Enantiomeric N-Substituted *N*-Normetazocines: A Comparative Study of Affinities at σ, PCP, and μ Opioid Receptors

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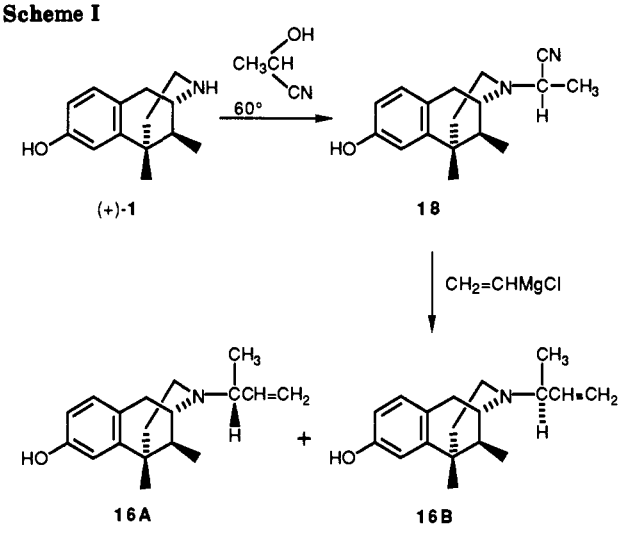
The optical antipodes of *N*-allyl-*N*-normetazocine (2; SKF 10047, NANM) were the original compounds used for the classification of the σ receptor as distinct from other receptors such as the PCP (NMDA), opioid, and dopamine receptors. Later studies showed that (+)-*N*-(dimethylallyl)-*N*-normetazocine [(+)-4, (+)-pentazocine] was more potent and selective for the σ receptor. In order to gain additional structure–activity relationship information, several N-substituted *N*-normetazocine analogs were prepared and evaluated for their σ-1 ([^{3}H]-(+)-3-PPP or [^{3}H]-(+)-pentazocine), PCP ([^{3}H]TCP), and μ opioid ([^{3}H]DAMGO) receptor binding affinities. (+)-*N*-Benzyl-*N*-normetazocine [(+)-10)] possessed subnanomolar affinities for the σ site, K_i = 0.67. The analog (+)-10 showed >14 000- and 2400-fold selectivity, respectively, for the σ receptor relative to the PCP and μ opioid receptors. The N-substituted *N*-normetazocines were enantioselective for the σ site. The (+)-*N*-benzyl analog, (+)-10, showed a 55-fold selectivity relative to (−)-10. Analysis of the data also revealed that (+)-normetazocine [(+)-1] [K_i = 30 nM] possessed the highest affinity for the PCP receptor. However, (+)-metazocine [(+)-5] (K_i = 41 nM) was the most selective compound for the PCP receptor relative to the σ (51-fold) and μ opioid (>200-fold) sites.

N-Alkyl-substituted analogs of *cis*-(±)-*N*-normetazocine (1) such as *N*-allyl-*N*-normetazocine [2, (±)-SKF 10,047, NANM], cyclazocine (3), and pentazocine (4) are reported to produce psychotomimetic effects in animals and in humans.[1-3] A σ (opiate) receptor subtype was postulated by Martin et al.[3] in 1976 to account for the psychotomimetic actions of (±)-SKF 10,047. Since the (−)-isomers of N-substituted *N*-normetazocines bind much stronger to μ and κ opiate receptors than the (+)-isomers, the behavioral effects of the compounds could be due to the interaction of the (−)-isomer at these opiate receptors.[4,5] However, the low affinity of (+)-SKF 10,047 for the μ and κ opiate receptors,[4-6] combined with the observation that most of the behavioral effect of (+)-SKF 10,047 cannot be antagonized by naloxone[7-11] suggested that other receptors may be responsible for the behavioral effects of (+)-SKF 10,047. Several studies have revealed three types of receptor interaction: (−)-SKF 10,047 binds primarily to μ and κ opiate receptors;[12] (+)-SKF 10,047 binds to the phencyclidine (PCP) site of the *N*-methyl-D-aspartate (NMDA) receptor complex[13] and to a non-opiate site that retains the designation σ receptor.[6,14-16] Little is known about the functions of this distinct σ binding site, but available data suggest it may underlie certain motor and perhaps antipsychotic effects of neuroleptics.[17,18] Furthermore, the existence of at least two putative subtypes (σ-1 and σ-2) of σ binding sites has further complicated the picture.[19,20] A paucity of selective ligands with high affinity for σ sites has stymied progress toward understanding their functions and potential role in novel therapies for psychiatric or neurological disease. Such compounds would also be useful for biochemical and neurophysiological investigations. Toward these goals, we present here the synthesis and receptor binding properties of several (+)- and (−)-N-substituted *N*-normetazocine analogs.

