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Original article

Synthesis, β-adrenoceptor pharmacology and toxicology of *S*-(−)-1-(4-(2-ethoxyethoxy)phenoxy)-2-hydroxy-3-(2-(3,4-dimethoxyphenyl)ethylamino)propane hydrochloride, a short acting β_1-specific antagonist

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Abstract

The synthesis of *S*-(−)-1-(4-(2-ethoxyethoxy)phenoxy)-2-hydroxy-3-(2-(3,4-dimethoxyphenyl)ethylamino)propane hydrochloride (D140S·HCl **6**), a novel short acting β_1-specific adrenoceptor antagonist, has been described. The antagonist potency for D140S·HCl **6** has been compared with esmolol, another short acting agent, and other well known β-adrenoceptor antagonists in isolated rat tissue preparations. The pharmacokinetics of D140S·HCl **6** in 7 day continuous intravenous infusions and 4 weeks intravenous bolus injection studies in conscious rats and dogs have been examined in toxicology studies. The effect on the isoprenaline-induced heart rate increase and the pharmacodynamic half-life of D140S·HCl **6** has been compared with esmolol in a conscious rat model. In addition, the results of a range of toxicological studies are presented. The results indicate that D140S·HCl **6** is a highly specific β_1-adrenoceptor antagonist ($pA_2 = 8.15 \pm 0.22$, β_1/β_2 selectivity > 4400). The in vitro studies suggest D140S·HCl is ca. ten times more potent and 60 times more β_1-specific than racemic esmolol. Pharmacokinetic non-linearity was seen when given as a 7 day intravenous infusion at toxicological doses above 10 mg kg^{-1} h^{-1} in the rat and 2.5 mg kg^{-1} h^{-1} in the dog. Both D140S·HCl **6** and esmolol have very short durations of action after intravenous infusion in the rat (pharmacodynamic half-life is < 15 min for D140S·HCl and 10 min for esmolol). The toxicological tests indicate that D140S·HCl **6** shows no unexpected toxicity and none of the tissue irritancy problems reported for esmolol formulations.

Keywords: β1-Adrenoceptor antagonist; Short acting antagonist; D140S·HCl; Synthesis; Pharmacology; Toxicology

1. Introduction

Short acting β-blocking drugs represent a special class of β-adrenoceptor antagonists suitable for parenteral use with a short half-life (< 30 min) in the circulation [1–3]. The short acting agents have an advantage over oral agents in clinical situations where a quick on/off effect is desirable. There is considerable parenteral use of short acting β-blocking drugs in anesthetic procedures, cardiac surgery and in intensive care to control tachycardia, arrhythmias and hypertension [1,2,4–6,8–10]. In addition, there is extensive preclinical and clinical data supporting the early use of β_1-adrenoceptor

Abbreviations: Co, maximum plasma concentration at time 0; D140S·HCl, *S*-(−)-1-(4-(2-ethoxyethoxy)phenoxy)-2-hydroxy-3-(2-(3,4-dimethoxyphenyl)ethylamino)propane hydrochloride; Homoveratrylamine, 3,4-dimethoxyphenethylamine; (±)-LK204-545, (±)-1-(2-(3-(2-cyano-4-(2-cyclopropylmethoxyethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea; NOEL, no effect level; $t_{1/2}$, Half-life; Vd, volume of distribution.

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antagonists in acute myocardial infarction [1,4,8–10]. Early introduction of β-blocker therapy following myocardial infarction would be facilitated by the availability of a safe non-irritant, short acting parenteral agent. There is a paucity of available compounds which have these properties and are significantly β_1-adrenoceptor specific with minimal negative hemodynamic effects. The only marketed short acting β-blocker is esmolol and its short pharmacodynamic half-life ($t_{1/2}$, ca. 10 min) can be attributed to the ester group in the para-substituent of the phenoxypropanolamine (Fig. 1), which undergoes rapid hydrolysis by esterases to a metabolite with relatively weak activity [4,7]. Esmolol formulations, however, are tissue irritants and cause profound hypotension in up to 50% of reported cases [1].

During our β_1-adrenoceptor antagonist design and synthesis program, we identified a series of antagonists specific for the β_1-adrenoceptor which had very short pharmacodynamic $t_{1/2}$ [11]. One of these compounds, *S*-(−)-1-(4-(2-ethoxyethoxy)phenoxy)-2-hydroxy-3-(2-(3,4-dimethoxyphenyl)ethylamino)propane hydrochloride (D140S·HCl **6**), is highly β_1-adrenoceptor selective, non-irritant, minimally cardio- and vasodepressive and is short acting, making it suitable for parenteral use. Since D140S·HCl **6** does not contain an ester moiety (Fig. 2) its short duration of action cannot be due to esterase hydrolysis, as in the case of esmolol. Unfortunately we have not yet elucidated the exact mechanism responsible for the short duration of action of D140S·HCl **6**. However, the pharmacokinetic and pharmacodynamic studies that we have carried out so far, and present here, suggest that non-linear kinetics and by inference saturation of metabolism only occur at the higher doses used in the toxicology studies. The synthesis of D140S·HCl **6** is presented here, along with the in vitro antagonist potency of this compound in rat atria (β_1-adrenoceptor) and trachea (β_2-adrenoceptor) and a summary of its toxicology. The pharmacokinetics of D140S·HCl **6** in rats and dogs, as well as the effect on the isoprenaline-induced heart rate increase and the pharmacodynamic $t_{1/2}$ of D140S·HCl **6** in a conscious rat model, are also presented.

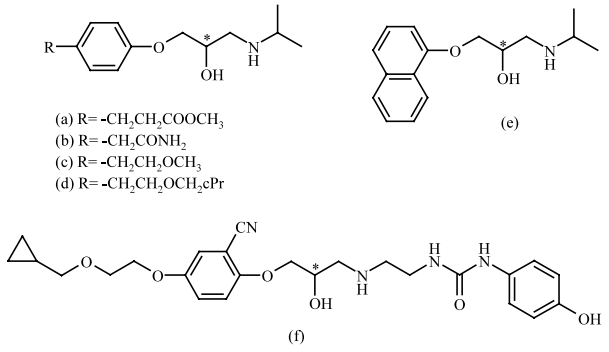

Fig. 1. Structures of (a) esmolol, (b) atenolol, (c) metoprolol, (d) betaxolol, (e) propranolol and (f) LK 204-545. The asterisks denote a chiral centre.

