g (1.55 mmoles) of the purified Boc-Pro-OH coupling product above (before Ts removal) in 50 mL of methanol was treated with 10 mL of water and 10 mL of 1 M aqueous potassium hydroxide, and the resulting clear solution was stirred at 25 °C for 1 h under nitrogen. (By TLC, the reaction was complete at this time. A small aliquot allowed to react under the same conditions for 18 h underwent no further change.) Most of the methanol was removed in vacuo, and the aqueous residue was saturated with solid sodium chloride and extracted with methylene chloride (5 × 100 mL) and chloroform (2 × 100 mL). The combined organic layer was dried over magnesium sulfate and concentrated. Chromatographic purification of the crude product on 180 g of silica gel (4 M NH_3–MeOH/$CHCl_3$, 12:88), followed by precipitation from CH_2Cl_2/hexane, afforded 1.27 g (87%) of pure THAM amide **30** as an easily handled, white, amorphous solid: ^1H NMR ($CDCl_3$) δ 3.80–3.60 (m, 6 H, CH_2OH), 1.45 (s, 9 H); R_f 0.28 (4 M NH_3–MeOH/$CHCl_3$, 15:85).

Biology. Inhibition of Human Plasma Renin Activity. The compounds in Table I were assayed for plasma renin inhibitory activity as follows: Lyophilized human plasma with 0.1% EDTA was obtained commercially (New England Nuclear). The angiotensin I generation step utilized 250 μL of plasma, 2.5 μL of phenylmethanesulfonyl fluoride, 25 μL of maleate buffer (pH 6.0), and 10 μL of an appropriate concentration of inhibitor in a 1% Tween 80 in water vehicle. Incubation was for 90 min at 37 °C. Radioimmunoassay for angiotensin I was carried out with a commercial kit (Baxter). Plasma renin activity values for inhibitor tubes were compared to control tubes to estimate percent inhibition. The inhibition results were expressed as IC_{50} values, which were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition.

Estimation of Solubility in Water. The solubility of each compound (Table I) in water was estimated visually. Each compound was added to a series of clear test tubes containing distilled water to make concentrations of compound ranging from 1–100 mg/mL. Each tube was shaken and observations initially made with the tubes at 25 °C and again after 30 min in a 37 °C water bath.

Evaluation in Recombinant Human Renin Infused Rats. Sprague-Dawley rats were anesthetized with methoxyflurane and bilaterally nephrectomized. Approximately 18 h later, each animal was reanesthetized with dial urethane, 100 mg/kg ip; tracheostomized, and bilaterally vagotomized. One carotid artery and both jugular veins were catheterized. In some animals that had fasted for 24 h prior to nephrectomy, an infant feeding tube was passed into the stomach through the mouth for the oral administration of compounds. Mecamylamine at 1.25 mg/kg iv was utilized to elicit ganglionic blockade. an intravenous infusion of recombinant human renin was administered at 2.4 GU/kg per min for 10 min and was followed by a sustained infusion at 0.6 GU/kg per min for the duration of the experiment. The compounds in Table I were dissolved in 0.1 M citric acid and infused intravenously at 0.05 mL/min for 10 min or administered into the stomach as a 5 mL/kg bolus approximately 30–35 min postinitiation of the renin infusion.

Resolution, Absolute Stereochemistry, and Enantioselectivity of 2-Methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol

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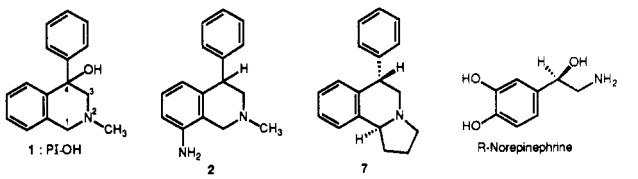
Racemic 2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (PI-OH) (1) was found to be an effective potentiator of the contractile response of norepinephrine (NE) on rat anococcygeus muscle. This paper describes the resolution of racemic PI-OH by an HPLC method to give the optically pure enantiomers (+)-1 and (−)-1. The absolute configuration of (+)-1 was *R* as determined by CD analysis and by single-crystal X-ray diffractometric analysis of the methiodide **6** derived from (+)-1. Examination of the effects of the enantiomers to potentiate the contraction of the rat anococcygeus muscle by NE showed a high degree of enantioselectivity. The NE potentiation was found to reside exclusively in (*R*)-(+)-1; the activity ratio being 21 at 3 × 10^{-6} M, whereas (*S*)-(−)-1 did not show any potentiating and inhibiting activity.

