Soft Drugs. XX. Design, Synthesis, and Evaluation of Ultra-Short Acting Beta-Blockers

Hee-Sun Yang,[1] Whei-Mei Wu,[1] and Nicholas Bodor[1,2]

Received May 23, 1994; accepted October 4, 1994

A new type of ultra-short acting β-blocker which might prove advantageous in treating acute arrhythmias was designed, synthesized and investigated. Based on the soft drug "inactive metabolite approach," the inactive phenylacetic acid metabolite of both metoprolol and atenolol was reactivated by esterification with sulfur-containing aliphatic alcohols. Since the sulfur-containing moieties are labile to the ubiquitous esterases, the new compounds should be inactivated by a one step enzymatic cleavage back to the inactive phenylacetic acid derivative. Pharmacological and pharmacokinetic profiles of the new compounds were evaluated in rats and rabbits. Isoproterenol-induced tachycardia was inhibited with short-term infusion of each compound. This tachycardia blocking effect rapidly disappeared upon termination of infusion, while β-blocking activity was 2–4-fold longer after comparable doses of the short-acting β-blocker, esmolol. The rapid recovery from the β-receptor blockade is believed due to fast hydrolysis of the soft drugs in the body. This is supported from *in vitro* results showing the $t_{1/2}$ of esmolol is about 10-fold longer than the new soft drugs in rat, rabbit, dog and human blood. Hydrolysis studies in phosphate buffered solutions indicated that the esters are labile to base-catalyzed hydrolysis. However, the relative $t_{1/2}$ values measured in biological media compared to phosphate buffered solution clearly support rapid enzymatic cleavage of the soft drugs. Interestingly, one of the soft β-blockers, the sulfonyl ester derivative, showed a unique property of exhibiting good β-receptor blocking activity without significant hypotensive action.

KEY WORDS: ultra-short acting β-blockers; soft drugs; inactive metabolite approach; metoprolol.

INTRODUCTION

Beta-blockers are widely used in the treatment of various cardiovascular disease (1–4) and very short acting β-antagonists are often used to control acute superventricular arrhythmias in critically ill patients. However, the currently available very short acting β-antagonist, esmolol, is limited because of the risk of long-lasting cardiac depression and occasional heart failure (5–7). Development of truly ultra-short acting β-blockers could circumvent limitations of currently available agents (8,9). Another limitation for β-blockers is the documented transformation to active and/or toxic metabolites (10). Several β-blockers undergo oxidative metabolic transformation to products with significant β-receptor blocking activities, but different biological half-lives. Bufuralol represents an example of this phenomenon with the alcohol and ketone metabolites demonstrating significant β-receptor blocking activities and longer half-lives (11). This can present a complex pharmacokinetic picture and lead to uncontrollable therapeutic dose monitoring. Design of novel soft β-blockers with an ultra-short duration of action and predictable metabolism offers the potential to overcome these current dosing problems.

The soft drug concept is particularly suitable for addressing these therapeutic problems, and the "inactive metabolite approach" was used in the present study (10,12,13). In our laboratory, the inactive acid metabolite of metoprolol was the precursor used in a series of soft β-blockers that were tested for cardiovascular and ophthalmic activity (10,14,15). We selected the inactive metabolite of metoprolol to design a new series of three ultra-short acting β-blockers in the present study.

In 1982, Erhardt et al. reported thirty-two compounds that were potential short acting β-blockers (16,17). Among these compounds, the methyl ester analog was described, but discarded as too long acting (17). In their report, esmolol, a homolog of metoprolol, was identified for therapeutic development (17). In the present work, we selected three sulfur-containing moieties to serve as the ester functionalities to reactivate the inactive metabolite of metoprolol to β-antagonist action. Design considered the observation that the rate of hydrolytic deactivation can be controlled by the ester structure. In 1981, we explored these sulfur-containing moieties for aspirin prodrugs and reported that the moieties demonstrate chemical stability, but are subject to rapid esterase hydrolysis (18,19).

The sulfur-containing aliphatic alcohols, methylthiomethyl and its oxidized derivatives were used to reactivate the inactive acid metabolite of metoprolol, 4-(2-hydroxy-3-isopropylamino)-propoxy phenylacetic acid. Soft drug design predicts that ubiquitous esterases should quickly hydrolyze the labile sulfur-containing ester functionalities to produce the corresponding inactive phenylacetic acid derivative (compound 9) as shown in Fig. 1. This study evaluated the selected pharmacodynamic and pharmacokinetic properties in rat and rabbit models and β-antagonist action was compared to esmolol (8,20–22).

METHODOLOGY

Chemistry

Chemicals were obtained from Aldrich Chemical Company and Fisher Scientific. Thin layer chromatography was carried out with EM Science DC-aluminum foil plates coated with silica gel or aluminum containing Fluorescent indicator. The ^1H NMR spectra were determined using Varian T-90 NMR spectrophotometer and are reported as parts per million (δ) relative to the internal standard, tetramethylsilane. Mass spectra were recorded with FAB mass spectrometry (Kratos MFC 500). Melting points were measured using Fisher Johns melting point apparatus and are uncorrected. Elemental analysis was performed by Atlantic Microlab Inc. (Atlanta, GA).

[1] Center for Drug Discovery, J. Hillis Miller Health Center, Box 100497, College of Pharmacy, University of Florida, Gainesville, Florida 32610.

[2] To whom correspondence should be addressed.

