Synthesis and β-Adrenergic Antagonism of 2-(Aryloxy)-1-(2-piperidyl)ethanols[1]

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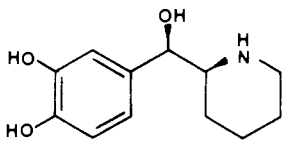
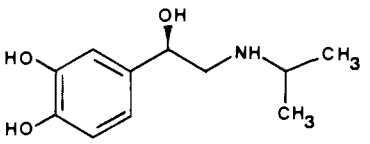
A series of *erythro*- and *threo*-2-(aryloxy)-1-(2-piperidyl)ethanol derivatives (**3**) was synthesized from 2-(2-oxiranyl)pyridine for evaluation as β-antagonists. Most compounds displayed high competitive β-blocking potency, but they lacked significant β_1/β_2 selectivity. The 1-naphthoxy derivative *erythro*-**3b** was 17 (β_1) and 33 (β_2) times more potent than its open-chain analogue, propranolol. Within the whole series, *erythro*-**3** diastereomers were more potent β-blockers than the *threo*-**3** isomers, and the potency of the latter seems to be rather insensitive to structural modification. The effect of N-methylation and of interposition of an alkyl chain between the aromatic ring and the side chain, while being detrimental to β-blocking activity, was less marked than in the classic (aryloxy)propanolamine blockers.

Rimiterol (**1**) is a β_2-selective adrenergic stimulant,[2] which can be considered as a cyclic analogue of isoprenaline (**2**). Although α-substituted arylethanolamines usually are less potent adrenergic agents than the unbranched compounds,[3] the above cyclization does not seem to have any detrimental effect upon the bronchial relaxant effect in rimiterol. To our knowledge, the piperidine moiety present in rimiterol has not been incorporated into a β-adrenergic blocking structure of the (aryloxy)propanolamine type.



1 rimiterol



2 isoprenaline

We report in this paper the synthesis and β-blocking activity of a new series of 2-(aryloxy)- or 2-(arylalkoxy)-1-(2-piperidyl)ethanols (**3a–h**, see structures in Table I), structurally related to the classic (aryloxy)propanolamine β-blockers. Due to the presence of two asymmetric centers in compounds **3**, these piperidines can exist in two diastereomeric erythro and threo forms, which must be investigated separately. Aiming to determine the effect of cyclization upon β-blocking potency, we have also synthesized and tested the open-chain analogues **4a–h**, bearing an *N*-isopropyl group.

Chemistry

2-(2-Oxiranyl)pyridine (**6**) was obtained in 64% yield from 2-vinylpyridine (**5**), through the method of Hanzlik et al.[4] Oxirane ring opening with the sodium salt of the appropriate phenol gave compounds **7a–e**. The best yields were obtained when the reaction was carried out in dimethylformamide solution and the aryloxide was generated in situ by treatment of the phenol with sodium hydride (method A).

The arylalkoxy derivatives **7f,g** were obtained by acid-promoted (BF_3·Et_2O) oxirane opening, in the presence of an excess of benzyl alcohol or 3-phenylpropanol, respectively (method B). This reaction is regiospecific, due to the electron-attracting effect of the pyridine ring, which renders the benzylic carbonium ion very unstable.

Catalytic hydrogenation of the pyridine ring in **7a–g** (PtO_2, HCl, method C) afforded a mixture of the erythro and threo diastereomers of **3a–g**, in approximate 2:1 to 3:1 ratios. All attempts at direct chromatographic separation of the diastereomeric pairs were unsuccessful, so less polar N-acetylated derivatives were prepared. The N-acetylated compounds *erythro*- and *threo*-**8a–g** were easily separated by silica gel column chromatography, and pure piperidinoethanols *erythro*-**3a–g** and *threo*-**3a–g** were obtained in good yields by alkaline hydrolysis of the corresponding acetamides (methods D and E).

For compounds **3h**, a slightly different approach was used. The mixture of isomers of **3a** reacted with formaldehyde and KOH (method F) to give perhydrooxazolo[3,4-*a*]pyridines *erythro*-**9** and *threo*-**9**, which could be isolated by column chromatography. Each diastereomer of **9** was separately reduced with lithium aluminum hydride[5] to the *N*-methyl analogue *erythro*- or *threo*-**3h**.

^{1}H NMR spectroscopy allowed stereochemical assignment of compounds **3a–h**. Thus, LAOCOON 3 analysis[6] of the four-spin system in the $OCH_2CHOHCHN$ fragment gave vicinal CHOCHN coupling constant values of 4.1 Hz for *erythro*-**3** and of 7 Hz for *threo*-**3** stereoisomers. These values are in good agreement with the coupling constants found in aryl(2-piperidyl)methanols and aryl(2-tetrahydropyridyl)methanols[7] closely related to our compounds.