Results

Chemistry. (−)-(1*R*,5*R*,9*R*)- and (+)-(1*S*,5*S*,9*S*)-*N*-normetazocine, (−)-1 and (+)-1, respectively, were the

Scheme I



starting materials for the preparation of the N-substituted *N*-normetazocine analogs listed in Table I.[21] The (+)- and

(1) Haertzen, C. A. Subjective Effects of Narcotic Antagonists Cyclazocine and Nalorphine on the Addiction Research Center Inventory (ARCI). *Psychophamacologia (Berlin)* **1970**, *18*, 366–377.
(2) Keats, A. S.; Telford, J. In *Molecular Modification in Drug Design*; Gould, R. F., Ed.; Advances in Chemistry Series 45; American Chemical Society: Washington, DC, 1964; p 170.
(3) Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. The Effects of Morphine- and Nalorphine-like Drugs in the Nondependent and Morphine-dependent Chronic Spinal Dog. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 517–532.
(4) Martin, B. R.; Katzen, J. S.; Woods, J. A.; Tripathi, H. L.; Harris, L. S.; May, E. L. Stereoisomers of [^{3}H]-N-Allylnormetazocine Bind to Different Sites in Mouse Brain. *J. Pharmacol. Exp. Ther.* **1984**, *231*, 539–544.
(5) Tam, S. W. (+)-[^{3}H]SKF 10,047, (+)-[^{3}H]Ethylketocyclazocine, μ, κ, σ, and Phencyclidine Binding Sites in Guinea Pig Brain Membrane. *Eur. J. Pharmacol.* **1985**, *109*, 33–41.
(6) Tam, S. W.; Cook, L. σ Opiates and Certain Antipsychotic Drugs Mutually Inhibit (+)-[^{3}H]SKF 10,047 and [^{3}H]-Haloperidol Binding in Guinea Pig Brain Membranes. *Proc. Nat. Acad. Sci. U.S.A.* **1984**, *81*, 5618–5621.
(7) Katz, J. L.; Spealman, R. D.; Clark, R. D. Stereoselective Behavioral Effects of N-Allylnormetazocine in Pigeons and Squirrel Monkeys. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 452–461.

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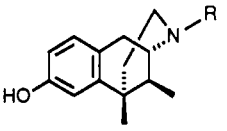
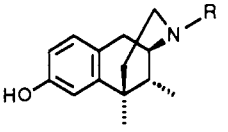
Table I. Physical Properties of New N-Substituted *N*-Normetazocine Analogs