0724-8741/95/0300-0329$07.50/0

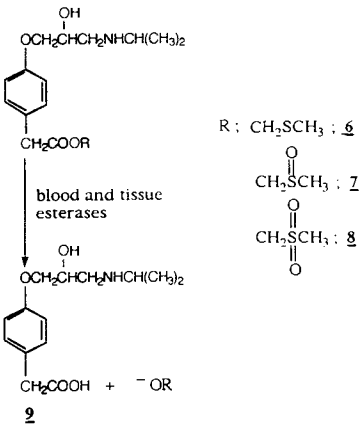
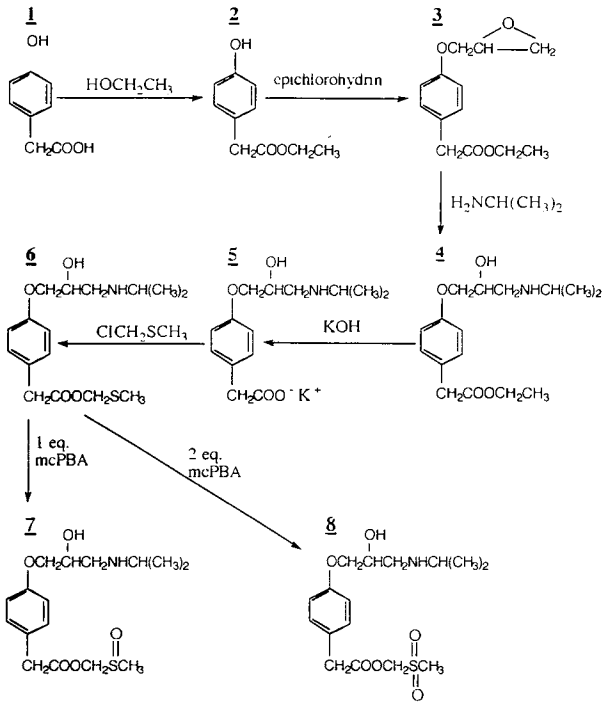


Fig. 1. Structural features of the soft drugs (6, 7 and 8) and their metabolic pathways.

Synthesis of Methylthiomethyl 4-(2-hydroxy-3-isopropylamino)-propoxy phenylacetate oxalate, 6

Intermediate, **4**, ethyl 4-(2-hydroxy-3-isopropyl-amino)-propoxy phenylacetate was synthesized following previously reported method with minor modification as shown in Scheme 1 (10). The intermediate 4 (10 g) and potassium hydroxide (1.72 g) were dissolved in 100 ml of the ethanol/water (1:1). The reaction mixture was refluxed for 10 hrs. The ethanol was removed *in vacuo* and the residue was washed with methylene chloride twice. The water was evaporated to give 9.48 g of potassium 4-(2-hydroxy-3-isopropylamino)-propoxy phenylacetate, 5. The intermediate 5 (7.8 g) dried with phosphorous pentoxide was suspended in 600 ml of dried benzene. Sodium iodide (0.96 g) and 18-crown-6 (1.35 g) were added to the reaction mixture. Chloromethylmethylsulfide (3.76 g) was added to the suspension, and refluxed for 15 hrs. After cooling, the reaction mixture was washed with 250 ml portions of saturated aqueous sodium carbonate solution, twice with brine, dried and evaporated to give 7.6 g of crude oily product (23). This was purified by column chromatography to give 5.4 g of the pure desired product (51.5%), which was isolated as an oxalate. Column chromatography: sorbent, silicagel; eluent, hexane:ethyl acetate = 1:2. Mass spectrum: (70 ev) m/z (relative intensity); 328 $[M + H]^+$(100), 307 (10), 154 (18), and 136 (10).



Scheme 1. Synthetic pathways of methylthiomethyl, 6, methylsulfinylmethyl, 7, and methylsulfonylmethyl, 8, esters.

Compound 9 (the predicted phenylacetic acid metabolite) was synthesized following the method reported by Arfwidsson (24). m.p. = 125 − 131° as an oxalate.

Synthesis of Methylsulfinylmethyl 4-(2-hydroxy-3-isopropylamino)-propoxy phenylacetate oxalate, 7

Compound 6 (as an oxalate) (1.0 g) was suspended in 5 ml of chloroform and 20 ml of a chloroform solution containing 0.6 g of m-chloroperoxy benzoic acid (purity, 80–85%) was added to the solution in portions over 20 min with stirring in ice-cooled water bath. After stirring for 30 min at 0°C, chloroform was evaporated *in vacuo* (25). The residue was triturated with 60 ml of ether/2-propanol (1:1) solution, crystallized in methylene chloride/ether, and washed with ether to give 0.85 g of the desired product (82%) as a white crystalline compound. Mass spectrum: (70 ev) m/z (relative intensity); 344 $[M + H]^+$(100), 268 (10), and 116 (20).

Synthesis of Methylsulfonylmethyl 4-(2-hydroxy-3-isopropylamino)-propoxy phenylacetate oxalate, 8

Compound 6 (as an oxalate) (1.0 g) was suspended in 5 ml of chloroform and 20 ml of chloroform solution containing 1.4 g of mcPBA (purity, 80–85%) was added to the solution in portions over 20 min with stirring, in an ice-cooled water bath. After stirring for 30 min at 0°C and for 5 hrs at room temperature (25), chloroform was evaporated *in vacuo*, and the residue was triturated with 60 ml of ether, crystallized in acetone, and washed with 2-propanol and ether to give 0.85 g of the desired product (78%) as a white crystalline compound. Mass spectrum: (70 ev) m/z (relative intensity); 360 $[M + H]^+$(100), 343 (3), and 275 (3).

Analytical Method

The HPLC system used for the analysis of the soft drugs and metabolites in buffers and biological media consisted of a Model SP 8810 precision isocratic pump, Model SP 8450 UV/VIS detector and Model SP 4290 integrator (Spectra-Physics, San Jose, CA). The soft drugs and esmolol were analyzed using a Waters reverse phase NOVA-PAK Phenyl column (10 cm × 5 mm i.d.). The acid metabolite of the soft drugs was analyzed by using a reverse phase ASI C-8 column (5 mm × 30 cm) fitted with a C-18 guard column (RAININ) packed with 7 μ packing material.