Results and Discussion

Compounds *erythro*- and *threo*-**3a–h** and **4a–h** were evaluated for their in vitro β-blocking activity against isoprenaline, in tracheal smooth muscle and electrically stimulated left atrium of guinea pigs. The resulting pA_2

(1) A preliminary account of this work was presented by Pujol, M. D.; Minguillón, C.; Rosell, G.; Mauleón; D., Miquel, J.; Barenys, B. at the XXII Rencontres Internationales de Chimie Thérapeutique, Clermont-Ferrand, France, September 1986; Communication C-19.

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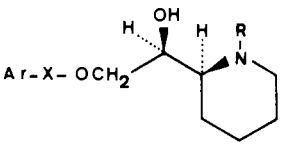
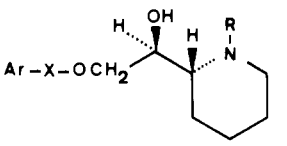
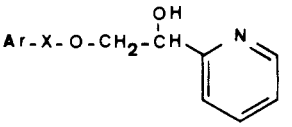
Table I. Structures and Preparation of Compounds 7, 3, and 9



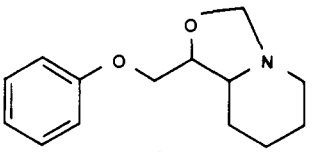
erythro-3



threo-3



7



9

compound	Ar–X	R	% yield (method)	formula[a]	mp, °C (purifn solvent)
7a	C_6H_5		58 (A)	$C_{13}H_{13}NO_2$·HCl	134–136 (Et_2O–EtOH)
7b	1-naphthyl		50 (A)	$C_{17}H_{15}NO_2$	102–104 (Et_2O–AcOEt)
7c	2-naphthyl		38 (A)	$C_{17}H_{15}NO_2$·HCl	165–167 (AcOEt–Et_2O)
7d	*m*-CH_3-C_6H_4		53 (A)	$C_{14}H_{15}NO_2$	oil[b]
7e	*p*-CH_3-C_6H_4		60 (A)	$C_{14}H_{15}NO_2$	oil[b]
7f	C_6H_5-CH_2		91 (B)	$C_{14}H_{15}NO_2$	123–126 (Et_2O–AcOEt)
7g	C_6H_5-$(CH_2)_3$		51 (B)	$C_{16}H_{19}NO_2$	oil[b,c]
erythro-**3a**	C_6H_5	H	31[d] (C, D, E)	$C_{13}H_{19}NO_2$	116–119 (Et_2O)
threo-**3a**	C_6H_5	H	16[d] (C, D, E)	$C_{13}H_{19}NO_2$	114–117 (Et_2O)
erythro-**3b**	1-naphthyl	H	22[d] (C, D, E)	$C_{17}H_{21}NO_2$	102–105 (AcOEt–hexane)
threo-**3b**	1-naphthyl	H	17[d] (C, D, E)	$C_{17}H_{21}NO_2$	124–129 (Et_2O–AcOEt)
erythro-**3c**	2-naphthyl	H	28[d] (C, D, E)	$C_{17}H_{21}NO_2$·HCl	139–141 (AcOEt)
threo-**3c**	2-naphthyl	H	18[d] (C, D, E)	$C_{17}H_{21}NO_2$	82–85 (AcOEt)
erythro-**3d**	*m*-CH_3-C_6H_5	H	37[d] (C, D, E)	$C_{14}H_{21}NO_2$	oil[b]
threo-**3d**	*m*-CH_3-C_6H_5	H	22[d] (C, D, E)	$C_{14}H_{21}NO_2$	oil[b]
erythro-**3e**	*p*-CH_3-C_6H_5	H	25[d] (C, D, E)	$C_{14}H_{21}NO_2$	112–115 (AcOEt–hexane)
threo-**3e**	*p*-CH_3-C_6H_5	H	14[d] (C, D, E)	$C_{14}H_{21}NO_2$	88–91 (Et_2O–AcOEt)
erythro-**3f**	C_6H_5-CH_2	H	22[d] (C, D, E)	$C_{14}H_{21}NO_2$	69–71 (AcOEt–hexane)
threo-**3f**	C_6H_5-CH_2	H	13[d] (C, D, E)	$C_{14}H_{21}NO_2$	80–82 (AcOEt–hexane)
erythro-**3g**	C_6H_5-$(CH_2)_3$	H	22[d] (C, D, E)	$C_{16}H_{25}NO_2$	98–102 (AcOEt–hexane)
threo-**3g**	C_6H_5-$(CH_2)_3$	H	14[d] (C, D, E)	$C_{16}H_{25}NO_2$	oil[b]
erythro-**3h**	C_6H_5	CH_3	70 (G)	$C_{14}H_{21}NO_2$·HCl	142–145 (Me_2CO)
threo-**3h**	C_6H_5	CH_3	49 (G)	$C_{14}H_{21}NO_2$	76–79 (AcOEt–hexane)
erythro-**9**			43 (C, F)	$C_{14}H_{19}NO_2$	oil[b]
threo-**9**			18 (C, F)	$C_{14}H_{19}NO_2$	oil[b]

[a] All compounds were analyzed for C, H, and N, and analytical values were within ±0.4% of calculated values. [b] Purified by silica gel column chromatography and analyzed as oil; prior biological testing this compound was dissolved in 1 N HCl. [c] Bp 220–230 °C (0.5 mmHg). [d] Yields calculated from the corresponding pyridine 7.

values are collected in Table II.