In the previous paper,[1] we reported a convenient synthesis of racemic 2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (PI-OH) (1), a compound with a chemical structure similar to that of nomifensine (2) (Chart I).[2] Racemic 1 potentiated the concentration-dependent responses of rat anococcygeus muscle to norepinephrine (NE) and electrical nerve stimulation. The potency of PI-OH was greater than that of cocaine, nomifensine (2), or desipramine in both responses.[3] Nomifensine and desipramine potentiated the response to NE at low concentration, which was progressively masked by an inhibitory effect as the concentration of the two drugs is increased.[3] However, PI-OH (1) had no side effects such as postsynaptic inhibition, and therefore it may be an ideal potentiator of the response to NE in adrenergically innervated tissues.[3] The potentiation produced by these drugs and by cocaine in NE-sensitive anococcygeus muscle to NE was consistent with the view that by inhibiting the neural uptake mechanism they cause supersensitivity in adrenergically innervated tissues.[4]

Chart I



PI-OH (1) has an asymmetric center at position 4, that structurally corresponds to β-hydroxyphenethylamines such as norepinephrine. Thus, the optical resolution of

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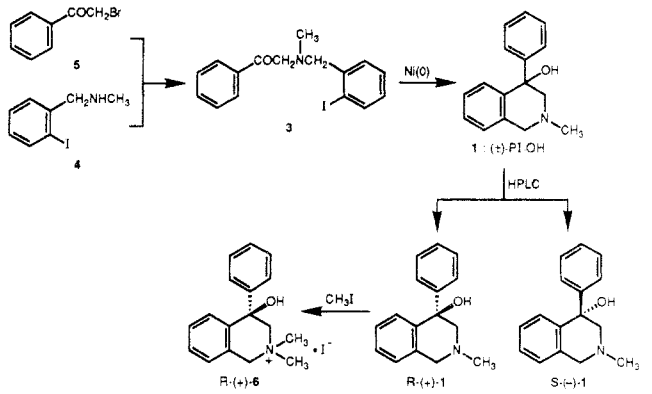
Scheme I



the racemic 1 and the biological evaluation of its enantiomers should proivde a valuable tool for analysis of drug effects on the amine uptake$_1$ mechanism, which is believed to be a stereoselective process.[5]

Naturally occurring epinephrine and norepinephrine have the *R* configuration. An important theory governing the adrenergic activity of phenethylamine possessing one point of asymmetry (at their β-carbon atoms) is the Easson–Stedman hypothesis,[6-8] which suggests that three-point attachment occurs in the binding of a sympathomimetic amine possessing an asymmetric β-carbon atom to the adrenergic receptors: (a) the basic nitrogen group, (b) the phenyl group, and (c) the benzylic hydroxy group of the β-carbon atom. According to the hypothesis, as modified by Blaschko[7] and Beckett,[8] these three groups of the *R*-(−) enantiomer of epinephrine are in a highly favorable stereochemical orientation for interaction with adrenergic receptors only if they are in the configuration that corresponds to (*R*)-(−)-epinephrine. For as far as (*S*)-(+)-epinephrine and its desoxy derivative are concerned, the β-hydroxy group is either incorrectly oriented or absent, and therefore they do not interact with the receptor. The present paper describes the optical resolution of PI-OH (1), the determination of the absolute configuration of the enantiomers, and the enantioselectivity of their NE potentiating activities.

Chemistry

In the previous paper,[1] we prepared PI-OH (1) in 24% yield by treating a phenacylamine (3) [obtained from a benzylamine (4) and phenacyl bromide (5)] with zerovalent nickel [Ni(0)] generated in situ in the $(Ph_3P)_2NiCl_2$–Zn–Ph_3P system, shown in Scheme I. Modification of the cyclization conditions[9] utilizing Ni(0) generated in the $NiCl_2$–Zn–Ph_3P system gave racemic 1 in 45% yield.