The mobile phase included various combinations of acetonitrile and 0.01 M KH_2PO_4 buffer (pH 3.0) containing 4 mM of tetramethyl ammonium perchlorate (25/75, 14/86, 10/

90, 3/97 and 20/80 for 6, 7, 8, 9 and esmolol, respectively). The detector wavelength was set at 272 nm. Compounds were detected at 272 nm in most cases. Compound 6 was detected at 235 nm for *in vivo* rabbit blood samples. The flow rate of the mobile phase was 1 ml/min, and the retention times for 6, 7, 8, 9 and esmolol were 6.3, 6.9, 6.5, 5.4, and 5.5 min, respectively. The calibration curve was linear ($r = 0.996 - 0.999$) for compounds injected over the range of 50 μM to 1 mM.

Sample Preparations for Blood and Liver Homogenate Samples. Each collected sample was mixed with two volumes of acetonitrile containing 5% DMSO, vortexed, and centrifuged at 24,000 g for 10 min. The supernatant was analyzed for the selected compounds by HPLC. Blood samples collected from rabbits required chloroform extraction as follow; samples (0.1 ml) were diluted 1:4 with deionized water and mixed 1.25 parts chloroform. The mixture was vortexed and centrifuged at 24,000 g for 10 min. A 0.5 ml of chloroform layer was evaporated under N_2 gas and the dried residue was reconstituted with mobile phase and analyzed (26). The average recovery of the compound in the chloroform layer over the concentration range of 0.4 to 20 μg/ml was 56 ± 2% with a detection limit of about 0.35 μg/ml for blood sample.

Stability in Aqueous Buffer Solutions

Isotonic phosphate buffer solutions (0.5 mM) were prepared at specific pHs ranging from 2.7 to 9.5 and held at 37°C. Solution of 6, 7 or 8 were added to buffered solutions to result an initial concentration of 300 μM and samples were withdrawn at selected times and analyzed for 9 and 6, 7 or 8 by HPLC. The pseudo first-order rate constant for hydrolysis of 6, 7 and 8 was determined by linear regression from the plot of natural logarithm of the HPLC peak area versus time.

Metabolism and Stability in Biological Media

The stability of 6, 7 and 8 was determined in blood and liver homogenate. Blood was collected from male Sprague Dawley rats, male New Zealand White rabbits and Mongrel dogs. Human blood was collected by venipuncture from healthy male and female volunteers. Heparinized blood was used within 30 min. Compound solutions in deionized water (40 μL, 15 mM) were mixed with blood (2 ml) at 37°C. Incubated samples (0.1 ml) were obtained at selected time intervals and analyzed for the compounds and their metabolite, 9 as described earlier. Rat, rabbit and dog liver homogenates were prepared by homogenizing freshly collected liver with pH 7.4 phosphate buffer to obtain a 20% w/v tissue suspension. An aliquot of stock solution of the compound was added into the prewarmed (37°C) liver homogenate. Sampling and analysis were done by the same method as described above.

Pharmacokinetic Studies in Rats and Rabbits

Male Sprague-Dawley rats weighing 250–300 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Compound (6, 7, or 8) was dissolved in normal saline at a concentration of 60 mg/ml, and infused in the animals for 1.5 min at a rate of 10 mg/kg/min through the tail vein. Blood samples (0.1 ml) were taken at various time intervals and analyzed for compound 6, 7 and 9 as mentioned earlier. Six male New Zealand White rabbits weighing 2.5–3.0 kg were used. Rabbits were anesthetized by i.m. injection of the combination of ketamine HCl and xylazine at a dose of 35 mg/kg and 5 mg/kg, respectively. Ear veins were cannulated for i.v. drug infusion and blood sampling took place from the contralateral ear vein. After a 20 min stabilization period, compound 6 was dissolved in normal saline and infused for 1.5 min at a rate of 10 mg/kg/min (cumulative dose, 15 mg/kg). Blood samples (0.1 ml) were at 1, 2, 3, 4, 5, 6, and 7 min post-infusion, and analyzed as mentioned earlier.

Pharmacological Studies in Rats

The effect of the soft β-blockers on isoproterenol-induced tachycardia was compared to esmolol in anesthetized rats. Groups of male Sprague-Dawley rats ($n \geq 5$) weighing 200–300 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). A Narco Bio System physiograph was connected to monitor heart rates. The heart rate transducer was implanted (s.c.) in each arterial leg. The physiologic signal was transduced to a Microdisplacement Myograph Linear-Core F-50 recorder (Narco Bio System, Austin, TX) and the heart rate was continuously monitored. Isoproterenol is commonly used to assess the potency of β-blockers on the cardiovascular system (27). In these experiments, unstimulated heart rate in rats under pentobarbital anesthesia was 420 ± 10 beats per min (bpm). Isoproterenol (50 μg/kg, s.c.) rapidly increased the heart rate to a steady state tachycardia of 595 ± 27 bpm within 1–1.5 min. This was sustained for 40–45 min and was used as the control tachycardia value to calculate percent inhibition after β-blocker administration. Inhibition of isoproterenol-induced tachycardia was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Control tachycardia(bpm)} - \text{Cmpd tachycardia(bpm)}}{\text{Control tachycardia(bpm)}} \times 100$$

After a 20 min of stabilization, isoproterenol (50 μg/kg) was administered to induce tachycardia. Five minutes after the administration of isoproterenol, drug solution (6) (30 mg/ml in saline) was infused at ranges from 4 to 24 mg/kg/min via the tail vein for 2.5 min and heart rate was monitored. Dose response of esmolol on the inhibition of isoproterenol-induced tachycardia was also studied at a dose range of 1–8 mg/kg/min using the same methods. The dose response curve indicated that 24 mg/kg/min of 6 is pharmacologically equipotent to 4 mg/kg/min of esmolol in this animal model. Five animals were infused with normal saline (0.8 ml/kg/min) after administration of isoproterenol to serve as the control group.