2. Chemistry

The synthesis of D140S·HCl **6** is relatively straightforward and achieved with the use of the appropriate chiral glycidyl-3-nitrobenzenesulfonate as shown in Fig. 2. The hydroxyl group of commercially available 2-ethoxyethanol **1** was protected using *p*-toluenesulfonyl chloride and the resulting compound was reacted with 4-benzyloxyphenol to give 1-benzyloxy,4-(2-ethoxyethoxy)benzene **3**. The benzyl protecting group was removed by hydrogenation to give the desired phenol **4**. The phenol **4** reacted with 2*S*-(+)-glycidyl-3-nitrobenzenesulfonate to produce the *S*-(+)-epoxide **5**. The ring opening addition reaction of the epoxide **5** with homoveratrylamine, followed by the conversion of the free base to the hydrochloride salt gave the final compound D140S·HCl **6**. The enantiomeric purity of the final product is >99%, as assessed by chiral HPLC, and essentially dependent on the enantiomeric purity of the glycidyl-3-nitrobenzenesulfonate. Under the conditions described in the Section 7.1.4 there appears to be no 'pseudoracemisation', which occurs when the phenoxide anion attacks the oxirane ring rather than directly displacing the nitrobenzenesulfonate (this phenomenon was a serious source of racemisation in our initial synthesis when using chiral glycidyl toluenesulfonate). The assigned structure of D140S·HCl **6** was fully confirmed by MS and ^{1}H-NMR spectra and elemental analysis. D140S·HCl **6** is very soluble in water and stable to treatment with hot acids and bases without degradation or racemisation.

3. Pharmacology

3.1. Isolated tissue preparations

The compounds listed in Table 1 were evaluated for their ability to antagonise β_1- and β_2-adrenoceptors in rat atria and tracheal rings, respectively. Cumulative concentration–response curves were obtained in each preparation as described by van Rossum [12] and curves were fitted by computer analysis according to the method of Zabrowsky et al. [13] using the sigmoidal fit function of the ORIGIN graphics package (MICROCAL ORIGIN, MicroCal Software Inc., One Roundhouse Plaza, Northampton, MA 01060, USA). The antagonist potencies, or pA_2 values, were calculated and represent the mean ± S.E.M. from three to five individual experiments.

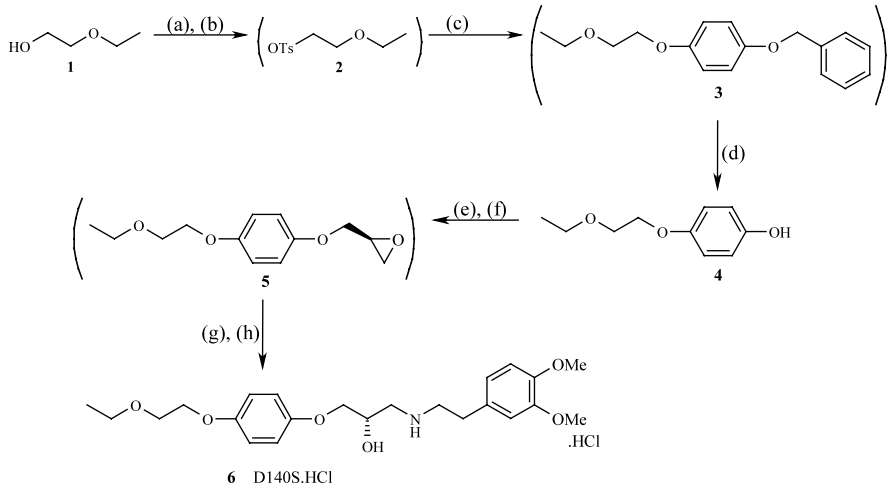

Fig. 2. Synthetic pathway for D140S·HCl **6**. Reagents: (a) *p*-toluenesulfonyl chloride; (b) NaOH; (c) 4-benzyloxyphenol, NaOH; (d) H_2, Pd/C; (e) NaH, DMF; (f) 2*S*-(+)-glycidyl-3-nitrobenzenesulfonate, DMF; (g) homoveratrylamine, dioxane; (h) diethylether, ethereal HCl. Note: the brackets surrounding intermediates **2**, **3** and **5** are to indicate that they were not isolated and characterised during the synthesis of D140S·HCl **6**.

3.2. D140S·HCl (*6*) pharmacokinetics

Plasma concentrations of D140S·HCl **6** for the following pharmacokinetic studies were estimated by HPLC using the assay described in the Section 7.2.3. The collection of plasma samples was carried out for us by the commercial laboratories whilst performing the toxicological studies (Section 4).

3.2.1. Seven day continuous intravenous infusion in conscious rats

Plasma samples were taken from 80 conscious Sprague–Dawley rats (ten males and ten female at each dose level, weighing 210–332 and 170–255 g, respectively), via indwelling catheters, at the end of D140S·HCl **6** infusions of 7 days duration. Doses studied were 0, 5.0, 10.0 and 15.0 mg kg^{-1} h^{-1}. The plasma concentrations of D140S·HCl **6** on day 7 of the continuous intravenous infusion were calculated and are given in Table 2.

3.2.2. Four week intravenous bolus injection study in conscious rats

Forty eight conscious Sprague–Dawley rats (six males and six females at each dose level, weighing 195–233 and 162–193 g, respectively) received intravenous bolus injection doses of 0, 10.0, 20.0 and 30.0 mg kg^{-1} bd D140S·HCl **6** for 4 weeks. Plasma samples were taken 10 min after the second dose on day 28. The plasma concentrations of D140S·HCl **6** after the bolus injection were calculated and are given in Table 3.

