBr, cm^{-1}): 3387–3294 O–H, N–H, stretch; 2976–2922 C–H stretch; 1658 C=O stretch; ^1H NMR δ ppm: 1.4 (m, 6H, 2 $-CH_3$), 2.1 (s, 2H, 2 –OH), 2.65 (m, 2H, –CH_2–CH_3–), 2.9 (s, 2H, $ArCH_2$–), 3.3 (m, 5H, 2 –NHCH_2–, –NHC*H*), 4.0 (d, 2H, $-OCH_2$–), 4.9 (m, 1H, –C*H* (OH)), 5.4 (d, 1H, –Ar–CH (OH)), 6.5–7.4 (m, 8H, Ar–H), 7.7 (s, 1H, –NH–), 8.8 (s, 2H, $-^+NH_2$–), 9.1 (s, 1H, Ar–OH); Elemental analysis (%): C 66.28 (66.37), H 7.29 (7.34), N 6.15 (6.18).

4.1.3. Compound 9c. Yield: 84%; mp 173–174 °C. IR (KBr, cm^{-1}): 3365–3295 O–H, N–H, stretch; 2962–2874 C–H stretch; 1660 C=O stretch; ^1H NMR δ ppm: 1.2 (m, 6H, 2 $-CH_3$), 2.1 (s, 2H, 2 –OH), 2.3 (m, 2H, –CH_2–CH_3–), 2.8 (m, 2H, $-COCH_2$–), 3.1 (m, 3H, –NHCH_2–, NHC*H*–), 3.8 (d, 2H, OCH_2), 5.0 (m, 1H, –C*H*(OH)), 5.7 (d, 1H, ArCH–), 6.9–7.6 (m, 8H, Ar–H), 7.9 (s, 1H, –NH–), 8.8 (s, 2H, $-^+NH_2$–), 9.1 (s, 1H, Ar–OH); Elemental analysis (%): C 60.10 (60.19), H 7.15 (7.11), N 6.38 (6.37).

4.1.4. Compound 9d. Yield: 84%; mp 187–188 °C. IR (KBr, cm^{-1}): 3387–3305 O–H, N–H, stretch; 2964–2926 C–H stretch; 1658 C=O stretch; ^1H NMR δ ppm: 1.4 (s, 12H, 4 $-CH_3$), 2.2 (s, 2H, 2 –OH), 3.2 (m, 3H, NHCH_2–, NHC*H*–), 3.95 (d, 2H, $-OCH_2$), 4.6 (m, 1H, –C*H* (OH)), 5.6 (d, 1H, ArCH–), 6.9–7.6 (m, 8H, Ar–H), 8.35 (s, 1H, –NHCO–), 8.8 (s, 2H, $-^+NH_2$–), 9.2 (s, 1H, Ar–OH); Elemental analysis (%): C 61.22 (60.98), H 7.45 (7.34), N 6.10 (6.18).

4.1.5. Compound 9e. Yield: 86%; mp 214–215 °C. IR (KBr, cm^{-1}): 3382–3329 O–H, N–H, stretch; 1647 C=O stretch; ^1H NMR δ ppm: 1.25 (d, 3H, $-CH_3$), 2.2 (s, 2H, 2 –OH), 3.2 (m, 3H, NHCH_2–, NHC*H*–), 4.5 (d, 2H, $-OCH_2$), 4.5 (m, 1H, –C*H*(OH)), 5.6 (d, 1H, ArCH–), 7.1–7.6 (m, 13H, Ar–H), 7.9 (s, 1H, Ar–NH), 8.75 (s, 2H, $-^+NH_2$–), 9.05 (s, 1H, Ar–OH); Elemental analysis (%): C 63.55 (63.48), H 6.02 (6.18), N 5.97 (5.92).

4.1.6. Compound 9f. Yield: 80%; mp 180–181 °C. IR (KBr, cm^{-1}): 3390–3323 O–H, N–H, stretch; 2928–2851 C–H stretch; 1640 C=O stretch; ^1H NMR δ ppm: 1.4 (d, 3H, $-CH_3$), 1.9 (m, 10H, 5 $-CH_2$–), 2.25 (s, 2H, 2 –OH), 3.2 (m, 4H, NHCH_2–, 2 –NHC*H*–), 3.9 (d, 2H, $-OCH_2$), 4.3 (m, 1H, –C*H*(OH)), 5.5 (d, 1H, ArCH–), 7.0–7.6 (m, 8H, Ar–H), 8.2 (s, 2H, 2 –CONH–), 8.9 (s, 2H, $-^+NH_2$–), 9.15 (s, 1H, Ar–OH); Elemental analysis (%): C 60.89 (60.77), H 7.53 (7.34), N 8.45 (8.50).