Except for the phenylpropyl derivative *threo*-**3g** in the trachea, all piperidines **3** were active as β-adrenergic antagonists. The cyclic analogue *erythro*-**3b** of propranolol (**4b**) is a very potent, nonselective β-blocker, whereas *erythro*-**3d**, the piperidine derivative of toliprolol (**4d**), is more potent in the trachea than the parent compound.

Although the *erythro* isomers of α-branched β-blockers or β-agonists are known to be more potent than their *threo*-**3** counterparts,[3] in our piperidylethanol series the *erythro*-**3**/*threo*-**3** potency ratios are small, with a mean value of only 3–4. Thus, an inspection of pA_2 values in Table II reveals that most *erythro*-**3** isomers can be considered as equally potent than the corresponding *threo*-**3** isomers. Nevertheless, the most active compounds **3a** and **3b** display a significant degree of stereoselectivity, which cannot be attributed to different physical properties. Thus, we have measured[8] octanol/phosphate buffer (pH 7.0) distribution coefficients and pK_b values for the pair *erythro*-**3a** and *threo*-**3a**, obtaining very similar values (log D = −1.08 and −1.12; pK_b = 9.42 and 9.34, respectively). In addition, the chromatographic behavior of these diastereomers is always identical.

As it has been described,[9] 2-naphthoxy derivative **4c** is 5–10 times less active than its isomer, propranolol (**4b**). In the piperidine series, introduction of a 2-naphthoxy group in **3c** has also a detrimental effect upon β-blocking potency, with respect to **3b** isomers. Another structural feature that caused a reduction in β-antagonism is the interposition of an alkyl chain between the aromatic ring and the ethereal oxygen atom in (aryloxy)propanolamines.[10] Thus, **4f,g** are 10–100 times less potent than **4a** as β_1- or β_2-blockers. Cyclic compounds **3f** and **3g** are also less active than the corresponding isomer of **3a**, but in this case the potency ratios are of lower value, and the benzyloxy derivative *threo*-**3f** turned out to be equally active to *threo*-**3a**. Moreover, piperidines **3f** and **3g** are more potent β-blockers than their open-chain analogues **4f** and **4g**, which suggests that the potency of 1-(2-piperidyl)ethanols, especially those with threo stereochemistry, is less affected by structural modifications than classic (aryloxy)propanolamines. Accordingly, the detrimental effect of N-methylation[9] is also less evident in the cyclic series than in the open-chain compounds: tertiary amine **4h** is 59 (β_1) and 16 (β_2) times less potent than **4a**, whereas the potency ratios of *erythro*-**3a**/*erythro*-**3h** are only 7 (β_1) and 6 (β_2). Compounds *threo*-**3a** and *threo*-**3h** are almost equally potent.

In conclusion, the erythro isomers of 2-(aryloxy)-1-(2-piperidyl)ethanols constitute a new series of very potent and competitive β-adrenergic antagonists, derived from (aryloxy)propanolamine β-blockers through introduction of a piperidine ring. This structural variation does not seem to markedly affect potency nor cardioselectivity, although compound *erythro*-**3b** is significantly more potent than propranolol. The cyclic compounds show little sen-

(8) For the determination of distribution coefficients we followed the method for Hellenbrecht, D.; Lemmer, B.; Wiethold, G.; Grobecker, H. *Naunyn-Scmiedeberg's Arch. Pharmacol.* **1973**, *277*, 211. The pK_b values were measured following the indications In Albert, A.; Serjeant, E. P. *Ionization Constants of Acids and Bases. A Laboratory Manual*; Methuen: London, 1962.