Attempted resolution of racemic 1 was unsuccessful by several methods, including recrystallization of the diastereomeric salts with di-*p*-toluoyl-D-tartaric acid,[10] dibenzoyl-L-tartaric acid,[11] and (*S*)-(+)-camphor-10-sulfonic acid,[12] and by the chromatographic separation of the diastereomeric esters of (+)-*N*-tosylvaline,[13] (+)-*O*-acetylmandelic acid,[14] and (*R*)-(+)-5-phenyl-1,3-dioxolane-2,4-dione.[15,16] Recently, optical resolution methods were developed by using HPLC on a chiral stationary phase.[17] Thus, we tried to separate the racemic 1 with Daicel Chiralcel OB, OC, OD, OF, OG, and OJ using *n*-hexane–2-propanol as an eluent. The best separation was achieved by Chiralcel OJ to give optically pure (+)-PI-OH $[\alpha]_D$ +38.9° (CH_3OH) and (−)-PI-OH $[\alpha]_D$ −38.9° (CH_3OH). ^1H NMR spectra of the racemic 1 and the two enantiomers were completely identical.

CD spectra of the (+) and (−) enantiomers of 1 showed typical split Cotton curves with $[\theta]_{220}$ −38 000, $[\theta]_{205}$ +16 000 and $[\theta]_{220}$ +38 000, $[\theta]_{205}$ −16 000, respectively. The negative exciton chirality[18] of the (+) enantiomer as shown in Figure

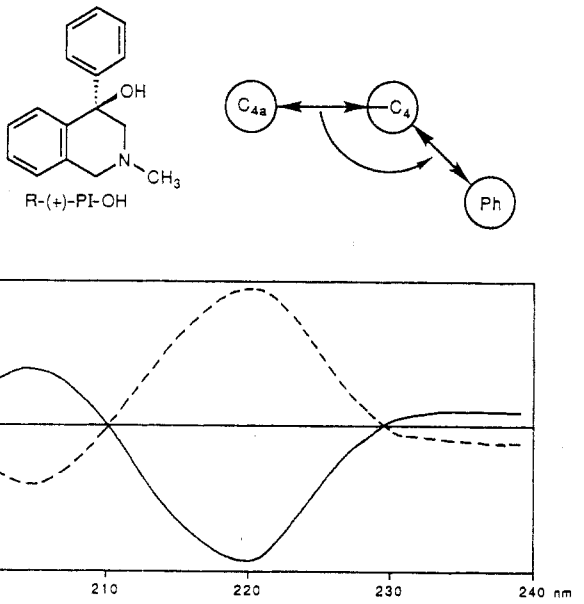


Figure 1. CD spectra of (*R*)-(+)- and (*S*)-(−)-1 in methanol (0.0103 mg/mL). Each spectrum was the result of four computer averaged scans: (—) (*R*)-(+)-1; (- - -) (*S*)-(−)-1.

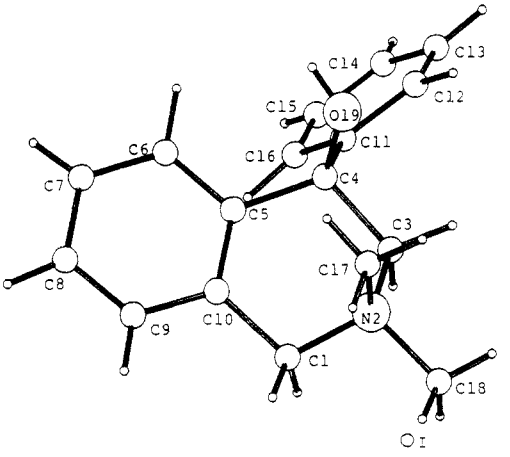


Figure 2. Single-crystal X-ray diffractometric determined structure of (*R*)-(+)-6; $[\alpha]_D$ +22.8° (*c* 1.67, MeOH).

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Table I. Potentiating Activities of the Enantiomers of 2-Methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (1) on the Responses of Rat Anococcygeus Muscle to Norepinephrine (NE)

concn of test compd	(±)-1		(R)-(+)-1		(S)-(−)-1	
	pD_2 value	activity ratio[a]	pD_2 value	activity ratio	pD_2 value	activity ratio
n[b]	6		6		6	
0	6.25 ± 0.06	1.0	6.23 ± 0.08	1.0	6.23 ± 0.06	1.0
10^{-7}	6.42 ± 0.04	1.5	6.60 ± 0.08	2.3	-	
3×10^{-7}	6.73 ± 0.05	3.0	7.01 ± 0.06	6.0	6.24 ± 0.05	1.0
10^{-6}	7.18 ± 0.03	8.5	7.28 ± 0.04	11.2	6.23 ± 0.05	1.0
3×10^{-6}	7.50 ± 0.04	17.8	7.56 ± 0.04	21.4	6.27 ± 0.03	1.1

[a] "Activity ratio" is calculated from the antilogalithm of the difference between the pD_2 values for NE obtained in the absence and presence of the test compounds, respectively; the activity in the absence of the potentiating compound is 1.0. [b] n is the number of experiments.