Pharmacological Studies in Rabbits

The effects of the soft β-blockers on isoproterenol-induced tachycardia and mean arterial pressure (MBP) were compared with esmolol. Six male New Zealand White rabbits weighing 2.5–3.5 kg were used in a randomized complete block design (including three soft drugs, esmolol and control). Each drug or control was tested on two rabbits

each time, thus every animals received 5 types of treatment with at least 7 days separating test periods. The experiments were completed in 2 months. Animals were anesthetized as described above. The vein in one ear and the artery in the other ear were cannulated for i.v. drug infusion, while the catheter in the artery was connected to a pressure transducer (RP-1500 Narco Bio System). The heart rate were continuously monitored as described for rats. After the cannulation and the stabilization for 20 min, each animal was injected with isoproterenol (30 μg/kg, s.c.). The drugs (6, 7, 8 or esmolol) were dissolved in normal saline and 15 min after isoproterenol injection. Test solution was infused at a rate of 2.5–10 mg/kg/min via the ear vein for 1.5 min at 0.5 ml/min. Animals infused with normal saline (0.5 ml/min) after the administration of isoproterenol were used as the control group.

The percentage inhibition of isoproterenol-induced tachycardia was calculated by the same method used in rats. Mean arterial pressure (MBP) was approximated using the following formula:

$$MBP = DP + PP/3 \quad (PP = SP - DP)$$

RESULTS

Syntheses

The soft drugs, 6, 7 and 8 were isolated as the neutral oxalates (amine$_2$ · oxalic acid$_1$). The physical and spectral properties of the soft drugs are listed in Table I. The racemic mixtures were obtained in all three cases.

Stability of the Soft Drug in Aqueous Buffer Solutions

The hydrolysis rates of the soft drugs at various pH values were investigated in order to assess their chemical stability. The apparent rate constants (K_{app}) were determined from the disappearance of 6, 7 and 8 and the corresponding pH-rate profiles are shown in Fig. 2. For all three compounds, the ester linkage undergoes base-catalyzed hydrolysis, which resulted in the predicted acid metabolite, 9. At physiological pH, pH 7.4, the half-lives of 6, 7, and 8 were 209, 214 and 155 min, respectively. By introducing a sulfoxide group (7) or a sulfone group (8), the stability in acidic conditions (pH < 6) increased, while in basic conditions (pH > 7), the compound was more susceptible to hydrolysis.

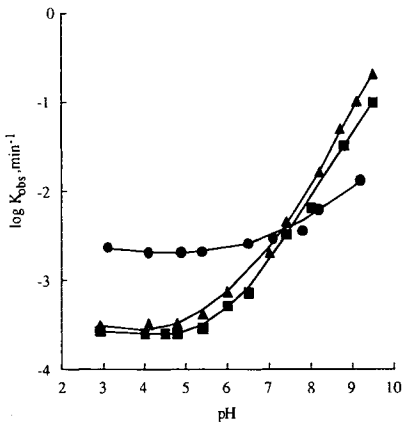


Fig. 2. Experimentally determined pH-rate profiles for the hydrolysis of the soft drugs. methylthiomethyl; 6 (●), methylsulfinylmethyl; 7 (■) and methylsulfonylmethyl; 8 (▲) esters at 37°C.

Metabolism of the Soft Drugs in Biological Media

The stability and the metabolic pathway of the soft drugs in biological media were examined in blood and liver homogenates obtained from different species. The hydrolysis rates of the soft drugs compared to esmolol are shown in Table II. In blood or liver homogenate, the metabolism of the soft β-blockers follows a pseudo first-order kinetics, and all three soft drugs were converted to the corresponding acid metabolite, 9. No other metabolic pathway was found in blood or liver homogenate studies. The recovery of the acid metabolite from the hydrolysis of the soft drugs in biological media was 100 ± 3%. There was a significant difference in the hydrolysis rate among species examined (rat > human and rabbit > dog), however, the relative order of stability for the three soft drugs in all species was constant. Compound 6 showed the longest half-life, followed by 7 and 8. Except in two cases, such as 6 and 7 in dog blood, the soft drugs exhibited much faster hydrolysis rates than esmolol, especially in rat and human blood. In human blood, compared to the half-life of esmolol, 22.8 min, significantly shorter half-lives of the soft drugs, <1 min, were obtained. Unlike esmolol, the soft drugs displayed a much shorter hydrolytic half-life in human blood than in dog and rabbit blood. The reason remains unknown, however, specie specific difference in hydrolytic enzymes might explain this result. In 20%

Table I. Spectral and Physical Properties of the Soft Drugs (6, 7, and 8)

Compound (oxalate)	m.p. (°C)	NMR Data Common signals: 1.0–1.3 (δ, 6H, CH_3-CH-CH_3), 6.8–7.4 (AB, 4H, aromatic)	Analysis for C, H, N, S
6	115–120	2.2 (s, 3H, -S-CH_3), 2.4–2.9 (m, 2H, -CH_2-N), 3.0–3.3 (m, 1H, CH_3-CH-CH_3), 3.6 (s, 2H, CH_2COO), 3.8–4.1 (ABX, 2H, -O-CH_2), 4.2–4.4 (m, 1H, -CH-OH), 5.1 (s, 2H, -O-CH_2-S)	54.82, 7.63, 3.79, 8.68.
7	102–104	2.7 (s, 3H, -S-CH_3), 2.8–3.1 (m, 3H, -CH_2-N and CH_3-CH-CH_3), 3.7 (s, 2H, -CH_2COO), 4.0–4.2 (ABX, 2H, -O-CH_2), 4.1–4.4 (m, 1H, -CH-OH), 4.9–5.5 (q, 2H, -O-CH_2-S)	52.29, 6.78, 3.61, 8.14.
8	90–93	2.6–3.4 (m, 3H, -CH_2-N and CH_3-CH-CH_3), 2.9 (s, 3H, -S-CH_3), 3.7 (s, 2H, -CH_2COO), 3.7–4.2 (m, 1H, -CH-OH), 3.8–3.9 (s, 2H, -O-CH_2), 5.2 (s, 2H, -O-CH_2-S)	50.49, 6.39, 3.53, 7.84.

liver homogenate, the hydrolytic half-life was too short to be estimated. In dog liver homogenate, the half-lives of the soft drugs were about 1–2 min. The results support a one-step metabolic inactivation to the corresponding acid metabolite.