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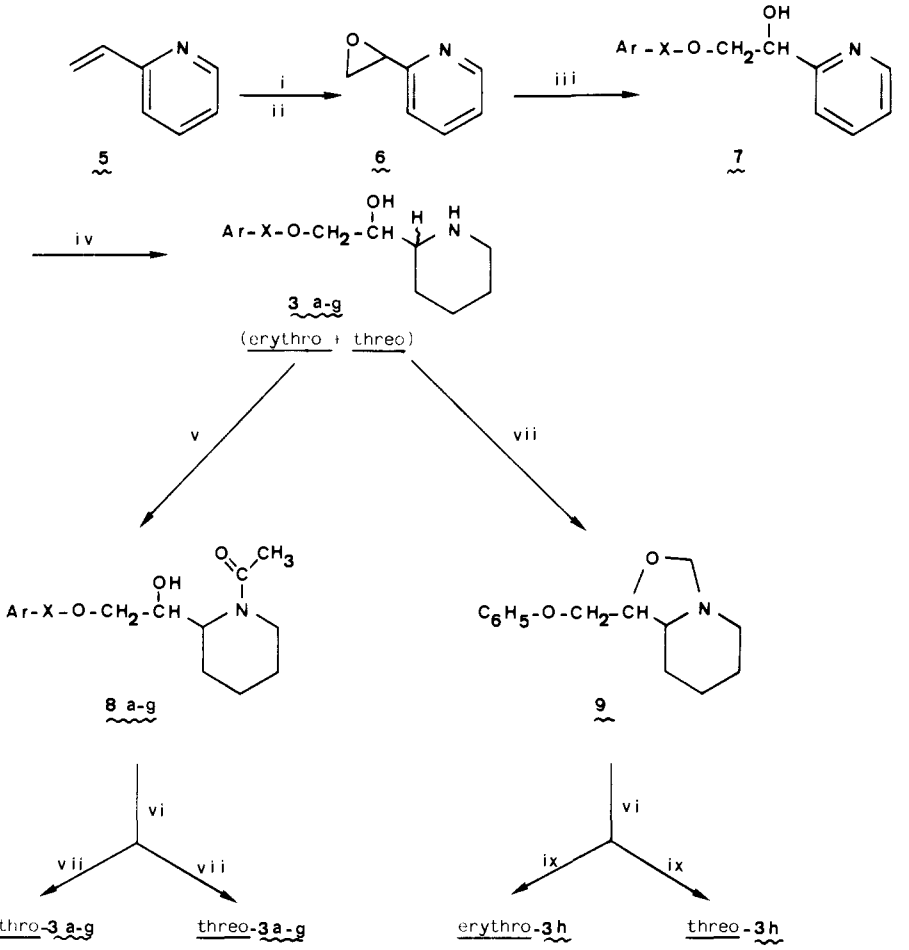
(10) Pujol, M. D.; Mauleón, D., unpublished results.

Scheme I[a]



[a] (i) NBS, dioxane–H_2O; (ii) Na_2CO_3; (iii) ArOH, NaH, DMF (compounds **3a–e**) or $Ar(CH_2)_nOH$, $BF_3{\cdot}Et_2O$ (compounds **3f–g**); (iv) H_2, PtO_2, HCl–MeOH; (v) AcCl, NaOH $H_2O/CHCl_3$; (vi) chromatography; (vii) KOH, $EtOH/H_2O$; (viii) HCHO, KOH, $MeOH/H_2O$; (ix) $LiAlH_4$, THF.

sitivity to structural changes that are clearly detrimental to potency in the open-chain series. Moreover, the erythro/threo potency in the piperidine series are of lower magnitude than those reported for open-chain diastereomers. The number of compounds tested is too small to allow definitive conclusions about these points.

Experimental Section

Melting points were determined in a capillary tube on a Büchi apparatus and are uncorrected. NMR spectra were measured at 200 MHz with a FT Varian XL-200 spectrometer, with TMS as an internal standard. Although only selected spectral data are presented, all isolated, purified compounds were fully characterized (IR, ^1H NMR, ^{13}C NMR) and their structural and stereochemical assignments are considered unambiguous. TLC was performed on silica gel 60 Merck HF_{254} (5–25 μm), and the spots were detected with UV light or iodoplatinate reagent. Column chromatography was conducted on silica gel Merck 60 (63–200 μm), and flash chromatography[11] was performed on silica gel Scharlau 60 (40–60 μm). All microdistillations were made on a Büchi GKR-50 Kugelrohr apparatus. Solutions in organic solvents were dried over anhydrous sodium sulfate and evaporations were made in vacuo (rotating evaporator). Elemental analysis were performed by Departamento de Química Orgánica (CSIC), Barcelona, Spain, and agreed with theoretical values to within ±0.4%.

Method A. 2-Phenoxy-1-(2-pyridyl)ethanol (7a). A solution of 1.88 g (20 mmol) of phenol in 80 mL of DMF was added to a suspension of 1.20 g (25 mmol, 50% dispersion in mineral oil, washed with hexane) of sodium hydride in 100 mL of DMF. When the evolution of gas ended, a solution of 2.42 g (20 mmol) of 2-(2-oxiranyl)pyridine (**6**)[4] in 10 mL of DMF was added dropwise under a nitrogen atmosphere. The resulting mixture was stirred at 60 °C for 12 h, poured into 500 mL of water, and extracted with ether. The ethereal layers were thoroughly washed with brine, dried, and evaporated to give 2.8 g of an oil. Column chromatography (ethyl acetate/hexane, 60:40) afforded 2.49 g (58% yield) of **7a**: ^1H NMR ($CDCl_3$) δ 4.20 (OCH_2), 4.88 (t, CHOH), 8.30 (d, pyridine-H_α).