3.2.3. Seven day continuous intravenous infusion in conscious dogs

Plasma samples were taken from 16 conscious beagle dogs (two males and two females at each dose level, weighing 6.8–9.8 kg) on days 2 and 7 of the study. D140S·HCl **6** intravenous infusion rates were 0, 2.5, 5.0 and 10.0 mg kg^{-1} h^{-1}, via indwelling catheters. The high dose was reduced to 7.5 mg kg^{-1} h^{-1} on day 4 because of side effects developing in all 4 dogs at the 10.0 mg kg^{-1} h^{-1} dose (see Sections 5.2.3 and 5.4). The

Table 1
In vitro potency (pA_2) and selectivity of D140S·HCl **6** and reference β-adrenoceptor antagonists in rat atria and trachea

Compound	β_1-Adrenoceptor atria [a] (pA_2)	β_2-Adrenoceptor trachea [b] (pA_2)	Selectivity [c] β_1/β_2
D140S·HCl **6**	8.15±0.22	<4.5	>4400
Esmolol	7.14±0.20	5.26±0.16	76
Atenolol	7.30±0.12	5.91±0.30	25
Metoprolol	7.60±0.17	6.43±0.13	15
Betaxolol	8.06±0.15	6.38±0.13	48
LK 204-545	8.53±0.08	4.73±0.17	6300
Propranolol	8.40±0.32	8.13±0.27	2

[a] Antagonist pA_2 value±S.E.M. determined in isolated spontaneously beating atria.
[b] Antagonist pA_2 value±S.E.M. determined in tracheal chain, previously contracted with 1.0 μM carbachol.
[c] Antilog (pA_2 atria-pA_2 trachea). $N = 3–5$ individual experiments.

Table 2
Plasma concentrations of D140S·HCl **6** at the end of 7 days of continuous intravenous infusion in conscious rats

Infusion rate of D140S·HCl **6** (mg kg^{-1} h^{-1})	Plasma concentration of D140S·HCl±S.E.M. (μg mL^{-1}) in males [a]	Plasma concentration of D140S·HCl±S.E.M. (μg mL^{-1}) in females [a]
5.0	0.91±0.38	1.22±0.18 [b]
10.0	2.64±1.22	2.64±0.25
15.0	13.25±1.94	15.84±1.56 [b]

[a] A two-way ANOVA of concentration/dose demonstrates non-linearity with dose, $P < 0.01$.
[b] Significantly different from male values, $P < 0.05$, Student's t-test. $N = 80$ Sprague–Dawley rats, ten of each sex at each dose level. Data for 0 mg kg^{-1} h^{-1} infusion rate have not been included in the table, the plasma blank values are given in Section 7.2.2.3.

D140S·HCl **6** plasma concentrations on days 2 and 7 of the study were calculated and are listed in Table 4.

3.2.4. Four week intravenous bolus injection study in conscious dogs

D140S·HCl **6** intravenous bolus injection doses were 0, 5.0, 10.0 and 15.0 mg kg^{-1} bd; 6 h apart in 16 conscious beagle dogs (two males and two females at each dose level, weighing 7.5–11.0 kg). Plasma samples were taken 1 h before the second dose of the day (predose) and 0.5, 1, 2, 4, 8 and 12 h after the second dose on days 1, 14 and 28. The pharmacokinetic $t_{1/2}$ of D140S·HCl **6** in dogs on days 1, 14 and 28 of the 4 weeks intravenous bolus injection study were determined and are given in Table 5.

3.3. Pharmacodynamics

Pilot studies have demonstrated that the $t_{1/2}$ for esmolol and D140S·HCl **6** at a dose of 20.0 μg kg^{-1} min^{-1} was ca. 10 min and for atenolol 2–5 h in a conscious cannulated rat model (unpublished results). The purpose of this study was to compare the pharmacodynamic $t_{1/2}$ of these three β-blockers when given by intravenous infusion for 30 min, i.e. ca. three esmolol half-lives, at 5.0, 10.0 or 20.0 μg kg^{-1} min^{-1}. The degree of β_1-adrenoceptor blockade and the $t_{1/2}$ of the pharmacodynamic response was assessed by monitoring the heart rate response to intravenous boluses of isoprenaline (0.1 μg kg^{-1}) before, during and after a 30 min infusion of each drug. The effect of atenolol is likely to be greater and the $t_{1/2}$ longer than seen in these experiments, since longer infusion periods are required to achieve steady state for this compound. The heart rate and the ability to inhibit the isoprenaline-induced heart rate increase (expressed as a percent of predrug response) at the end of the 30 min drug infusion for D140S·HCl **6**, esmolol and atenolol are listed in Table 6. The pharmacodynamic $t_{1/2}$, determined from the heart rate increase in response to bolus doses of isoprenaline given at 10 min intervals for up to 150 min after the drug infusion, for the three β-blockers are also given in Table 6.

4. Toxicology

The results of the toxicological studies are summarised in Table 7 and were performed by Hazelton Laboratories, Madison, WI, USA (HWI), Inveresk Research International Limited, Tranent, Scotland (IRI) and Biodevelopment Laboratories, Cambridge, MA, USA (BDL).

5. Results and discussion

5.1. Isolated tissue preparations

The functional potency of the compounds (Table 1) was determined for inhibiting (−)-isoprenaline-induced: (i) β_1-adrenoceptor mediated chronotropic effects in spontaneously beating rat atria; and (ii) β_2-adrenoceptor mediated relaxation of rat tracheal chain previously

Table 3
Peak plasma concentrations of D140S·HCl **6** 10 min after last intravenous bolus injection in conscious rats in a 4 weeks study

D140S·HCl **6** dose (mg kg^{-1} bd)	Plasma concentration of D140S·HCl±S.E.M. (μg mL^{-1}) in males [a]	Plasma concentration of D140S·HCl±S.E.M. (μg mL^{-1}) in females [a]
10.0	2.65±0.31	2.82±0.24
20.0	5.30±0.76	5.40±0.43
30.0	9.34±1.00	8.07±1.01 [b]

[a] Linear regression demonstrates a linearity with dose, for males $r^2 = 0.99$ and for females $r^2 = 1.00$.
[b] Significantly different from male value, $P = 0.04$, Student's t-test. $N = 48$ Sprague–Dawley rats, six of each sex at each dose level. Data for 0 (mg kg^{-1} bd) dose have not been included in the table, the plasma blank values are given in Section 7.2.2.3.