(+)-cis



(−)-cis

compd	R	method (% yield)[a]	molecular formula[b]	mp, °C	$[\alpha]^{25}_D$, deg (*c*, EtOH)
(+)-**8**	$(CH_2)_2CH(CH_3)_2$	B (16)	$C_{19}H_{29}NO$	142.0–142.9	+119 (0.1)
(−)-**8**	$(CH_2)_2CH(CH_3)_2$	B (32)	$C_{19}H_{29}NO$	142.0–142.8	−119 (0.1)
(+)-**9**	$CH_2C{\equiv}CCH_3$	A (45)	$C_{18}H_{23}NO$	205–207	+131.5 (1.0)
(−)-**9**	$CH_2C{\equiv}CCH_3$	A (41)	$C_{18}H_{23}NO$	205–207	−131.7 (1.0)
(+)-**10**	$CH_2C_6H_5$	B (67)	$C_{21}H_{25}NO{\cdot}HCl$[c]	161.0–162.5	+79 (0.1)
(−)-**10**	$CH_2C_6H_5$	B (10)[d]	$C_{21}H_{25}NO{\cdot}HCl$[c]	161.5–162.8	−79 (0.1)
(+)-**11**	$(CH_2)_3C_6H_5$	B (44)	$C_{23}H_{29}NO{\cdot}HCl$[c]	131–133	+80 (0.1)
(−)-**11**	$(CH_2)_3C_6H_5$	B (17)	$C_{23}H_{29}NO{\cdot}HCl$[c]	129.3–130.5	−76.0 (0.1)
(+)-**12**	$(CH_2)_4C_6H_5$	B (20)	$C_{24}H_{31}NO$	130.6–130.9	+98 (0.1)
(−)-**12**	$(CH_2)_4C_6H_5$	B (26)	$C_{24}H_{31}NO$	129.8–130.4	−98 (0.1)
(+)-**13**	$(CH_2)_5C_6H_5$	B (21)	$C_{25}H_{33}NO$	124.0–124.4	+93 (0.1)
(−)-**13**	$(CH_2)_5C_6H_5$	B (30)	$C_{25}H_{33}NO$	123.9–124.3	−93 (0.1)
(+)-**14**	(*trans*)-$CH_2CH{=}CHCH_3$	A (48)	$C_{18}H_{25}NO$	153–154	+120.4 (1.0)
(−)-**14**	(*trans*)-$CH_2CH{=}CHCH_3$	A (47)	$C_{18}H_{25}NO$	155–157	−124.0 (1.0)
(+)-**15**	(*cis*)-$CH_2CH{=}CHCH_3$	C (53)	$C_{18}H_{25}NO$	140–141	+122.2 (1.0)
(−)-**15**	(*cis*)-$CH_2CH{=}CHCH_3$	C (75)	$C_{18}H_{25}NO$	138–140	−121.2 (1.0)
(+)-**16A**	$CH(CH_3)CH{=}CH_2$	D (e)	$C_{16}H_{25}NO$	70.2–72.2	+102 (0.34)
(+)-**16B**	$CH(CH_3)CH{=}CH_2$	D (e)	$C_{16}H_{25}NO$	72.1–73.5	+145.2 (1.0)
(−)-**17A**	$CH(CH_3)CH{=}CH_2$	D (f)	$C_{16}H_{25}NO$	70.4–72.4	−102 (1.0)
(−)-**17B**	$CH(CH_3)CH{=}CH_2$	D (f)	$C_{16}H_{25}NO$	69.4–70.9	−146 (1.0)

[a] A general preparation for each type of synthesis is given in the Experimental Section. The yields were not optimized. [b] All compounds were analyzed for C, H, N; the results agreed to within ±0.4% of the theoretical values. [c] These salts were hydrated with $0.5H_2O$. [d] Sodium carbonate was used in place of sodium hydrogen carbonate. [e] A 57% yield of a mixture of **16A** and **16B** was obtained. [f] A 70% yield of a mixture of **17A** and **17B** was obtained.

(−)-isomers of *N*-allyl-*N*-normetazocine (**2**), pentazocine (**4**), metazocine (**5**), cyclazocine (**3**), phenazocine (**6**), and *N*-(3-chloropropyl)-*N*-normetazocine (**7**) were prepared as previously reported.[21,22] Alkylation of (+)- and (−)-*N*-normetazocine with the appropriate alkylating agent in DMF or EtOH using potassium hydrogen carbonate as the proton acceptor gave the analogs (+)- and (−)-**8–14** shown in Table I. Subjection of (+)- and (−)-**9** to catalytic reduction using a poisoned catalyst gave the cis analogs (+)- and (−)-**15**. The synthesis of analogs **16A** and **16B** is shown in Scheme I. When (+)-**1** was heated with lactonitrile, the intermediate **18** was obtained. Treatment of **18** with vinylmagnesium chloride gave **16A** and **16B**, which were separated. Subjection of (−)-**1** to the same sequence as shown in Scheme I provided **17A** and **17B**.