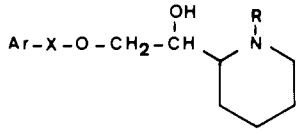
Method B. 2-(Benzyloxy)-1-(2-pyridyl)ethanol (7f). A solution of 3.25 g (30 mmol) of benzyl alcohol, 2.33 g (16.4 mmol) of $BF_3{\cdot}Et_2O$, and 0.907 g (7.5 mmol) of 2-(2-oxiranyl)pyridine (**6**) in 50 mL of CH_2Cl_2 was heated at reflux under N_2 for 12 h. The solvent was removed in vacuo, the residue was suspended in 2 N HCl and extracted with ether. The aqueous solution was made alkaline with 2 N NaOH and extracted with CH_2Cl_2. Evaporation of the dried extracts and crystallization from Et_2O–AcOEt gave 1.58 g (91% yield) of **7f**: ^1H NMR δ 3.62 (OCH_2), 4.51 (s, CH_2 Ar), 4.67 (t, CHOH), 8.30 (d, pyridine-H_α).

Method C. Catalytic Hydrogenation of Compounds 7. A solution of 400 mg (1.86 mmol) of **7a** in 20 mL of anhydrous ether was treated with excess ethereal HCl. After evaporation of the solvent, the residue was dissolved in 30 mL of CH_3OH and 20 mg of PtO_2 was added. The mixture was shaken in the presence of H_2 at atmospheric pressure, until >130 mL of H_2 were taken up. The catalyst was filtered off, and the solvent was removed in vacuo. The residue was dissolved in water, made alkaline with 2 N NaOH, and extracted with CH_2Cl_2. Evaporation of the dried extracts afforded 390 mg (95%) of an oil, which was identified as a mixture of *erythro*-**3a** and *threo*-**3a**.

Method D. 1-(*N*-Acetyl-2-piperidyl)-2-phenoxyethanol (8a). A solution of 663 mg (3 mmol) of **3a** (mixture of stereoisomers) in 35 mL of $CHCl_3$ was mixed with a solution of 1.20 g (30 mmol) of NaOH in 10 mL of water. To the stirred and

(11) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

Table II. β-Blocking Activities of 1-(2-Piperidyl)ethanols **3** and Their Open-Chain Analogues **4**



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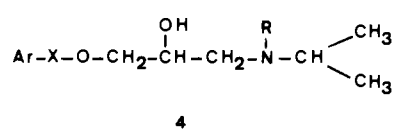

4

compound	Ar–X	R	pA_2[a] β_1, atrium[b]	pA_2[a] β_2, trachea[c]	selectivity β_1/β_2
erythro-**3a**	C_6H_5	H	8.10 ± 0.30 (22)[d]	7.86 ± 0.32 (18)[f]	1.7
threo-**3a**	C_6H_5	H	6.99 ± 0.18 (17)[d]	7.12 ± 0.30 (18)[d]	0.7
erythro-**3b**	1-naphthyl	H	9.82 ± 0.33 (23)[d]	10.20 ± 0.32 (21)[d]	0.4
threo-**3b**	1-naphthyl	H	8.75 ± 0.27 (20)[f]	8.91 ± 0.43 (17)[d]	0.7
erythro-**3c**	2-naphthyl	H	8.06 ± 0.30 (18)[d]	7.81 ± 0.34 (20)[f]	1.8
threo-**3c**	2-naphthyl	H	7.39 ± 0.22 (16)[d]	7.09 ± 0.35 (16)[f]	2.0
erythro-**3d**	*m*-CH_3-C_6H_4	H	7.02 ± 0.21 (18)[d]	8.47 ± 0.35 (21)[d]	0.03
threo-**3d**	*m*-CH_3-C_6H_4	H	6.59 ± 0.28 (17)[d]	6.90 ± 0.43 (17)[d]	0.5
erythro-**3e**	*p*-CH_3-C_6H_4	H	7.24 ± 0.37 (19)[d]	7.32 ± 0.32 (20)[d]	0.8
threo-**3e**	*p*-CH_3-C_6H_4	H	7.15 ± 0.33 (16)[d]	7.07 ± 0.36 (18)[d]	1.2
erythro-**3f**	C_6H_5-CH_2	H	7.31 ± 0.37 (20)[d]	6.92 ± 0.31 (18)[d]	2.5
threo-**3f**	C_6H_5-CH_2	H	7.09 ± 0.22 (16)[d]	7.47 ± 0.35 (20)[d]	0.4
erythro-**3g**	C_6H_5-$(CH_2)_3$	H	6.56 ± 0.38 (21)[f]	7.60 ± 0.38 (17)[d]	0.1
threo-**3g**	C_6H_5-$(CH_2)_3$	H	6.42 ± 0.32 (17)[f]	inactive (12)	
erythro-**3h**	C_6H_5	CH_3	7.25 ± 0.33 (20)[e]	7.10 ± 0.27 (19)[d]	1.4
threo-**3h**	C_6H_5	CH_3	6.80 ± 0.37 (18)[f]	6.95 ± 0.12 (18)[d]	0.7
4a	C_6H_5	H	8.77 ± 0.38 (23)	7.86 ± 0.34 (21)	8.1
4b (propranolol)	1-naphthyl	H	8.60 ± 0.32 (28)	8.47 ± 0.25 (27)	1.5
4c	2-naphthyl	H	7.71 ± 0.24 (19)	7.78 ± 0.39 (20)	0.9
4d (toliprolol)	*m*-CH_3-C_6H_4	H	8.75 ± 0.40 (27)	7.94 ± 0.25 (28)	6.5
4e	*p*-CH_3-C_6H_4	H	8.40 ± 0.34 (21)	8.15 ± 0.35 (20)	1.8
4f	C_6H_5-CH_2	H	5.98 ± 0.24 (18)	5.96 ± 0.36 (20)	1.0
4g	C_6H_5-$(CH_2)_3$	H	6.60 ± 0.28 (17)	6.94 ± 0.38 (16)	0.5
4h	C_6H_5	CH_3	7.00 ± 0.32 (19)	6.65 ± 0.13 (17)	2.2