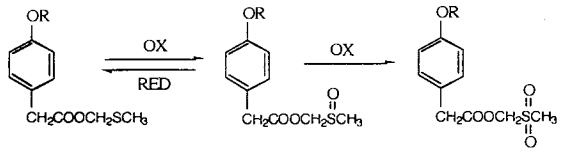
Redox Biotransformation of the Sulfur-Containing Moieties

In addition to direct hydrolysis, the redox biotransformation of the sulfur-containing alkyl groups could occur in the body as shown in Scheme 2. Thus, redox biotransformation was investigated in rat, rabbit and dog liver homogenates.

In rat and rabbit 20% liver homogenates, after incubation of the soft drugs (600 μM) at 37°C, no redox biotransformation product was detected by HPLC. In the case of 20% dog liver homogenate, when 6 (600 μM) was incubated at 37°C, only a trace of the oxidation product, 7 was detected in the first sample (15 sec). Similarly, when 7 was incubated, a trace of its oxidation product (but not 6, the reduction product of 7) was detected in the first sample (15 sec). No 7 or 6, were detected after incubating 8. These results indicate that oxidation precedes reductive bio-transformation. Although no redox biotransformation of the sulfur-containing esters could be detected in rat and rabbit liver homogenates, it can be assumed that because of the fast hydrolysis, the redox products could not be detected.

Pharmacokinetic Studies in Rats and Rabbits

In rats, disappearance of 6, 7 and 8 from blood was too rapid to estimate $t_{1/2}$ values after i.v. infusion (15 mg/kg). In contrast, esmolol could be measured through 12 min. The distribution and elimination half-lives of esmolol were estimated at 0.4 and 2.3 min, respectively. *In vitro* studies indicated that soft drugs were relatively stable in rabbit blood compared to rat blood. Therefore, the disappearance profile of 6 was studied in rabbits during the isoproterenol-induced tachycardia studies. Results showed that the blood concentrations of 6 reached 14.3 ± 0.6 μg/ml during the infusion, while the blood concentration rapidly decreased to 2.1 ± 0.4 μg/ml and 0.3 ± 0.0 μg/ml at 0.5 and 1.5 min, respectively after terminating the infusion. Because of the rapid metabolism, pharmacokinetic parameters could not be estimated.



Scheme 2. Redox biotransformation of the sulfur-containing esters.

Effect of the Soft Drugs on Isoproterenol-Induced Tachycardia in Rats

As shown in Fig. 3, infusion of saline did not show any effect on isoproterenol-induced tachycardia, while significant inhibition occurred with infusion of 6 (24 mg/kg/min). During 2.5 min infusion, heart rate began to decrease within 15 sec and continued to decrease throughout the infusion with a heart rate of 446 ± 21 bpm at 2.5 min. After terminating the infusion, the heart rate immediately started to increase and reached the original isoproterenol-induced tachycardia level within 10 min. The other two soft drugs, 7 and 8, were less potent compared to 6 with maximum % inhibition of about 2/3 of 6. All three soft drugs demonstrated rapid onset which was similar to esmolol, but studies showed significantly faster recovery from the soft drug β-receptor blockade than esmolol.

The dose of esmolol selected for comparison in these studies was 4 mg/kg/min, which effected the same maximal tachycardial % inhibition compared to 24 mg/kg/min of 6. Fig. 3 illustrates that esmolol had the same fast onset of action compared to the soft drugs, however, the β-receptor blockade persisted for approximately 20 min after terminating the infusion.

Effect of the Soft Drugs on Isoproterenol-Induced Tachycardia and Mean Arterial Pressure in Rabbits

The heart rate and MBP of the anesthetized (ketamine & xylazine) rabbits determined were 156 ± 9 bpm and 92 ± 6 mmHg, respectively. Injection of isoproterenol (30 μg/kg,

Table II. The *in Vitro* Hydrolytic Half-Lives (min) of the Soft Drugs and Esmolol in the Biological Media and in the Buffer Solution (pH 7.4)

	6	7	8	Esmolol
Human blood	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	22.9 ± 0.3
Rat blood	<0.25[a]	<0.25[a]	<0.25[a]	0.9 ± 0.1
Rabbit blood	6.1 ± 0.6	3.6 ± 0.1	3.4 ± 0.1	9.9 ± 0.1
Dog blood	109 ± 18	37 ± 3	14 ± 2	40 ± 5
Rat liver homogenate (20%)	UD[b]	UD[b]	UD[b]	UD[b]
Rabbit liver homogenate (20%)	UD[b]	UD[b]	UD[b]	UD[b]
Dog liver homogenate (20%)	0.9 ± 0.1	1.7 ± 0.3	1.0 ± 0.2	1.5 ± 0.3
Buffer (pH = 7.4)	209	214	155	—[c]

[a] A half-life of <0.25 indicates that more than 95% of the compound was metabolized in 0.25 min.

[b] Undetectable. The compound was immediately metabolized after incubation.

[c] Not determined.

** Data presented in the text and table are mean ± S.E.M. (3 samples for the *in vitro* studies, and 4–6 samples for the *in vivo* studies). A student t-test for paired and unpaired data was used for the statistical analysis.