Receptor Binding. Table II lists the σ, PCP, and μ opioid binding data for (+)- and (−)-*N*-normetazocine and several (+)- and (−)-*N*-substituted *N*-normetazocine analogs. Examination of the data in Table II reveals the following relationships. The (+)-isomer of all the compounds were more potent than the corresponding (−)-isomer for the σ site labeled by [^{3}H]-(+)-pentazocine or [^{3}H]-(+)-1-propyl-3-(3-hydroxyphenylpiperidine ([^{3}H]-

(8) Vaupel, D. B. Naltrexone Fails to Antagonize the Sigma Effect of PCP and SKF 10,047 in the Dog. *Eur. J. Pharmacol.* **1983**, *92*, 269–274.

(9) Brady, K. L.; Balster, R. L.; May, E. L. Stereoisomers of N-Allylnormetazocine: Phencyclidine-Like Behavioral Effects in Squirrel Monkeys and Rats. *Science* **1982**, *215*, 178–180.

(10) Iwamoto, E. T. Locomotor Activity and Antinociception after Putative Mu, Kappa and Sigma Opioid Receptor Agonists in the Rat: Influence of Dopaminergic Agonists and Antagonists. *J. Pharmacol. Exp. Ther.* **1981**, *217*, 451–460.

(11) Cowan, A. Simple in Vivo Tests that Differentiate Prototype Agonists at Opiate Receptors. *Life Sci.* **1981**, *28*, 1559–1568.

(12) Zukin, S. R. Differing Stereospecificities Distinguish Opiate Receptor Subtypes. *Life Sci.* **1982**, *31*, 1307–1310.

(13) Zukin, S. R.; Tempel, A.; Gardner, E. L.; Zukin, R. S. Interation of [^{3}H](−)-SKF-10,047 with Brain σ Receptors: Characterization and Autoradiographic Visualization. *J. Neurosci.* **1986**, *46*, 1032–1041.

(14) Quirion, R.; Chicheportiche, R.; Contreras, P. C.; Johnson, K. M.; Lodge, D.; Tam, S. W.; Woods, J. H.; Zukin, S. R. Classification and Nomenclature of Phencyclidine and Sigma Receptor Sites. *Trends Neurosci.* **1987**, *10*, 444–446.

(15) Su. T.-P. Evidence for Sigma Opioid Receptor: Binding of [^{3}H]SKF-10047 to Etorphine-inaccessible Sites in Guinea-pig Brain. *J. Pharmacol. Exp. Ther.* **1982**, *223*, 284–290.

(16) Tam, S. W. Naloxone-inaccessible Sigma Receptor in Rat Central Nervous System. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6703–6707.

(17) Ferris, C. D.; Hirsch, D. J.; Brooks, B. P.; Snyder, S. H. Sigma Receptors: from Molecule to Man. *J. Neurochem.* **1991**, *57*, 729–737.

(18) Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; de Costa, B.; Rice, K. C. Sigma Receptors: Biology and Function. *Pharmacol. Rev.* **1990**, *42*, 355–402, for a review on sigma receptors.

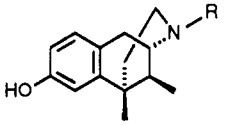
(19) Hellewell, S. B.; Bowen, W. D. A Sigma-like Binding Site in Rat Pheochromocytoma (PC12) Cells: Decreased Affinity for (+)-Benzomorphans and Lower Molecular Weight Suggest a Different Sigma Receptor Form from that in Guinea Pig Brain. *Brain Res.* **1990**, *527*, 244–253.

(20) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T.-P.; Tam, S. W.; Taylor, D. P. Classification of Sigma Binding Sites: A Proposal. *Trends Pharmacol. Sci.* **1992**, *13*, 85–86.

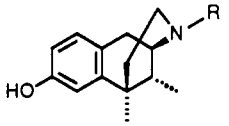
(21) Brine, G. A.; Berrang, B.; Hayes, J. P.; Carroll, F. I. An Improved Resolution of (±)-cis-N-Normetazocine. *J. Heterocycl. Chem.* **1990**, *27*, 2139–2143.