Table 4
Plasma concentrations of D140S·HCl **6** during 7 days continuous intravenous infusions in conscious dogs

Infusion rate of D140S·HCl **6** (mg kg^{-1} h^{-1})	Plasma concentration of D140S·HCl ± S.E.M. on day 2 [a] (μg mL^{-1})	Plasma concentration of D140S·HCl ± S.E.M. on day 7 [a] (μg mL^{-1})
0	0	0
2.5	0.54 ± 0.05 [b]	0.67 ± 0.07 [b]
5.0	1.49 ± 0.19 [b]	2.13 ± 0.51 [b]
10.0	4.13 ± 0.36	–
7.5 [c]		5.08 ± 0.61

[a] ANOVA of concentration/dose demonstrates non-linearity with dose, $P < 0.01$ for day 2 and $P < 0.02$ for day 7.
[b] For both the 2.5 and 5.0 mg kg^{-1} h^{-1} dose, the D140S·HCl plasma concentrations were higher on day 7 than on day 2, $P < 0.03$, Paired t-test.

contracted with 1 μM carbachol. In rat atria (β_1-adrenoceptor) D140S·HCl **6** was more potent than esmolol, atenolol and metoprolol; of similar potency to betaxolol and propranolol; and less potent than LK 204-545 (Table 1). Like LK 204-545 [14], D140S·HCl **6** was highly cardioselective and the order of selectivity (i.e. β_1/β_2) was LK 204-545 ≃ D140S·HCl **6** > esmolol > betaxolol > atenolol > metoprolol > propranolol. It should be acknowledged that the comparison is of a single D140·HCl enantiomer with racemic forms of the other compounds. In the guinea pig the β_1/β_2 pA_2 values for D140S·HCl **6**, racemic D140·HCl, racemic esmolol and racemic propranolol were 7.7/ < 4.5, 7.25/ < 4.5, 6.8/5.3 and 8.3/8.0, respectively (unpublished data).

5.2. D140S·HCl (**6**) pharmacokinetics

5.2.1. Seven day continuous intravenous infusion in conscious rats

The plasma concentrations for rats at the end of a 7 day continuous infusion of 5.0, 10.0 and 15.0 mg kg^{-1} h^{-1} D140S·HCl **6** are listed in Table 2. Samples taken from the rats on the last day of the study showed a small but significant increase in D140S·HCl **6** plasma concentrations at 5.0 and 15.0 mg kg^{-1} h^{-1} in females compared with males ($P < 0.05$; Student's t-test) but no significant difference at 10.0 mg kg^{-1} h^{-1} ($P = 0.99$, Student's t-test). There was evidence of non-linearity with dose ($P < 0.01$, two-way analysis of variance (ANOVA) on steady state concentration/dose) and the plasma concentrations increased ca. 5-fold when the dose was increased from 10.0 to 15.0 mg kg^{-1} h^{-1}. The relationship between D140S·HCl **6** dose and plasma concentration was linear to 10.0 mg kg^{-1} h^{-1}, above this the relationship is non-linear ($P < 0.01$, paired t-test). This non-linearity is characteristic of many β-blockers at higher doses and has been particularly well studied with propranolol [15].

5.2.2. Four week intravenous bolus injection study in conscious rats

D140S·HCl **6** plasma concentrations for rats 10 min after receiving the final dose of 10.0, 20.0 and 30.0 mg kg^{-1} bd are listed in Table 3. At 10.0 and 20.0 mg kg^{-1} bd dose levels there was no significant difference in D140S·HCl **6** plasma concentrations between males and females ($P > 0.1$, Student's t-test). A difference was observed at 30 mg kg^{-1} bd ($P = 0.04$, Student's t-test) but in contrast to the previous experiment the levels were greater in males when compared with females (see Section 5.2.1) suggesting there were no consistent gender differences in these two studies. In contrast to the previous study (Section 5.2.1), there appeared to be a dose linearity with plasma D140S·HCl concentrations (for males $r^2 = 0.99$, for females $r^2 = 1.00$) at all doses studied which may reflect the lower overall doses studied. The maximum dose studied was 60.0 mg kg^{-1} per day, as a bi-daily dosage, whereas the total daily dose administered in the continuous infusion studies were 120, 240 and 360 mg kg^{-1} per day (Section 5.2.1).

Table 5
Mean $t_{1/2}$ of D140S·HCl **6** after intravenous bolus injection in conscious dogs in a 4 weeks study

D140S·HCl **6** dose (mg kg^{-1} bd)	$t_{1/2}$ ± S.E.M. day 1 (min) [a]	$t_{1/2}$ ± S.E.M. day 14 (min) [a]	$t_{1/2}$ ± S.E.M. day 28 (min) [a]
10.0	25.8 ± 3.0	25.2 ± 2.4	24.0 ± 1.2
15.0	27.0 ± 1.2	28.2 ± 3.0	25.2 ± 1.8

[a] A two-way ANOVA found no difference in $t_{1/2}$ between dogs (male and female), dose level or day of sampling, $P > 0.1$. $N = 16$ beagle dogs, two of each sex at each dose level. Data for 0 and 5.0 mg kg^{-1} bd dose have not been included in the table as the plasma levels of D140S·HCl **6** were below the assay levels of detection (refer to Section 7.2.2.3), except for the 0.5 and 1 h levels at the 5.0 mg kg^{-1} bd dose.

Table 6
The effect of D140S·HCl **6**, esmolol and atenolol on heart rate responses before and after 0.1 μg kg^{-1} isoprenaline intravenous bolus,% inhibition of isoprenaline-induced heart rate increase and the pharmacodynamic $t_{1/2}$ of D140S·HCl **6**, esmolol and atenolol

Compound	Dose (μg kg^{-1} h^{-1}) [a]	Control resting heart rate (bpm) [b]	Control heart rate increase in response to isoprenaline (bpm) [c]	Heart rate increase in response to isoprenaline after 30 min infusion (bpm) [d]	Percent Inhibition of isoprenaline-induced heart rate increase after 30 min infusion [e]	Pharmacodynamic $t_{1/2}$ (min)
D140S·HCl **6**	5.0	389±26	169±19	143±22	16±6	<10
	10.0	378±31	150±17	107±12	29±5	<10
	20.0	403±27	147±21	89±25	40±12	10–15
Esmolol	5.0	406±24	134±19	131±23	2±11	<10
	10.0	397±22	143±35	119±27	16±8	<10
	20.0	386±21	136±13	104±16	24±14	<10
Atenolol	5.0	382±39	157±14	96±19	39±8	>60 [f]
	10.0	397±23	136±39	42±19	70±8	>60 [f]
	20.0	392±31	160±39	57±29	65±10	>60 [f]