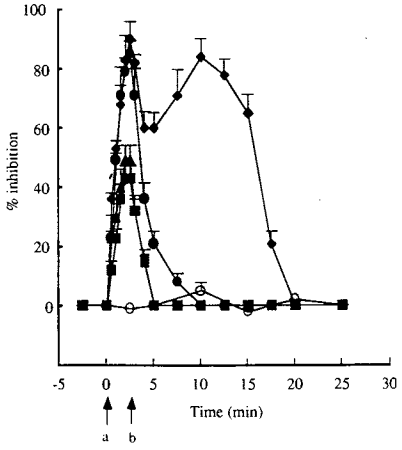


Fig. 3. Effects of the soft drugs, 6 (●), 7 (■), 8 (▲), esmolol (♦), and control (○)on isoproterenol-induced tachycardia in anesthetized rats. Doses for soft drugs and esmolol were 24 mg/kg/min and 4 mg/kg/min, respectively. a, infusion started; b, infusion stopped.

s.c.) immediately produced an increase in heart rate and decrease in mean arterial pressure that reached steady-state within 10–15 min at 324 ± 10 bpm and 87 ± 6 mmHg, respectively. β-stimulation was sustained for 30–35 min. Infusion of normal saline (control) did not show any effect on the heart rate and mean arterial pressure.

Each of the soft drugs (total dose, 15 mg/kg) inhibited isoproterenol-induced tachycardia within 30 sec after beginning the infusion and greatest blockade was reached at the end of infusion (1.5 min) as shown in Fig. 4. At greatest observed inhibition, the heart rate was reduced from 324 ± 19 bpm to 235 ± 11 bpm for compound 6. The blockade (50–60% inhibition) persisted for 3–4 min and heart rate recovered to the pre-infusion value within 9–11 min after terminating the infusion. Compound 7 and 8 showed lower β-blocking activity compared to 6 or esmolol indicating decreased β-blocking potency in this animal model. The same dose of esmolol showed highest β-blockade comparable to compound 6 however, the duration of action was nearly twice as long.

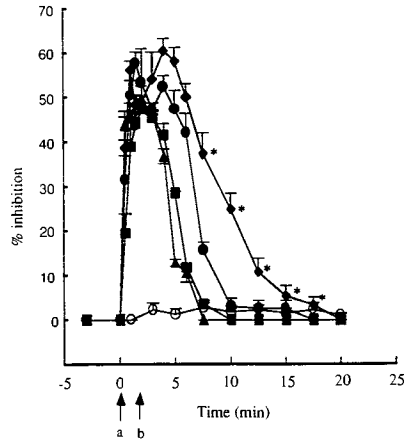


Fig. 4. Effects of the soft drugs, 6 (●), 7 (■), 8 (▲), esmolol (♦), and control (○) on isoproterenol-induced tachycardia in rabbits at a dose of 10 mg/kg/min. a, infusion started; b, infusion stopped. *; Significantly different from those of the soft drugs at $p < 0.002$.

The dose-response (2.5–10 mg/kg/min) of 6 on isoproterenol-induced tachycardia in rabbits is shown in Fig. 5. Maximal inhibition was achieved at 5 and 10 mg/kg/min (7.5 and 15 mg/kg total dose), while duration of β-receptor blocking activity of 6 increased with increasing doses.

The effects of 6 and esmolol on isoproterenol-induced tachycardia were also compared at a dose of 5 mg/kg/min (Fig. 6). In contrast to the results in rats, compound 6 showed greater β-blocking activity compared to esmolol in rabbits. Maximal % inhibition attained after esmolol (5 mg/kg/min; 7.5 mg/kg total dose) was about one-half the peak activity after the same dose of 6. However, the duration of activity was much longer than 6.

Fig. 7 shows that identical doses (15 mg/kg over 1.5 min) of esmolol, compound 6 and 7 induced immediate and substantial effects on MBP. Infusion of 6 or esmolol produced significant decreases in MBP from 87 ± 6 mmHg to 35 ± 5 mmHg or 48 ± 3 mmHg, respectively. The recovery of MBP started immediately upon termination of infusion and returned to the pre-infusion range within 4–4.5 min for all three compound. In contrast, compound 8 had almost no effect on MBP and was not significantly different from saline.

Fig. 8 shows the dose response of 6 on MBP for animals treated with 3.75 to 15 mg/kg over 1.5 min. In contrast to the maximal effects on heart rate shown in Fig. 4, a change in dose caused a significant change in the maximal MBP decreases.

The effects of 6 and esmolol on MBP at a dose of 5 mg/kg/min were almost the same, although 6 showed a significantly higher β-receptor blockade on heart rate and a shorter duration of action than esmolol.

DISCUSSION

Three novel soft drugs (6, 7 and 8) were synthesized to further improve the safety and selectivity of β-antagonists for acute treatment of cardiac emergencies. These active soft drugs were designed to undergo a facile, predicted metabolism *in vivo* to nontoxic metabolites after exerting their therapeutic action.

In vitro, each of the soft drugs were unstable in rat,

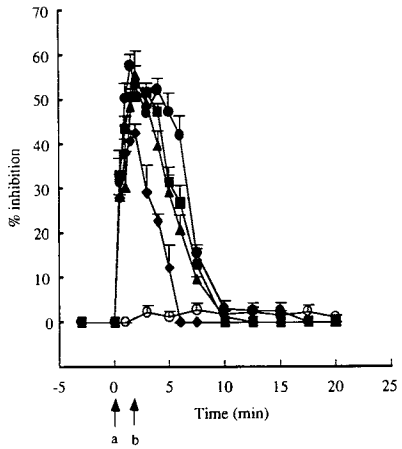


Fig. 5. Effect of the soft drugs, 6, on isoproterenol-induced tachycardia in rabbits at doses of 0 (○), 2.5 (♦), 5 (▲), 7.5 (■), and 10 (●) mg/kg/min. a, infusion started; b, infusion stopped.