(22) Aceto, M. D.; May, E. L.; Harris, L. S.; Jacobson, A. E.; Awaya, H. Agonist-Antagonist Actions and Stereoselectivity of Optical Pairs of Some N-Substituted α-N-Normetazocines. *J. Pharm. Sci.* **1984**, *73*, 1867–1868.

Table II. Receptor Binding Data for (+)- and (−)-Substituted *N*-Normetazocine Analogs



(+)-cis



(−)-cis

compd	R	σ:[a-c] [^{3}H]-(+)-pentazocine[d] or [^{3}H]-(+)-3-PPP[e] K_i (nM)	PCP:[b] [^{3}H]TCP[f] K_i (nM)	opioid (μ):[b] [^{3}H]DAMGO[g] K_i(nM)	PCP/σ ratio
(+)-1	H	3380 ± 503[d]	30 ± 3.0	470 ± 110	0.009
(−)-1	H	5580 ± 387[d]	197 ± 16	23 ± 6	0.035
(+)-2	$CH_2CH{=}CH_2$	59.7 ± 2.5[d]	225 ± 11	>10000	3.77
(−)-2	$CH_2CH{=}CH_2$	>3000[d]	504 ± 22	3.6 ± 0.05	<0.17
(+)-3	CH_2-c-C_3H_5	17.2 ± 0.03[d]	153 ± 22	1240 ± 80	8.90
(−)-3	CH_2-c-C_3H_5	2650 ± 385[d]	107 ± 34	0.18 ± 0.02	0.04
(+)-4	$CH_2CH{=}C(CH_3)_2$	3.1 ± 0.3[d]	4040 ± 616	1700 ± 166	1303
(−)-4	$CH_2CH{=}C(CH_3)_2$	83.1 ± 6.2[d]	842 ± 124	13 ± 3	10.1
(+)-5	CH_3	2100 ± 269[d]	41 ± 11	4970 ± 906	0.020
(−)-5	CH_3	>6000[d]	1190 ± 230	11.0 ± 2.3	<0.20
(+)-6	$(CH_2)_2C_6H_5$	3.90 ± 1.26[d]	>10000	55 ± 8	>2564
(−)-6	$(CH_2)_2C_6H_5$	61.7 ± 10.9[d]	1880 ± 176	0.2 ± 0.04	30.5
(+)-7	(cis)-$CH_2CH{=}CHCl$	10.5 ± 1.4[d]	250 ± 21	1980 ± 100	24
(−)-7	(cis)-$CH_2CH{=}CHCl$	403 ± 30[d]	470 ± 40	1.5 ± 0.03	1.2
(+)-8	$(CH_2)_2CH(CH_3)_2$	2.25 ± 0.23[d]	>10000	3500 ± 1000	>4444
(−)-8	$(CH_2)_2CH(CH_3)_2$	8.74 ± 0.72[d]	2740 ± 196	4.9 ± 0.1	313
(+)-9	$CH_2C{\equiv}C$-CH_3	93.7 ± 19[e]	1230 ± 110	1000 ± 129	13
(−)-9	$CH_2C{\equiv}C$-CH_3	10600 ± 2940[e]	360 ± 68	5.1 ± 0.2	0.034
(+)-10	$CH_2C_6H_5$	0.67 ± 0.10[d]	>10000	1610 ± 505	>14925
(−)-10	$CH_2C_6H_5$	36.5 ± 6.3[d]	6500 ± 800	97 ± 17	178
(+)-11	$(CH_2)_3C_6H_5$	1.15 ± 0.02[d]	9700 ± 800	343 ± 74	8435
(−)-11	$(CH_2)_3C_6H_5$	38.6 ± 4.4[d]	3600 ± 600	20 ± 3.5	93.3
(+)-12	$(CH_2)_4C_6H_5$	3.23 ± 0.69[d]	>10000	270 ± 30	3096
(−)-12	$(CH_2)_4C_6H_5$	85.2 ± 19.0[d]	5960 ± 526	4.0 + 0.5	70.0
(+)-13	$(CH_2)_5C_6H_5$	2.64 ± 0.35[d]	>10000	260 ± 40	>3788
(−)-13	$(CH_2)_5C_6H_5$	38.5 ± 0.25[d]	>10000	68 ± 6	260
(+)-14	(*trans*)-$CH_2CH{=}CHCH_3$	14.5 ± 4.7[e]	381 ± 138	1690 ± 132	26
(−)-14	(*trans*)-$CH_2CH{=}CHCH_3$	460 ± 4.4[e]	1120 ± 387	11 ± 1.6	2.4
(+)-15	(*cis*)-$CH_2CH{=}CHCH_3$	52.5 ± 5[e]	179 ± 52	5620 ± 1170	3.4
(−)-15	(*cis*)-$CH_2CH{=}CHCH_3$	1130 ± 25[e]	347 ± 94	9.0 ± 1.5	0.31
(+)-16A	$CH(CH_3)CH{=}CH_2$	1340 ± 167[e]	200 ± 20	690 ± 35	0.149
(+)-16B	$CH(CH_3)CH{=}CH_2$	159 ± 7.3[e]	90 ± 6	2270 ± 60	0.566
(−)-17A	$CH(CH_3)CH{=}CH_2$	1840 ± 496[e]	1900 ± 60	5.9 ± 1.8	1.03
(−)-17B	$CH(CH_3)CH{=}CH_2$	3698 ± 78[e]	2200 ± 200	3.0 ± 0.5	0.596