1 suggests the stereochemistry to be the *R* configuration. Thus, the (−) enantiomer has an *S* configuration.

In order to confirm this stereochemistry, (+)-1 was converted to (+)-PI-OH methiodide (**6**), $[\alpha]_D$ +22.8° (CH_3OH). This quaternary ammonium salt was examined by single-crystal X-ray diffractometric analysis. Figure 2 shows its molecular structure.[19] The absolute configuration of **6** was determined by the combination of the Bijvoet pairs that showed the structure of (*R*)-**6**. The two phenyl groups are planar within 0.09 Å, and the dihedral angle between their planes is 60.2°. The two substituents attached to the asymmetric carbon atom are the hydroxy group with a pseudoequatorial arrangement and the phenyl group with a pseudoaxial arrangement. The tetrahydropyridine ring adopts a half-chair conformation at the nitrogen atom since the methyl group attached to the nitrogen atom seems to be pushed to its opposite site by the bulky phenyl group.

Results and Discussion

The potentiating effects of (*R*)- and (*S*)-PI-OH on the contraction of rat anococcygeus muscle to NE were determined by the methods reported in our previous paper.[3] These results are shown in Table I. Clearly, enantioselectivity was observed with PI-OH in its ability to potentiate the response to NE. The *R* enantiomer shows a maximum sensitizing activity to NE at the concentration of 3×10^{-6} M and the pD_2 value of NE was 7.56 ± 0.03 (activity ratio 21), whereas the *S* enantiomer did not show any potentiating and inhibiting activities in the rat anococcygeus system. Therefore, the *S* enantiomer does not have any action on pre- and postsynaptic responses to NE.

High enantioselectivity in potentiating the action of NE has not been commonly reported:[20] there are a few examples such as oxaprotiline,[21] viloxazine,[22] mianserine,[23] and pyrroloisoquinoline compound **7**,[20] which are not β-hydroxyphenethylamine analogues. Compound (*R*)-(+)-1 having the hydroxy group[24] at position 4 showed high enantioselectivity similar to that of natural (*R*)-(−)-norepinephrine, which shows the importance of the β-hydroxy group and of its absolute configuration for the activity of NE uptake.[5] (*R*)-PI-OH incorporates both a fused and an attached aromatic ring. The fused benzene ring correlates with the *R* configuration of NE, while the attached phenyl substituent correlates with the *S* configuration of NE. Therefore, it is suggested that the fused aromatic ring is critical to NE potentiation by PI-OH and may occupy the site occupied by the phenyl substituent in (*R*)-(−)-norepinephrine. These results show that there may be a specific receptor for NE uptake, and that PI-OH (1) may be antagonistic at this NE uptake receptor(s). From these findings, (*R*)-(+)-PI-OH should prove to be a valuable tool in the analysis of the mechanisms of NE uptake.

Experimental Section

Chemistry. All melting points are given as uncorrected values. IR spectra were taken with a Perkin-Elmer 1720 infrared Fourier transform spectrophotometer and are given in cm^{-1}. ^1H NMR spectra were recorded on a JEOL JNM-FX 200 and a JEOL JNM-GSX 400 spectrometers with tetramethylsilane as a standard and are given as δ values. Optical rotations were determined with a Union PM-201 polarimeter. CD spectra were recorded on a JEOL J-600 spectropolarimeter. HPLC was run on a Shimazu LC-6A liquid chromatograph equipped with a chiral stationary phase column (Daicel Chiralcel OJ).