[a] pA_2 values ± SD. The values in parentheses are the number of antagonist concentrations used in the calculation of pA_2. The slope of Schild plot[13] was 1 ± 0.15 in all cases. [b] Antagonism of the isoprenaline-induced positive inotropic effect in left atrium. [c] Antagonism of the isoprenaline-induced relaxation of guinea pig tracheal chains, contracted with carbachol. [d] $P < 0.001$ (Student's t test) for the pA_2 difference between the cyclic compound and the corresponding open-chain analogue. [e] $P < 0.05$, see footnote *d*. [f] Nonsignificant pA_2 difference, see footnote *d*.

ice-cooled mixture was added dropwise 785 mg (10 mmol) of acetyl chloride, and stirring was continued at room temperature for 2 h. The mixture was diluted with 100 mL of water and extracted with $CHCl_3$, and the organic layers were washed with brine. Silica gel chromatography of the resulting residue allowed diastereomer separation. On elution with hexane/AcOEt (60:40), 275 mg (35% yield) of *erythro*-**8a** was obtained: IR (KBr) 1600 and 1500 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 2.08 (CH_3), 4.20–4.42 (CHOH), 4.60 (C^2HN, rotamer A), 4.36 (C^2HN, rotamer B), 3.81–4.00 (CH_2O); ^{13}C NMR ($CDCl_3$) δ 21.8 (CH_3, rotamer A), 22.0 (CH_3, rotamer B), 43.2 (C^6N, rotamer A), 37.2 (C^6N, rotamer B), 51.5 (C^2N, rotamer A), 56.4 (C^2N, rotamer B), 171.9 (C=O, rotamer A), 171.7 (C=O, rotamer B). On elution with hexane/AcOEt (30:70), 135 mg (17% yield) of *threo*-**8a** were obtained: IR (NaCl) 1650 and 1500 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 2.13 (CH_3, rotamer A), 2.20 (CH_3, rotamer B), 4.10–4.40 (CHOH), 4.88 (C^2HN, rotamer A), 4.36 (C^2HN, rotamer B); ^{13}C NMR ($CDCl_3$) δ 21.9 (CH_3, rotamer A), 21.4 (CH_3, rotamer B), 43.7 (C^6N, rotamer A), 37.9 (C^6N, rotamer B), 51.0 (C^2N, rotamer A), 55.1 (C^2N, rotamer B), 170.0 (C=O, rotamer A), 169.9 (C=O, rotamer B).

Method E. *erythro*- and *threo*-2-Phenoxy-1-(2-piperidyl)ethanols (*erythro*-3a and *threo*-3a). A soloution of 263 mg (1 mmol) of *erythro*-**8a** and 912 mg (16 mmol) of KOH in 15 mL of EtOH and 5 mL of H_2O was heated at reflux under N_2 for 5 h. The mixture was poured into 100 mL of water, the EtOH was removed in vacuo, and the aqueous layer was extracted with $CHCl_3$. Evaporation of the dried extracts afforded a yellow solid, which was crystallized from Et_2O, yielding 212 mg (96%) of *erythro*-**3a**: ^1H NMR ($CDCl_3$) δ (from LAOCOON 3 analysis) 3.96 (1 H, CH_2O, J_{gem} = 10.2 Hz, J_{vic} = 5.4 Hz), 3.98 (1 H, CH_2O, J_{vic} = 6.1 Hz), 3.88 (1 H, CHOH, J_{CHOCHN} = 4.1 Hz), 2.8 (1 H, C^2HN); ^{13}C NMR ($CDCl_3$) δ 27.3 (C^3H_2), 69.2 (CH_2O), 72.6 (CHOH).