[a] Unlabeled test ligand (0.005–1000 nM or 0.05–10000 nM) was incubated with 3 nM [^{3}H]-(+)-pentazocine or 3 nM [^{3}H]-(+)-3-PPP as described in Methods. IC_{50} values were determined using the iterative curve-fitting program CDATA (EMF Software, Baltimore, MD). All curves were best fit to a one-site model. [b] K_i values were calculated using the Cheng–Prusoff equation (see ref 34). A [^{3}H]-(+)-pentazocine K_d of 4.8 nM (see ref 30, 31), and a [^{3}H]-(+)-3-PPP K_d of 27.4 nM as determined previously (see ref 19) for σ, a [^{3}H]TCP K_d of 7.7 ± 0.4 nM for PCP, and [^{3}H]DAMGO K_d of 1.9 ± 0.36 nM for μ opiate was used. Values are averages of two to three experiments ± SEM, each carried out in duplicate. >3000 and >6000 = less than 30% inhibition at concentrations of 5000 nM and 10000 nM, respectively. [c] For reference, the prototypic σ ligands haloperidol, DTG, and (+)-3-PPP exhibited K_i vs [^{3}H]-(+)-pentazocine of 3.7 ± 0.6 nM, 27.7 ± 4.3 nM, and 29.5 ± 0.9 nM, respectively. Nearly identical values were obtained vs [^{3}H]-(+)-3-PPP. [d] [^{3}H]-(+)-Pentazocine. [e] [^{3}H]-(+)-1-Propyl-3-(3-hydroxyphenyl)piperidine. [f] [^{3}H]-1-[(2-Thienyl)cyclohexyl]piperidine. [g] [^{3}H]Tyr-D-Ala-Gly-NMe-Phe-Gly-ol.

(+)-3-PPP). (+)-*N*-Benzyl-*N*-normetazocine [(+)-10] with a K_i = 0.67 nM is the most potent compound. This compound also possesses the greatest selectivity for the σ site (as evidenced by the >14 000 PCP/σ ratio and K_i (1610 nM) for the μ opioid receptor. The phenylpropyl analog (+)-11 is also more potent and more selective for the σ site relative to the PCP than (+)-pentazocine [(+)-4], which was the most potent and selective *N*-normetazocine analogs reported before this study. The (+)-*trans*-butenyl analog, (+)-14, is ca. 3 times more potent than the (+)-*cis*-butenyl analog, (+)-15. Only one of the 1-methylallyl isomers of 16 and 17 showed appreciable affinity for the σ receptor.