2-Methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (PI-OH) (1). Ph_3P (739 mg, 2.8 mmol), $NiCl_2$ (178 mg, 1.4 mmol), and Zn (72 mg, 1.4 mmol) were placed in a two-necked flask. The flask was evacuated and filled with N_2. Dry oxygen-free DMF (10 mL) was added through a syringe. The mixture was stirred at 55 °C for 5 min. A solution of **3**[1] (243 mg, 0.7 mmol) in dry oxygen-free DMF (2 mL) was added, and the mixture was stirred at 55–60 °C for 10 h. Then, 2% HCl (15 mL) was added, and the aqueous layer was washed with ether. The aqueous layer was made basic with concentrated NH_4OH and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated to give a crude product (123 mg). This was subjected to preparative TLC on Al_2O_3 in benzene–ethyl acetate (3:1) to give 1 as colorless needles (72 mg, 45%): mp 105–106 °C (from 2-propanol); IR (KBr) 3273, 2799, 1491, 1443. This was identical with an authentic 1[1] by comparison of the ^1H NMR spectra and a mixed melting point test.

Resolution of (±)-PI-OH (1). (±)-PI-OH (100 mg) was submitted to semipreparative HPLC with a hexane–2-propanol (25:1) mixture at a flow rate of 4 mL/min and detected at 220 nm to give completely separated two fractions. The first fraction at 10.0 min retention time afforded the (+) enantiomer (45.8 mg) as a white solid. Recrystallization from 2-propanol gave colorless needles: mp 119.5–120 °C; $[\alpha]^{22}_D$ +38.9° (*c* 1.98, CH_3OH); CD (*c* 0.00103, CH_3OH) $[\theta]^{23}$ (nm) 0 (230), −38 000 (220), 0 (210), +16 000 (205); ^1H NMR ($CDCl_3$, 200 MHz) 6.86–7.40 (9 H, m, aromatic H), 3.27 and 3.48 (each 1 H, d, J = 15 Hz, $ArCH_2N$), 2.56 and 2.87 (each 1 H, d, J = 12 Hz, CCH_2N), 2.31 (3 H, s, NCH_3); IR (KBr) 3199, 2793, 1490, 1449. Anal. ($C_{16}H_{17}NO$) C, H, N.

The second fraction at 12.9 min retention time gave the (−) enantiomer (47.1 mg) as a white solid. Recrystallization from 2-propanol afforded colorless needles: mp 120 °C; $[\alpha]^{23}_D$ −38.9° (*c* 1.95, CH_3OH); CD (*c* 0.00103, CH_3OH), $[\theta]^{23}$ (nm) 0 (230), +38 000 (220), 0 (210), −16 000 (205). Anal. ($C_{16}H_{17}NO$) C, H, N.

(*R*)-(+)-2-Methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol Methiodide (6). A solution of (+)-PI-OH (25 mg, 0.10 mmol)

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(24) The potentiating activity ratio of the desoxy compound of PI-OH at 3×10^{-6} M was 6.2 (unpublished results).

and methyl iodide (1 mL, 16.0 mmol) in CH_3OH (2 mL) was refluxed for 30 min. The reaction mixture was evaporated in vacuo to give crude **6** (52 mg). Recrystallization from 2-propanol afforded the methiodide (**6**) as colorless needles (30 mg, 77.8%): mp 235 °C; $[\alpha]^{23}_D$ +22.8° (*c* 1.67, CH_3OH); ^1H NMR (Pyridine-d_5, 400 MHz) 7.26–7.82 (9 H, m, aromatic H), 5.44 and 5.82 (each 1 H, d, J = 15 Hz, $ArCH_2N$), 4.30 and 4.56 (each 1 H, d, J = 14 Hz, CCH_2N), 3.76 and 3.89 (each 3 H, s, $N(CH_3)_2$); IR (KBr) 3338, 3010, 2970, 1476, 1449, 1346. Anal. ($C_{17}H_{20}INO$) C, H, N.

Single-Crystal X-ray Analysis of (+)-PI-OH·CH_3I (6). (+)-PI-OH·CH_3I was recrystallized by the evaporation of the 2-propanol solution at room temperature. Crystal size was 0.3 × 0.3 × 0.3 mm. Cell parameters were determined by least-squares methods for 25 reflections ranged in 15° < 2θ < 25°, on a Rigaku four-circle diffractometer equipped monochromatized Mo Kα radiation. The conditions of intensity measurements: $2\theta_{max}$ = 50°, o scan technique, scan speed 2° min^{-1}, and small drifts of three reference reflections. Reflections (1783) were collected, and 1639 were cited as observed reflections ($F > 3\sigma_F$) and corrected for Lorentz polarization, but not for absorption.