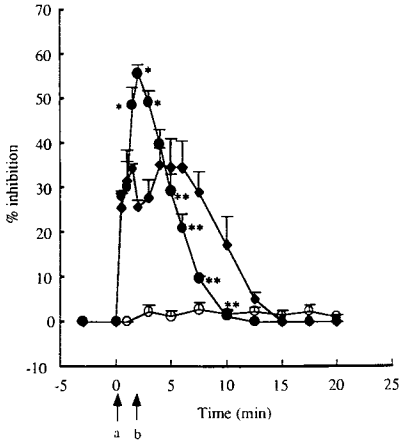


Fig. 6. Effects of the soft drugs, 6 (●), esmolol (♦), and control (○) on isoproterenol-induced tachycardia in rabbits at a dose of 5 mg/kg/min. a, infusion started; b, infusion stopped. *; $p < 0.002$ compared to that of esmolol at 1–2.5 min. **; $p < 0.005$ compared to that of esmolol at the same time points.

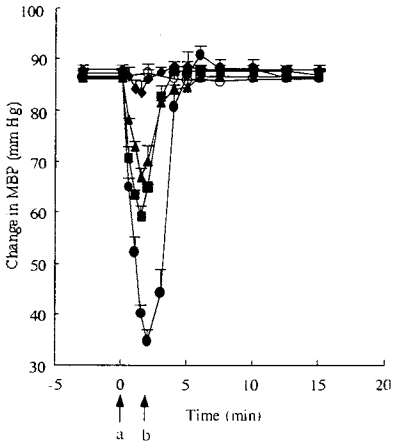


Fig. 8. Effect of the soft drug, 6, on mean arterial pressure in rabbits at doses of 0 (○), 2.5 (♦), 5 (▲), 7.5 (■) and 10 (●) mg/kg/min. a, infusion started; b, infusion stopped.

rabbit and human blood, and relatively stable in dog blood. The hydrolytic half-lives of the soft drugs in human and rat blood were much shorter than that of esmolol (<1 min and <0.25 min, compared to 23 min and 0.9 min, respectively), which indicates that these soft drugs should be metabolized faster than esmolol after clinical administration. In 20% liver homogenates from rats, rabbits and dogs, the soft drugs were hydrolyzed even faster than in blood and $t_{1/2}$ could not be estimated. Therefore, it is reasonable to conclude that hepatic metabolism may contribute to inactivation. While redox biotransformation is possible, but it is unlikely to affect the hydrolytic behavior of these esters because the rate of ester hydrolysis is very rapid in biological matrices.

In isoproterenol-stimulated rabbits, the soft drugs induced β-blocking effects that were generally comparable to esmolol in potency, but showed much shorter duration of activity. This rapid decrease in activity corresponded with fast disappearance from blood which is believed to be the result of the rapid hydrolysis of the compound by both blood and hepatic esterases. The facile *in vivo* hydrolysis is undoubtedly due to the sulfur-containing ester functionalities. A sulfinyl or sulfonyl ester instead of thio ester can further accelerate the rate of hydrolysis. The results correspond very well with the soft drug concepts.

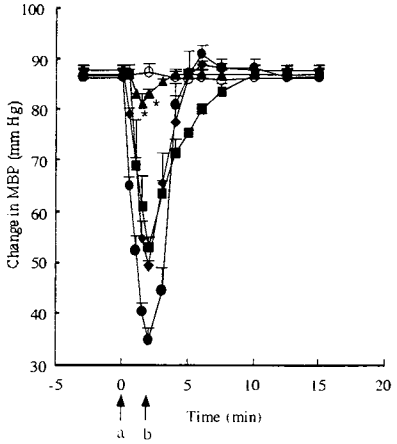


Fig. 7. Effects of the soft drugs, 6 (●), 7 (■), 8 (▲), esmolol (♦), and control (○) on mean arterial pressure in rabbits at a dose of 10 mg/kg/min. a, infusion started; b, infusion stopped. *; $p < 0.0001$ compared to that of 6, 7, or esmolol at 1–2.5 min.

In rats, the potency of the soft drugs was relatively low compared to esmolol (for 2.5 min infusion, the equi-potent doses were 24 mg/kg/min and 4 mg/kg/min, respectively). While species differences in receptor sensitivity might provide an explanation, difference in the stability of the soft drugs in the body between rats and rabbits is also a likely reason. Interestingly, differences between soft drugs were more apparent in the MBP response. Dose response differences suggest that this is a less sensitive parameter for detecting β-blocking activity. In rabbits, a 15 mg/kg dose of 6 significantly decreased MBP (−50 mmHg), while lower doses of 6 (7.5 or 9.75 mg/kg) produced the same β-receptor blocking activity with very little effect on MBP (−20 to −25 mmHg). At a dose of 15 mg/kg, 7 showed about the same effect as esmolol (−35 to −40 mmHg), and 8 showed almost no effect (−5 mmHg) on MBP.

These studies were able to identify a dose for methylsulfonylmethyl ester, 8 that exhibited good β-receptor blocking activity with minimal effects on MBP. This suggests that compound 8 might have therapeutic potential for treating severe acute arrhythmias without exerting significant hypotensive effects. Full dose response studies should be conducted to characterize the relative potencies and selectivities of compound 6 and 8. The most frequently reported adverse effect associated with esmolol was hypotension (28, 29). In conclusion, this study suggests that sulfur-containing ester type soft drugs may have significant advantages as ultrashort acting β-blockers.

REFERENCES

1. W. H. Frishman, J. Stormand, M. Kirschner, M. Poland, N. Klein, S. Halprin, T. H. LeJemtel, M. Kram and E. H. Sonnenblick. Labetalol Therapy in Patients With Systemic Hypertension and Angina Pectoris: Effects of Combined Alpha and Beta Adrenoceptor Blockade. *Am. J. Cardiol.* 48:917–928 (1981).