[a] Infusion period was 30 min.
[b] Control resting heart rate±S.E.M. bpm.
[c] Increase in the control resting heart rate±S.E.M. bpm in response to a 0.1 μg kg^{-1} bolus dose of isoprenaline.
[d] Maximum increase in heart rate in response to the bolus dose of isoprenaline±S.E.M.. Isoprenaline was given after the 30 min drug infusion ceased.
[e] Expressed as the percentage change from predrug values±S.E.M.
[f] It was difficult to calculate $t_{1/2}$ for atenolol because at the end of the follow up time (2 h) isoprenaline responses had not recovered to basal levels, in contrast to D140S·HCl and esmolol. $N = 4–6$ individual experiments for each dose studied.

Table 7
Summary of the toxicological studies carried out on D140S·HCl **6**

Type of study	Laboratory [a]	Result
Acute toxicity, intravenous injection in albino CD-1 mice [b]	HWI	$LD_{50} > 60$ mg kg^{-1} NOEL 30 mg kg^{-1}
Acute toxicity, intravenous injection In Sprague–Dawley rats [b]	HWI	$LD_{50} > 40$ mg kg^{-1} NOEL 20 mg kg^{-1}
Acute toxicity, oral (gavage) dose in CD-1 mice [c]	IRI	LD_{50} ca. 1000 mg kg^{-1}
Acute toxicity, oral (gavage) dose in Sprague–Dawley rats [c]	IRI	LD_{50} ca 516 mg kg^{-1}
Seven days continuous intravenous infusion in conscious Sprague–Dawley rats [b]	HWI	NOEL 10 mg kg^{-1} h^{-1}
Seven days continuous intravenous infusion in conscious beagle dogs [b]	HWI	NOEL 5 mg kg^{-1} h^{-1}
Four weeks intravenous bolus injection study in conscious Sprague–Dawley rats [b]	HWI	NOEL 40 mg kg^{-1} per day
Four weeks intravenous bolus injection study in conscious beagle dogs [c]	HWI	NOEL 10 mg kg^{-1} per day
Two weeks oral (gavage) dose in male Sprague–Dawley rats [c]	IRI	NOEL > 400 mg kg^{-1} per day
Five days ocular toxicity study in beagle dogs [b]	IRI	NOEL > 8 mg per day
Four weeks ocular toxicity study in New Zealand White rabbits [b]	BDL	NOEL > 5 mg per day
Buehler sensitisation test in albino Dunkin–Hartley guinea pigs [c]	IRI	negative
Mutagenic activity with *S. typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia Coli* WP2*uvr* A [c]	IRI	negative
Chromosomal aberrations assay with Chinese Hamster ovary cells in vitro [c]	IRI	positive
Micronucleus test in bone marrow of CD-1 mice [c]	IRI	negative

[a] HWI, Hazelton laboratories, Madison, WI, USA. IRI, Inveresk Research International Limited, Tranent, Scotland. BDL, Biodevelopment Laboratories, Cambridge, MA, USA.
[b] D140S·HCl **6** was made up as a 4% parenteral formulation in phosphate buffered saline, pH 7.4.
[c] D140S·HCl **6** was used as the unformulated crystalline solid.

5.2.3. Seven day continuous intravenous infusion in conscious dogs

The measured D140S·HCl **6** plasma concentrations on days 2 and 7 of the 7 day study are tabulated in Table 4. After day 4, the 10.0 mg kg^{-1} h^{-1} dose was reduced to 7.5 mg kg^{-1} h^{-1} due to the four dogs developing side-effects, which included hypoactivity, tremors, heart rate reduction and decreased food consumption. Plasma concentrations on days 2 and 7 increased disproportionately with D140S·HCl **6** dose ($P < 0.01$ for day 2 and $P < 0.02$ for day 7, ANOVA on concentration/dose). Above 2.5 mg kg^{-1} h^{-1} the relationship between dose and plasma concentration is non-linear ($P < 0.03$ for both days 2 and 7, paired t-test on plasma concentration/dose). For both the 2.5 and 5.0 mg kg^{-1} h^{-1} D140S·HCl doses, which remained constant for the 7 days study, the plasma concentrations were higher on day 7 than on day 2 ($P < 0.03$, paired t-test for both the 2.5 and 5.0 mg kg^{-1} h^{-1} doses).

5.2.4. Four week intravenous bolus injection study in conscious dogs

The predose concentration of D140S·HCl **6** was measured 5 h after the first dose on day 28 when at 5.0 and 10.0 mg kg^{-1} bd no D140S·HCl was detected. At the highest dose (15.0 mg kg^{-1} bd) the predose concentration of D140S·HCl **6** was close to the limit of detection (0.1 μg mL^{-1}). The $t_{1/2}$ post dose for D140S·HCl **6** was estimated from plasma samples taken 0.5, 1, 2, 4, 8 and 12 h post dose for each day and the mean results are given in Table 5, where the average $t_{1/2}$ at these very high doses ranged from 24 to 28 min. Two way ANOVA found no difference in $t_{1/2}$ between dogs (male and female), dose level or day of sampling at each dose ($P > 0.1$). Maximum D140S·HCl **6** plasma concentration appeared to be linearly related to dose on all three study days (data not shown, $r^2 = 0.88$–0.96, $P < 0.01$). There was no evidence of saturation kinetics in the doses studied.