Crystal data: molecular formula $C_{16}H_{17}NO·CH_3I$, M_r 381.26; orthorhombic; space group $P2_12_12_1$; unit cell a = 12.667 (3), b = 16.119 (3), c = 8.159 (1) Å; V = 1665.9 (6) Å^3; Z = 4, D_{calcd} = 1.520 Mg m^{-3}; (Mo Kα) = 0.71069 Å; μ (Mo Kα) = 1.943 mm^{-1}; F(000) = 760; room temperature.

The structure was solved by the Patterson heavy atom method, which revealed the position of the iodine atom. The remaining atoms were located in succeeding difference Fourier syntheses. Their atomic parameters were refined by block-diagonal least-squares methods, minimizing w $(|F_o| - |F_c|)^2$, $w^{-1} = \sigma_F + 0.0001 \times F_o^2$, anisotropically. All H atoms were located on difference Fourier syntheses and refined isotropically. The final R value was 0.029 (R_w = 0.034, S = 4.306). In order to determine the absolute configuration of PI-OH·CH_3I, an anomalous dispersion factor for iodine atom was introduced. Then, the 101 Bijvoet pairs which have greatest values for the function: $[F_o(hkl) - F_o(hkl)]/[F_o(hkl) + F_o(hkl)]$ had as the same sign as those of the corresponding $F_c(hkl)$ calculated by the structure of **6**. Atomic scattering factors taken from *International Tables for X-Ray Crystallography*.[25] All calculations were performed by applications of the program packages; RASA,[26] X-SRANP,[27] and PLUTO[28] on PANAFACOM U1400 and micro VAX II computers of Tokushima Bunri University.

Pharmacology. Detailed methods used in evaluating these compounds were reported in the previous paper[3] from our laboratory. The isolated rat anococcygeus muscles were used for the assay of potentiating activity of PI-OH on the response to NE, which was evaluated from a shift in the concentration–response curves at low concentration of NE. The ability of a drug in potentiating the action of NE is expressed as the activity ratio, which was determined from the antilogarithm of the difference between the pD_2 values for NE (negative logarithm of the molar concentration of the agonist producing 50% of the maximum response) in the presence and absence of the test compounds.

Supplementary Material Available: Fractional coordinates, bond lengths, bond angles, anisotropic parameters, torsion angles, Bijvoet pairs, and fractional coordinates of hydrogen atoms (8 pages); a listing of structure factors (9 pages). Ordering information is given on any masthead page.

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Electrophilic α-Methylene-γ-lactone and Isothiocyanate Opioid Ligands Related to Etorphine

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Isothiocyanate and α-methylene-γ-lactone analogues of 6,14-*endo*-ethenotetrahydrothebaine and -oripavine were prepared with the electrophilic groups being located at C-19 in the C-7α-side chain. Isothiocyanates were prepared in the *N*-Me and *N*-CPM (*N*-cyclopropylmethyl) series, both as the phenols and 3-*O*-methyl ethers from the diastereomeric amines formed from reductive amination of thevinone (**2**) and *N*-(cyclopropylmethyl)northevinone (**13**). Although addition of the organozinc reagent from methyl α-bromomethacrylate to **25** failed, addition to 3-O-protected aldehydes **27** and **35** produced, after subsequent deprotection, α-methylene-γ-lactones **29** and **37**, respectively. In the opioid receptor displacement assays against [^{3}H]bremazocine as the radiolabeled ligand, the phenolic compounds were most potent with *N*-CPM isothiocyanates **20** and **21** showing IC_{50}s of 0.32 and 0.76 nM, respectively, and *N*-CPM α-methylene-γ-lactone **37** having an IC_{50} = 1.0 nM. Compound **37** showed irreversible effects in the binding assay which were μ-selective, as demonstrated by analogous experiments using [^{3}H]DAGO, and naloxone was found to protect against the irreversible effects. This observation suggests that a receptor-bound nucleophile is located at a position where it can readily reach the α-methylene group of lactone **37**.

Electrophilic ligands derived from opioid agonist and antagonist molecules have provided an important approach to aid in characterization of opioid drug–receptor interactions.[1] Ligands with an isothiocyanate group have been made in several series including 6α- and 6β-isothiocyanato-4,5-epoxymorphinans,[2] fentanyl- and etonitazene-derived isothiocyanates FIT, SUPERFIT, and BIT,[3] *N*-alkyl-6,14-*endo*-ethenotetrahydrooripavine-derived isothiocyanates,[4] and a derivative of U-50,488H.[5] Some of these are bound irreversibly in opioid-receptor prepa-

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