Via method E, 263 mg (1 mmol) of *threo*-**8a** afforded 201 mg (91% yield) of *threo*-**3a**: ^1H NMR ($CDCl_3$) δ (from LAOCOON 3 analysis) 4.05 (1 H, CH_2O, J_{gem} = 9.5 Hz, J_{vic} = 3.5 Hz), 3.96 (1 H, CH_2O, J_{vic} = 5.9 Hz), 3.73 (1 H, CHOH, J_{CHOCHN} = 7.0 Hz), 2.5 (1 H, C^2HN); ^{13}C NMR ($CDCl_3$) δ 28.9 (C^3H_2), 69.9 (CH_2O), 72.9 (CHOH).

Method F. *erythro*- and *threo*-1-(Phenoxymethyl)-3,5,6,7,8,8a-hexahydro-1*H*-oxazolo[3,4-*a*]pyridine (*erythro*-9 and *threo*-9). A solution of 1.8 g (8.1 mmol) of **3a** (stereoisomer mixture), 0.74 g (13 mmol) of KOH, and 1.6 g of formaldehyde (45% aqueous solution, 24 mmol) in 50 mL of MeOH was heated at reflux under N_2 for 2 h. The solvent was evaporated in vacuo, the residue was taken up in 100 mL of water and extracted with $CHCl_3$, and the organic layers were washed with brine. Evaporation of the dried extracts gave an oil (1.72 g, 91%), which was chromatographed on silica gel. On elution with hexane/AcOEt (70:30), 820 mg (43% yield) of *erythro*-**9** was obtained: ^1H NMR ($CDCl_3$) δ 3.76 (d, 1 H, OCH_2N, J = 1.8 Hz), 4.64 (d, 1 H, OCH_2N, J = 1.8 Hz); ^{13}C NMR ($CDCl_3$) δ 24.5 (C^3H_2), 86.1 (NCH_2O). On elution with hexane/AcOEt (50:50), 340 mg (18% yield) of *threo*-**9** was obtained: ^1H NMR ($CDCl_3$) δ 4.04 (d, 1 H, OCH_2N, J = 1.9 Hz), 4.58 (d, 1 H, OCH_2N, J = 1.9 Hz); ^{13}C NMR ($CDCl_3$) δ 22.0 (C^3H_2), 86.7 (OCH_2N).

Method G. *erythro*-2-Phenoxy-1-(*N*-methyl-2-piperidyl)ethanol (*erythro*-3h). A suspension of 190 mg (5 mmol) of $LiAlH_4$ in 3 mL of THF was cooled to 0 °C, and a solution of 400 mg (1.7 mmol) of *erythro*-**9** was added under N_2. The resulting mixture was stirred for 4 h at reflux, quenched with 10 N NaOH (1 mL), and poured into 100 mL of water. The aqueous layer was filtered and extracted with CH_2Cl_2, and the organic extracts were washed with water, dried, and evaporated to give 140 mg (70 % yield) of *erythro*-**3h**, which was purified as its hydrochloride.

Biological Test Procedures. (a) Isolated Guinea Pig Tracheal Chain. Guinea pigs, weighing 300–350 g, were sacrificed by a blow to the head. The trachea was dissected out, transferred to a dish containing Krebs solution, and cut transversally between the segments of cartilage. Five of the tracheal rings were tied together and mounted in a 30-mL organ bath containing modified

Krebs solution, maintained at 37 °C and gassed with 95% O_2/5% CO_2 throughout the whole experiment. An initial basal tension of 1 g was applied to each tracheal chain, and the tissue was allowed to stabilize for 90 min. Isometric force was recorded from the preparations by a force-displacement transducer and an Omni-Scribe recorder. A constant level of tone was induced by the addition of carbachol chloride (5.5×10^{-7} M, Sigma) to the bath, and, after 15 min, a control concentration–response curve for isoprenaline was obtained. The tissue was washed thoroughly, and 30 min later a single concentration of antagonist (**3a–h**, **4a–h**) was added to the bath and allowed to act for 30 min. During the last 15 min of antagonist incubation, carbachol chloride (5.5×10^{-7} M) was added to the bath, and the cumulative concentration–response curve for isoprenaline was again determined. All responses to different concentrations of isoprenaline were expressed as percentages of the maximal relaxation recorded for the control curve. β-Blockade was evaluated by determining the pA_2 as described by Van Rossum;[12] the slope of Schild plot was calculated in each case, according to Arunlakshana.[13]

(b) Isolated Guinea Pig Left Atria. Guinea pigs of either sex, weighing 300–350 g, were sacrificed by a blow to the head. The heart was removed as quickly as possible and placed in a dish containing carbogenated Krebs solution. Left atrial preparations were subjected to electric pulses delivered by a Cibertec stimulator through platinum electrodes on the muscle holder. The tissue was mounted under 1.0 g of tension in a 30-mL organ bath containing modified Krebs solution at 37 °C, continuously bubbled with carbogen. The tissue was allowed to equilibrate for 60 min before eliciting responses to drugs. After 45-min equilibration, the tissue was stimulated to contract by pulses of 5 ms and submaximal voltage. Contractions were recorded isometrically with a force-displacement transducer connected to an Omni-Scribe recorder. Cumulative concentration–response curves of isoprenaline were recorded, the solution in the muscle chamber was removed and after a 15 min period, and the preparation was exposed to a single concentration of antagonist for additional 15 min, after which another cumulative concentration–response curve for isoprenaline was reelicited. pA_2 values were calculated as described above.