5.3. Pharmacodynamics

In conscious cannulated rats, the effects of D140S·HCl **6**, esmolol and atenolol on; (i) heart rate; (ii) isoprenaline-induced increase in heart rate and (iii) their pharmacodynamic $t_{1/2}$ at the end of the β-blocker infusion were determined and are listed in Table 6. D140S·HCl and esmolol had little effect on resting heart rate. In contrast, atenolol caused a significant fall in resting heart rate at all three doses (average of 24 bpm at 5 μg kg^{-1} min^{-1} increasing to 48 bpm at 20 μg kg^{-1} min^{-1}, $P < 0.05$, 2 way ANOVA on control resting heart rate and heart rate after 30 min infusion). In these studies, the increase in control heart rate responses to 0.1 μg kg^{-1} isoprenaline ranged from 134 ± 19 to 169 ± 19 bpm. Inhibition of this heart rate increase at the maximum dose studied (20.0 μg kg^{-1} min^{-1}) ranged from an average of 32 bpm for esmolol, 56 bpm for D140S·HCl and 103 bpm for atenolol. Inhibition of the isoprenaline heart rate response was significant at all doses for D140S·HCl and atenolol but not at the 5.0 μg kg^{-1} min^{-1} infusion rate for esmolol ($P < 0.01$ for both

atenolol and D140S·HCl at all three doses and esmolol for the 10.0 and 20.0 μg kg^{-1} min^{-1} doses, two-way ANOVA on heart rate increase in response to isoprenaline in control and infusion periods). Moreover there was evidence of plateauing of the inhibition above 10.0 μg kg^{-1} min^{-1} for all three β-blockers. At the two higher doses studied (10.0 and 20.0 μg kg^{-1} min^{-1}), D140S·HCl **6** inhibited the isoprenaline response by ca. 29 and 40% which was nearly double the effect of racemic esmolol (16 and 24%). Once the D140S·HCl **6**, or esmolol, infusion ceased the effect on heart rate wore off quickly (pharmacodynamic $t_{1/2} < 15$ min for D140S·HCl and < 10 min for esmolol). The pharmacodynamic $t_{1/2} < 10$ min observed for esmolol was consistent with previously reported values [4,7]. Atenolol was the most potent in reducing the heart rate and the duration of action was much longer (pharmacodynamic $t_{1/2} > 60$ min at all doses). The present study probably underestimates the $t_{1/2}$ because a 30 min infusion does not achieve steady state for atenolol (see Section 3.3).

Esmolol and D140S·HCl **6** both behaved as short acting β_1-adrenoceptor blocking drugs when administered intravenously. Atenolol, as expected, was less β_1-selective, more potent in reducing the heart rate response to isoprenaline and had a much longer duration of action. The endogenous neurotransmitter noradrenaline is more β_1-specific and is a weak β_2-adrenoceptor agonist compared with isoprenaline and it is likely that D140S·HCl **6**, like esmolol, will be more effective at inhibiting tachycardia resulting from endogenous sympathetic over-activity involving noradrenaline.

5.4. Toxicology

The results of the toxicological tests (Table 7) indicate that D140S·HCl **6** showed no unexpected toxicity. The no effect level (NOEL) for the intravenous dose in rodents of 20–30 mg kg^{-1} is about 5% of the NOEL for the oral dose (516–1000 mg kg^{-1}) and is almost identical to that reported for betaxolol and similar to other β-blockers [16]. D140S·HCl **6** also shows a high level of safety in subacute intravenous, oral and ocular studies of between 7 and 28 days duration in rats, dogs and rabbits. For example in the 4 weeks intravenous bolus injection study in conscious beagle dogs, the NOEL for D140S·HCl **6** was 10 mg kg^{-1} per day compared with a reported figure of 3 mg kg^{-1} per day for betaxolol in a similar study [16]. Similarly, the NOEL for D140S·HCl **6** in the 4 weeks intravenous bolus injection study in conscious Sprague–Dawley rats was 40 mg kg^{-1} per day compared with betaxolol 6 mg kg^{-1} per day [16]. It is well recognised that dogs are more sensitive than rats to the toxic effects of β-blockers and the toxic effects seen at the 10 mg kg^{-1} min^{-1} dose in the 7 days continuous intravenous infusion conscious beagle dog study (i.e. hypoactivity, tremors, excessive salivation, decreased food consumption, heart rate reduction and liquid, few or no faeces) are consistent with those seen with high doses of other β-blockers [16]. D140S·HCl **6** was not mutagenic in a number of tests and there was no evidence of chromosomal damage in these studies (Table 7). Ocular doses of 5–8 mg per eye per day were well tolerated in a 5 days study in dogs and a 4 weeks study in rabbits (Table 7). In the latter study eye observations were similar to the control group at all doses tested, there were no microscopic changes in any of the eye tissues and adnexa examined and D140S·HCl **6** did not cause any systemic toxicity. The absence of local irritancy in all parenteral studies, ocular studies and in the Beuhler sensitisation test is an important feature of D140S·HCl **6**, unlike esmolol formulations which produce local tissue irritation [1].

6. Conclusions

The results demonstrate that D140S·HCl **6** is a short acting, highly specific β_1-adrenoceptor antagonist which appears to lack the local tissue irritancy seen with esmolol formulations. D140S·HCl $t_{1/2}$ was of the order of 10–15 min in the pharmacodynamic studies in the rat and up to 28 min in the toxicology studies in the dog. Pharmacokinetic non-linearity was seen in the toxicology studies when given as a 7 days continuous intravenous infusion above 10 mg kg^{-1} h^{-1} in the rat and above 2.5 mg kg^{-1} h^{-1} in the dog. There was no evidence of saturation kinetics in the other dose ranges studied. The high degree of β_1-specificity exhibited by D140S·HCl **6** should help to avoid adverse effects on the β_2-adrenoceptors present in lung and other tissues.

7. Experimental

7.1. Chemistry

7.1.1. General

Melting points (m.p.) were determined using a manual Gallenkamp electrothermal apparatus (range 0–400 °C) in glass capillary tubes and are uncorrected. IR spectra were recorded on a Perkin–Elmer FTIR 1600 spectrometer. ^{1}H-NMR spectra were recorded on a 60 MHz Varian EM 360 spectrometer. Chemical ionisation (CI, methane gas) mass spectra were recorded on either a Finnigan GCQ^{TM} or a Finnigan 4000 series GCMS mass spectrometer. All spectra were consistent with the assigned structures. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values and were carried out by The Campbell Microanalytical Laboratory, Department of Chemistry,

University of Otago, Dunedin, New Zealand. No attempts were made to maximise yields.