(+)-*N*-Normetazocine [(+)-1] with a K_i of 30 nM for the [^{3}H]-1-[(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP) labeled site has the highest affinity for the PCP receptor. It is also highly selective for the PCP site relative to the σ site (PCP/σ = 0.009). However, (+)-metazocine [(+)-5], which is only slightly less potent, K_i = 41 nM, has the highest selectivity for the PCP site when both σ and μ opioid sites are considered. It is interesting to note that none of the other analogs which possess larger N-substituents showed appreciable affinity for the PCP site. In addition, binding to the PCP site did not follow a consistent pattern of enantioselectivity. Thus, in contrast to NANM (2), metazocine (5), 14, and 15 where the (+)-isomer is more potent than the (−)-isomer, the (−)-isomer of cyclazocine (3), pentazocine (4), and 9 is more potent than the corresponding (+)-isomer.

The (−)-isomers were much more potent than the (+)-isomers for the μ opioid site labeled by [^{3}H]Tyr-D-Ala-Gly-NMe-Phe-Gly-ol ([^{3}H]DAMGO). (−)-Phenazocine [(−)-6] and (−)-cyclazocine [(−)-3] had the highest affinity for the μ opioid receptor. The 2-chloroallyl analog (−)-7 was the next most potent analog. It is interesting to note that (+)-phenazocine [(+)-6] exhibited affinity (K_i = 55 nM) that may have some pharmacological relevance.

Discussion

σ binding sites have been shown to be different from phencyclidine and dopamine D_2 receptors.[18] The site is distinguished from opioid sites by being insensitive to opioid antagonists and has also been defined by the hal-

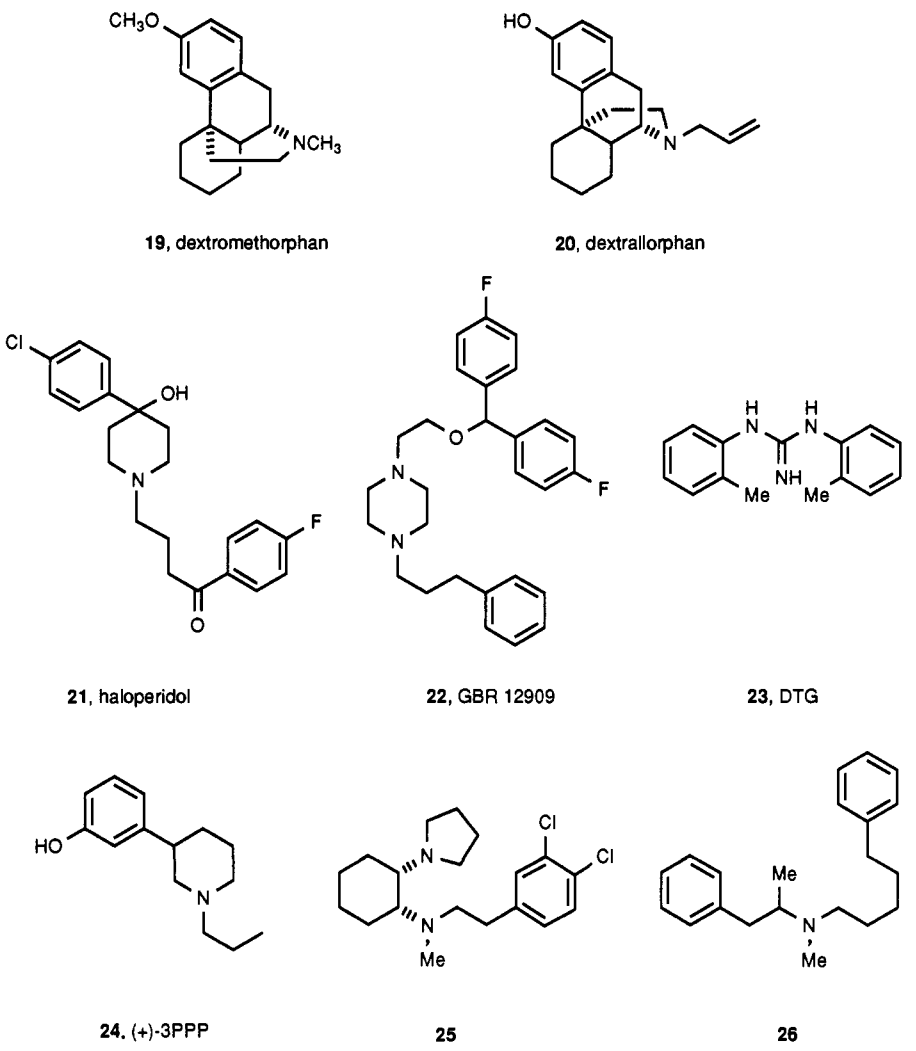


Figure 1. Selected σ binding ligands.