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Registry No. *erythro*-**3a**, 115462-42-5; *threo*-**3a**, 115462-43-6; *erythro*-**3b**, 88624-96-8; *threo*-**3b**, 88624-97-9; *erythro*-**3c**, 115462-44-7; *erythro*-**3c**·HCl, 115462-74-3; *threo*-**3c**, 115462-45-8; *erythro*-**3d**, 115462-46-9; *threo*-**3d**, 115462-47-0; *erythro*-**3e**, 115462-48-1; *threo*-**3e**, 115462-49-2; *erythro*-**3f**, 115462-50-5; *threo*-**3f**, 115462-51-6; *erythro*-**3g**, 115462-52-7; *threo*-**3g**, 115462-53-8; *erythro*-**3h**, 115462-54-9; *erythro*-**3h**·HCl, 115462-75-4; *threo*-**3h**, 115462-55-0; **4a**, 7695-63-8; **4b**, 525-66-6; **4c**, 2007-72-9; **4d**, 2933-94-0; **4e**, 5790-46-5; **4f**, 19343-24-9; **4g**, 80617-74-9; **4h**, 62372-04-7; **6**, 55967-94-7; **7a**, 115462-36-7; **7a**·HCl, 115462-72-1; **7b**, 88624-92-4; **7c**, 115462-37-8; **7c**·HCl, 115462-73-2; **7d**, 115462-38-9; **7e**, 115462-39-0; **7f**, 115462-40-3; **7g**, 115462-41-4; *erythro*-**8a**, 115462-58-3; *threo*-**8a**, 115462-59-4; *erythro*-**8b**, 115462-60-7; *threo*-**8b**, 115462-61-8; *erythro*-**8c**, 115462-62-9; *threo*-**8c**, 115462-63-0; *erythro*-**8d**, 115462-64-1; *threo*-**8d**, 115462-65-2; *erythro*-**8e**, 115462-66-3; *threo*-**8e**, 115462-67-4; *erythro*-**8f**, 115462-68-5; *threo*-**8f**, 115462-69-6; *erythro*-**8g**, 115462-70-9; *threo*-**8g**, 115462-71-0; *erythro*-**9**, 115462-56-1; *threo*-**9**, 115462-57-2; C_6H_5OH, 108-95-2; H_3C-*m*-C_6H_4OH, 108-39-4; H_3C-*p*-C_6H_4OH, 106-44-5; $C_6H_5CH_2OH$, 100-51-6; $C_6H_5(CH_2)_3OH$, 122-97-4; 1-naphthol, 90-15-3; 2-naphthol, 135-19-3.

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A Pyrimidine-Based "Flexible" Bisubstrate Analogue Inhibitor of Human Thymidylate Synthase

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The synthesis and characterization of two "flexible" bisubstrate analogues of the intermediate in the thymidylate synthase reaction are reported. Steric constraints are minimized and diasteromeric mixtures avoided by using a pyrimidine-based analogue as the folate portion of the inhibitor while retaining all known important binding sites. A preliminary assessment of certain conformational parameters by NMR is presented. The compounds are shown to be potent competitive inhibitors with respect to dUMP or 5,10-CH_2-H_4PteGlu but gave mixed kinetics with respect to 5,10-CH_2-H_4PteGlu$_5$ for human thymidylate synthase.

Thymidylate synthase (EC 2.1.1.45) plays a unique role in cellular biochemistry as the sole de novo source for the production of 2′-deoxythymidylate (dTMP). The reaction utilizes 2′-deoxyuridylate (dUMP) and 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4PteGlu) as substrate and cofactor, respectively. A unique feature of the reaction in comparison with other one-carbon transfer reactions is that 5,10-CH_2-H_4PteGlu acts both as one-carbon donor and as reductant, leading to the formation of dTMP and dihydrofolate. Although some details of the nature of this mechanism remain fuzzy, enough information has been developed about the molecular mechanism that structure **1** can be drawn as representing a ternary complex among substrate, cofactor, and enzyme.[1-5]

Efforts from a number of laboratories over several years[6-8] culminated in the synthesis of the deaza bisubstrate analogue **2** in this laboratory.[9] Compound **2** proved to be a potent competitive inhibitor of thymidylate synthase, and NMR studies suggested that the compound was

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