7.1.2. Materials

3,4-Dimethoxyphenethylamine (homoveratrylamine), 2*S*-(+)-glycidyl-3-nitrobenzenesulfonate, *p*-toluenesulfonyl chloride and dimethylformamide (DMF) were purchased from Aldrich Chemicals. 10% Palladium on carbon, 4-benzyloxyphenol (hydroquinone monobenzylether) and sodium hydride were obtained from Fluka Chemicals. 2-Ethoxyethanol and inorganic reagents were supplied by Ajax Chemicals. Analytical grade and HPLC grade solvents were purchased from Rhone Poulenc Australia. Silica plates (5 × 10 cm, Silica F_{254}) were purchased from Merck. Silica for column chromatography (100 Å, 50 μm) was supplied by Amicon Inc., MA 01923, USA.

7.1.3. 4-Ethoxyethoxyphenol (**4**)

2-Ethoxyethanol **1** (298 g, 3.3 mol) was placed in a 2 L round bottomed flask and *p*-toluenesulfonyl chloride (381 g, 2.0 mol) added with stirring. The mixture was cooled to 15 °C and a 25% aqueous solution of NaOH (320 mL) was added dropwise over 15 min, the mixture was then stirred for 3 h at 15 °C. 4-Benzyloxyphenol (320 g, 1.6 mol) was added to the mixture with stirring, followed by NaOH (64 g, 1.6 mol in 120 mL water) and the solution heated to reflux for 30 min. NaOH (22.5 g, 0.56 mol as a 25% aqueous solution) was again added and the mixture refluxed for a further 30 min. After the solution had cooled, crude 1-benzyloxy,4-(2-ethoxyethoxy)benzene (**3**) was filtered, washed with 5% aqueous NaOH and three times with distilled water and then dried.

The crude 1-benzyloxy,4-(2-ethoxyethoxy)benzene (**3**) was suspended in 4 l of EtOH and hydrogenated over 10% Pd/C (2.5 g) at room temperature (r.t.) and atmospheric pressure. When hydrogen absorption ceased, the reaction mixture was filtered through celite and evaporated to dryness. The solid residue was then dissolved in toluene (1 L) and filtered. The solution was transferred to a separating funnel and extracted with 5% aqueous NaOH (3 × 400 mL). The extracts were combined and acidified using dilute HCl. The precipitate was extracted with diethyl ether (2 × 500 mL), the extracts were combined and dried over anhydrous $MgSO_4$. The diethyl ether was removed under reduced pressure to leave a brown oil, which was then distilled under reduced pressure.

Yield = 190–235 g (65–81%); b.p. = 125–130 °C at 1.5 mmHg; ^{1}H-NMR ($CDCl_3$) δ 6.8 (4H, s) δ 3.9(4H, m) δ 3.6(2H, q), δ 1.2 (3H, t).

7.1.4. S-(−)-1-(4-(2-Ethoxyethoxy)phenoxy)-2-hydroxy-3-(2-(3,4-dimethoxyphenyl)ethylamino)propane hydrochloride (**6**)

NaH, 60% suspension in mineral oil (32 g, 0.8 mol), was washed with dry ether (3 × 100 mL) to remove the oil and suspended in anhydrous DMF (400 mL) under nitrogen. 4-Ethoxyethoxyphenol **4** (141 g, 0.8 mol) in anhydrous DMF (200 mL) was added dropwise over 30–60 min to the NaH suspension while stirring. Stirring continued at r.t. for a further 30 min. The resulting mixture was cooled to 5 °C with the aid of an ice bath and treated dropwise over 45–90 min with a solution of 2*S*-(+)-glycidyl-3-nitrobenzenesulfonate (200 g, 0.8 mol) in DMF (200 mL) while ensuring that the temperature did not rise above 10 °C. The solution was brought to r.t. and stirred for 2 h under nitrogen. Distilled water (50 mL) was added cautiously to destroy any excess NaH and the mixture diluted with 3 L of water and extracted with ethyl acetate (3 × 750 mL). The extracts were combined, washed with brine (2 × 250 mL) and dried over anhydrous $MgSO_4$. The filtrate was freed from solvent under reduced pressure giving the epoxide **5** as an oil (homogenous by TLC).

The epoxide **5** and homoveratrylamine (3,4-dimethoxyphenylethylamine, 141 g, 0.8 mol) in dioxane (400 mL) were heated to reflux for 2 h while stirring. The reaction mixture was then evaporated to dryness under reduced pressure. The residue was dissolved in anhydrous diethyl ether (300 mL) and excess saturated ethereal HCl was added with stirring. The mixture was then stirred overnight and the precipitate subsequently isolated by filtration. The precipitated hydrochloride **6** was washed with ether, excess solvent removed by suction and then recrystallised from hot isopropanol (800 mL). Yield of fine white crystals = 206–212 g (63–65%); m. p. = 147–149 °C; MS *m/e* 151 ($(CH_3O)_2PhCH_2^+$), 165 ($(CH_3O)_2$-$PhCH_2$ CH_2^+) and 268 ($CH_3CH_2OCH_2CH_2OPhO$-$CH_2CH(OH)$–$CH_2NHCH_2^+$) from loss of $(CH_3O)_2$-$PhCH_2$. On treatment with D_2O the *m/e* 268 ion shifts to *m/e* 269 and 270 from mono and dideuteration; ^{1}H-NMR (D_2O) δ 7.1 (7H, s), δ 3.0–4.3 (13H, m), δ 3.96 (3H, s), δ 3.93 (3H, s), δ 3.7 (2H, q, J = 7 Hz), δ 1.27 (3H, t, J = 7 Hz); $[\alpha]_D^{23} = -31.5$ to 32.5° (4.0% aqueous solution); Anal.: $C_{23}H_{34}ClNO_6$(C, H, N, Cl).

7.1.5. Enantiomeric purity of D140S·HCl (**6**)

Solutions of D140S·HCl **6** were injected onto a column of Chiracel OD (4 × 250 mm, Daicel Chemical Industries) using a mobile phase of isopropanol–hexane (9:1) with 0.02% triethylamine at a flow rate of 0.8 mL min^{-1}. Detection was by UV absorbance at 280 nm. Samples were dissolved in EtOH–hexane–isopropanol (2:1:1), at a concentration of ca. 0.5 mg mL^{-1}, and 10 μL was injected onto the column. The *R*-isomer eluted at 15 min and the *S*-isomer **6** at 24 min. The isomers were well resolved and the ratio of the peak areas was

used to estimate enantiomeric purity. D140S·HCl **6** consistently assayed at >99% enantiomeric purity. Neither acid nor alkaline conditions have been found to promote racemisation of D140S·HCl **6**.