2. J. G. Gerber and A. S. Nies. Beta-Adrenergic Blocking Drugs. *Annu. Rev. Med.* 36:145–164 (1985).
3. D. G. McDevit. Adrenoceptor Blocking Drugs: Clinical Pharmacology and Therapeutic Use. *Drugs* 17:267–288 (1979).
4. D. J. Greenblatt and J. Koch-Weser. Adverse Reactions to β-Adrenergic Receptor Blocking Drugs: A Report from Boston Collaborative Drug Surveillance Program. *Drugs* 7:118–129 (1974).
5. H. M. Beumer. Adverse Effects of β-Adrenergic Receptor Blocking Drugs on Respiratory Function. *Drugs* 7:130–138 (1974).
6. W. Frishman, R. Silverman, J. Strom, U. Elkayam and E. Sonnenblick. Clinical pharmacology of the new beta-adrenergic blocking drugs. Part 4. Adverse effects. Choosing a β-adrenoceptor blocker. *Am. Heart J.* 98, 2:256–262 (1979).
7. W. Frishman and R. Siverman. Clinical pharmacology of the new beta-adrenergic blocking drugs. Part 3. Adverse effects. Choosing a β-adrenoceptor blocker. *Am. Heart J.* 98:119–131 (1979).
8. V. S. Murphy and W. H. Frishman. Controlled Beta-receptor Blockade with Esmolol and Flestolol. *Pharmacotherapy,* 5, 3: 168–1821 (1988).
9. W. H. Frishman, V. H. Murphy, and J. A. Storm. Ultra-Short-Acting β-Adrenergic Blockers. *Med. Clin. of North America,* 72:359–372 (1988).
10. N. Bodor, Y. Oshiro, T. Loftsson, M. Katovich, and W. Caldwell. Soft Drugs VI. The Application of the Inactive Metabolite Approach for Design of Soft β-Blockers. *Pharm. Res.* 3:120–124 (1984).
11. P. J. Machin, D. N. Hurst, and J. M. Osbond. β-Adrenoceptor Activity of the Stereoisomer of the Bufuraolol Alcohol and Ketone Metabolites. *J. Med. Chem.* 28:1648–1651 (1985).
12. N. Bodor. Prodrugs versus soft drugs. In H. Bundgaard (ed). *Design of Prodrugs.* Elsevier, Chap. 11, p 333–353 (1985).
13. N. Bodor. Novel Approaches to the Design of Safer Drugs: Soft Drugs and Site Specific Chemical Delivery System. In Advances in Drug Research, 13, Ed: B. Testa, Academic Press, London, 255–331 (1987).
14. N. Bodor, A. Elkoussi, M. Kano, and M. Khalifa. Soft Drugs. 7. Soft β-Blockers for Systemic and Ophthalmic Use. *J. Med. Chem.* 81:1651–1656 (1988).
15. N. Bodor and A. Elkoussi. Novel 'soft' β-blockers as potential safe antiglaucoma agents. Current Eye Research 7:369–374 (1988).
16. P. W. Erhardt, C. M. Woo, R. J. Gorzynski, and W. G. Anderson. Ultra-Short-Acting β-Adrenergic Blocking Agent. 1. (Aryloxy)propanolamines Containing Esters in the Nitrogen Substituent. *J. Med. Chem.* 25:1402–1407 (1982).
17. P. W. Erhardt, C. M. Woo, W. G. Anderson, and R. J. Gorzynski. Ultra-Short-Acting β-Adrenergic Blocking Agent. 2. (Aryloxy)propanolamines Containing Esters on the Aryl Function. *J. Med. Chem.* 25:1408–1412 (1982).
18. T. Loftsson, J. Kaminski, and N. Bodor. Improved Delivery through Biological Membranes IX: Kinetics and Metabolism of Hydrolysis of Methylsulfinylmethyl 2-Acetoxybenzoate and Related Aspirin Prodrugs. *J. Pharm. Sci.* 70, 7:750–755 (1981).
19. T. Loftsson and N. Bodor. Improved Delivery through Biological Membranes X: Percutaneous Absorption and Metabolism of Methylsulfinylmethyl 2-Acetoxybenzoate and Related Aspirin Prodrugs. *J. Pharm. Sci.* 70, 7:756–758 (1981).
20. P. Turlapaty, A. Laddu, V. S. Merphy, B. Singh, and R. Lee. Esmolol: A Titratable Short-Acting Intravenous Beta-Blocker for Acute Critical Care Settings. *Am. Heart J.* 114:866–885 (1987).
21. P. Benfield and E. M. Sorkin. Esmolol. A Preliminary Review of its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Efficacy. *Drugs* 33:392–412 (1987).
22. D. M. Angran, N. J. Schultz, and V. H. Tschida. Esmolol hydrochloride: An ultrashort-acting, β-adrenergic blocking agent. *Clinical Pharmacy* 5:288–303 (1986).
23. L. G. Wade, J. M. Gerdes, and R. P. Wirth. Protection of Carboxylic Acids as Methylthiomethyl Esters. *Tet. Lett.* 8:731–732 (1978).
24. A. Arfwidsson, K. O. Borg, K. Hoffman, and I. Skanberg. Metabolism of Metoprolol in the Rat *in vitro* and *in vivo*. *Xenobiotica* 6, 11:697–711 (1976).
25. T. Loftsson, J. Kaminski, and N. Bodor. Improved Delivery through Biological Membranes VIII: Design, Synthesis, and *In Vivo* Testing of True Prodrugs of Aspirin. *J. Pharm. Sci.* 70, 7:743–749 (1981).
26. C. Y. Quon and H. F. Stampli. Biochemical Properties of Blood Esmolol Esterase. *Drug Meta. Dispo.* 13, 4:420–424 (1985).
27. N. Weiner. Norepinephrine, Epinephrine, and The Symphatomimetic Amines. In L. S. Goodman, A. G. Gilman, T. W. Rall, and F. Murad (eds). *The Pharmacological Basis of Therapeutics.* New York. 1990. pp 201–202.
28. B. E. Bleski. Esmolol. *Conn. Med.* 51:669–671 (1987).
29. P. Turlapaty, A. Laddu, V. S. Murphy, B. Singh, and R. Lee. Esmolol: A Titratable Short-Acting Intravenous Beta-Blocker for Acute Critical Care Setting. *Am. Heart J.* 114:866–885 (1987).