4.1.7. Compound 9g. Yield: 78%; mp 202–203 °C. IR (KBr, cm^{-1}): 3366–3294 O–H, N–H, stretch; 1641 C=O stretch; ^1H NMR δ ppm: 1.4 (d, 3H, $-CH_3$), 2.2 (s, 2H, 2 –OH), 3.2 (m, 3H, NHCH_2–, –NHC*H*–), 3.9 (d, 2H, $-OCH_2$), 4.6 (m, 1H, –C*H*(OH)), 5.6 (d, 1H, ArCH–), 6.8–7.6 (m, 15H, Ar–H), 8.3 (s, 2H, 2 –CONH–), 8.8 (s, 2H, $-^+NH_2$–), 9.2 (s, 1H, Ar–OH); Elemental analysis (%): C 64.57 (64.73), H 5.80 (5.99), N 7.65 (7.80).

Table 2. In vivo, uterine relaxant activity

Compound	Delay in onset of labour (h)[a]		Average weight of pups (g)	
	At 10 mg (μM)	At 15 mg (μM)	At 10 mg (μM)	At 15 mg (μM)
9a	30.07 ± 1.56 (23.4)	36.19 ± 1.06 (35.16)	7.34 ± 1.03 (23.4)	7.66 ± 0.71 (35.16)
9b	25.83 ± 1.19 (22.1)	32.59 ± 0.95 (33.15)	7.15 ± 1.12 (22.1)	7.30 ± 0.81 (33.15)
9c	27.16 ± 1.32 (22.8)	33.37 ± 1.83 (34.21)	7.16 ± 0.64 (22.8)	7.36 ± 1.07 (34.21)
9d	28.03 ± 0.93 (22.1)	34.21 ± 0.78 (33.15)	6.74 ± 1.15 (22.1)	7.04 ± 0.98 (33.15)
9e	29.25 ± 1.01 (21.16)	35.51 ± 1.50 (31.7)	7.28 ± 1.18 (21.16)	7.34 ± 0.59 (31.7)
9f	30.97 ± 0.87 (20.26)	36.42 ± 1.21 (30.39)	7.48 ± 1.50 (20.26)	7.76 ± 0.92 (30.39)
9g	31.07 ± 1.20 (21.66)	36.60 ± 1.36 (32.5)	7.54 ± 1.05 (21.66)	7.80 ± 0.87 (32.5)
9h	29.74 ± 1.55 (24.78)	35.91 ± 1.10 (37.17)	6.82 ± 0.89 (24.78)	7.38 ± 0.82 (37.17)
Isoxsuprine HCl	31.33 ± 1.08 (29.6)	—	7.32 ± 0.54 (29.6)	—

[a] $P < 0.001$.

Table 3. In vivo cardiac activity

Compound	Heart rate at different doses (beats per minute)[b]	
Dose (mg/kg)	1.0	1.5
Control	61.11	60.46
9a	57.84	60.29
9b	61.82	61.96
9c	60.66	62.53
9d	64.20	67.90
9e	62.87	64.15
9f	63.58	67.82
9g	64.59	68.31
9h	56.66	57.34
Dose (mg/kg)	0.05	
Isoxsuprine HCl	87.38[a]	

[a] Clinically significant tachycardia (heart rate > 75 bpm).
[b] Standard deviation is ±1–2 bpm.

4.1.8. Compound 9h. Yield: 82%; mp 164–165 °C. IR (KBr, cm^{-1}): 3368–3300 O–H, N–H, stretch; 3072 C–H stretch; ^1H NMR δ ppm: 1.25 (d, 3H, $-CH_3$), 2.2 (s, 2H, 2 –OH), 3.1 (m, 3H, $NHCH_2$–, –NHC*H*–), 3.95 (d, 2H, $-OCH_2$), 4.3 (m, 1H, –C*H*(OH)), 5.5 (d, 1H, ArCH–), 6.6–7.2 (m, 11H, Ar–H), 8.6 (s, 2H, $-^+NH_2$–), 9.25 (s, 1H, Ar–OH); Elemental analysis (%): C 65.26 (65.41), H 6.41 (6.48), N 3.48 (3.46).

5. Cardiac stimulant activity

All animal experiments had Institutional Ethics Committee clearance.

Adult dogs of either sex weighing 8–15 kg were anaesthetized with pentobarbitone sodium (75 mg/kg iv). A dog was laid on the operation table and its limbs were tied. It was intubated with a cuffed endotracheal tube and placed on a Harvard respirator (20 ml/kg, 10–15 cycles/min). Femoral vein and artery were cannulated using polyethylene tubing for drug administration and for determination of arterial blood pressure, respectively. The arterial cannula was connected to a Statham model P23 Gb transducer. Recordings were made on a polygraph. The heart rate was measured with a Bechman Cardiotech connected to a voltage/pressure-pulse coupler.

Test compounds (**9a**–**h**) were evaluated at 1.0 and 1.5 mg/kg body weight doses, while standard isoxsuprine hydrochloride was evaluated at 0.05 mg/kg body weight dose. Test doses were fixed by preliminary evaluation study. The heart beats measured are given in Table 3.

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