7.2. Pharmacology

All studies were performed in agreement with and according to the Prevention of Cruelty to Animals Act (1986), the NH and MRC/CSIRO/AAC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) and with the approval of the Animal Welfare Committee at the Austin and Repatriation Medical Centre.

7.2.1. Drugs and chemicals

(−)-Isoprenaline and (±)-propranolol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). (±)-Esmolol (Brevibloc® injection 100 mg per 10 mL esmolol hydrochloride) was purchased from Du Pont Pharmaceuticals (Aguadilla, PR, USA). (±)-Atenolol, (±)-metoprolol, (±)-betaxolol and (±)-LK204-545 ((±)-1-(2-(3-(2-cyano-4-(2-cyclopropylmethoxyethoxy)phenoxy)-2-hydroxypropylamino)-ethyl)-3-(4-hydroxyphenyl)urea) [14] were synthesised as the hydrochloride salts within our department by Dr. D. Iakovidis. All other chemicals were of reagent grade from BDH Chemicals (Kilsyth, Australia).

7.2.2. Isolated tissue preparations

Male and female Sprague–Dawley rats, 200–300 g, were used and killed by a blow to the neck followed by cervical dislocation and/or decapitation. Studies were carried out on rat isolated atria and tracheal rings as we described previously [14]. All tissues were allowed to equilibrate for 45 min with Krebs Ringer physiological salt solution; the composition of which in mmol L^{-1} was NaCl, 120; KCl 5.6; $MgSO_4$ 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.4; $NaHCO_3$, 25; glucose 11.2 and EGTA, 0.0025. Cumulative concentration–response curves were obtained from the non-selective β-adrenoceptor agonist (−)-isoprenaline in each preparation [14]. (−)-Isoprenaline was dissolved and diluted in 1 mg mL^{-1} ascorbic acid to prevent oxidation. For the measurement of antagonist activity the appropriate agent was added to the organ bath at least 30 min after the first control concentration–response curve was completed and allowed to equilibrate for 10 min before the next concentration–response curve established. The shift in this curve to the right was calculated as a pA_2 value [17]. At least three concentrations of each antagonist were examined to verify the pA_2.

7.2.2.1. Rat isolated spontaneously beating atria. Rat hearts were removed from adult animals and placed in Krebs Ringer salt solution (pH 7.4) aerated with 5% CO_2 in O_2. The atria were dissected free of the ventricles and overlying tissue and placed in a 20 mL bath maintained at 37 °C and connected to an isometric transducer. A tension of 1 g was applied and chronotropic activity was amplified and recorded on a Grass Polygraph.

7.2.2.2. Rat isolated tracheal chains. Tracheas were excised from adult rats, dissected free of overlying tissue and cut transversely into segments about 2 mm wide. Five segments were mounted in a 20 mL bath maintained at 37 °C at a tension of 1 g. Relaxation of the segments by (−)-isoprenaline were recorded by an isotonic transducer connected to a Grass Polygraph after tone had been established by administration of 1 μM carbachol (45 min prior to concentration–response curves).

7.2.2.3. Plasma assay for pharmacokinetic studies. D140S·HCl **6** was extracted from plasma, after addition of 1 μg of an internal standard (the methoxyethoxy analogue of D140S·HCl), by passage through BondElut Certify cartridges (Varian Pty. Ltd.). The cartridges were then washed with 2 mL distilled water, 1 mL of 0.1 M sodium acetate pH 4 and 2 mL MeOH–water (4:2). The drug was eluted with 2 mL of CH_2Cl_2–MeOH–HCl (80:20:1). The eluates were reduced to dryness at 60 °C before being reconstituted in 150 μL of the mobile phase. Recoveries of D140S·HCl **6** and the internal standard were ca. 90%. The extracts were analysed by HPLC with a Novapak C18, 5 μm, 3.9 × 150 mm column (Waters Assoc.) and a mobile phase of water–acetonitrile–70% perchloric acid–triethylamine (70:30:0.1:0.02) and a flow rate of 1 mL min^{-1}. Detection was by UV absorbance at 224 nm. D140S·HCl **6** elutes at ca. 7 min. Plasma blanks in rats and dogs gave readings of 0.08 ± 0.04 μg mL^{-1} and 0.10 ± 0.07 μg mL^{-1}, respectively, and only values exceeding twice these readings were used in the calculations.

7.2.3. Pharmacodynamic studies

The pharmacodynamic $t_{1/2}$ of D140S·HCl **6** was compared with esmolol (a short acting β-blocker) and atenolol (a conventional cardioselective β-blocker) by the inhibition of cardiovascular responses to a standard dose of (−)-isoprenaline in a conscious rat model.

Healthy male Sprague–Dawley rats (200–300 g) were prepared under anesthesia with indwelling catheters in the femoral artery and femoral vein and allowed to recover for at least 24 h. On the day of the experiment, the arterial catheter was attached to a flexible swivel and thence to a pressure transducer for the continuous recording of heart rate and blood pressure. The venous catheters were attached to an infusion pump for drug administration or to a syringe for bolus injections of (−)-isoprenaline (0.1 μg kg^{-1}). The catheters were suspended out of the animal's way and the rat was

allowed to move freely in a small covered enclosure isolated from outside disturbance. When the blood pressure and heart rate were stable, isoprenaline boluses (0.1 μg kg^{-1}) were given at intervals of 10 min until maximum responses in heart rate were reproducible (±10%). The doses were repeated for at least 40 min and the last five readings were considered to constitute the baseline response. The doses were continued at the same interval throughout the test period of a further 150 min. Ca. 3.5 min before the next dose the drug infusion was started, to allow for dead volume in the infusion line, and continued for a further 33.5 min. Rats were used for more than one experiment if their catheters remained patent with daily flushing with heparinised saline, but at least 24 h separated each experiment. D140S·HCl **6**, esmolol and atenolol were prepared in normal saline and infused at 5.0, 10.0 or 20.0 μg kg^{-1} min^{-1}.

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