operidol-sensitive binding of [^{3}H]-(+)-*N*-allyl-*N*-normetazocine [(+)-**2**]. Various biochemical studies have shown that there are at least two classes of σ binding sites.[18-20] A large number of compounds possessing a variety of structural features are reported to bind to one or both the σ site(s) with moderate (IC_{50} ~100 nM) to high affinity (IC_{50} <10 nM). Among the classes of drugs that bind to the σ site(s) (Figure 1) are opiates [dextromethorphan (**19**), dextrallorphan (**20**)], antipsychotics [haloperidol (**21**)], and dopamine uptake inhibitors [GBR 12909 (**22**)].[18] Some of the other compound classes that bind to the σ site(s) include guanidines [DTG (**23**)], 3-phenylpiperidines [(+)-3-PPP (**24**)],[18] cyclohexyldiamines [*cis*-*N*-methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1′-pyrrolidinyl)cyclohexylamine (**25**)],[18,23,24] and amphetamines [*N*-methyl-*N*-(1-methyl-2-phenylethyl)-5-phenyl-1-aminopentane (**26**)].[25,26]

Even though (+)-*N*-allyl-*N*-normetazocine [(+)-**2**] was used to initially show that the PCP and σ sites were different, there has been no systematic structure–activity relationship (SAR) study on the *N*-normetazocine class of compounds to determine if more potent and/or selective compounds could be prepared. This is even more surprising since reports had shown that (+)-cyclazocine [(+)-**3**] and (+)-pentazocine [(+)-**4**] were more potent than (+)-*N*-allyl-*N*-normetazocine [(+)-**2**] at σ sites.[18] (+)-Pentazocine was also much more selective for the σ site relative to the PCP site.[18] The above information, combined with the fact that *N*-normetazocine is a relatively rigid structure which would lend itself to computer-aided molecular modeling studies, led us to undertake a study to determine the structural features of N-substituted *N*-normetazocines requires for potent and selective binding to the σ sites. The present study provides new information concerning the effect of the N-substituent as well as the stereochemistry of the *N*-normetazocine on σ binding. Receptor selectivity was determined by also determining the affinity of the new analogs for the PCP and μ opioid receptors.

We found that the binding potency and selectivity of N-substituted *N*-normetazocine to the σ and the PCP receptor sites are sensitive to changes in the N-substituent and to the stereochemistry of the benzomorphan. For example, only (+)-*N*-normetazocine and the analog with the smallest N-substituent, (+)-metazocine, showed reasonable affinity for the PCP receptor. In contrast, analogs with larger, more lipophilic N-substituents were more

(23) de Costa, B. R.; Rice, K. C.; Bowen, W. D.; Thurkauf, A.; Rothman, R. B.; Band, L.; Jacobson, A. E.; Radesca, L.; Contreras, P. C.; Gray, N. M.; Daly, I.; Iyengar, S.; Finn, D. T.; Vazirani, S.; Walker, J. M. Synthesis and Evaluation of N-Substituted cis-N-Methyl-2-(1-pyrrolidinyl)cyclohexylamines as High Affinity σ Receptor Ligands. Identification of a New Class of Highly Potent and Selective σ Receptor Probes. *J. Med. Chem.* **1990**, *33*, 3100–3110.

(24) Radesca, L.; Bowen, W. D.; Di Paolo, L.; de Costa, B. R. Synthesis and Receptor Binding of Enantiomeric N-Substituted *cis*-N-[2-(3,4-Dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamines as High Affinity Sigma Receptor Ligands. *J. Med. Chem.* **1991**, *34*, 3058–3065.

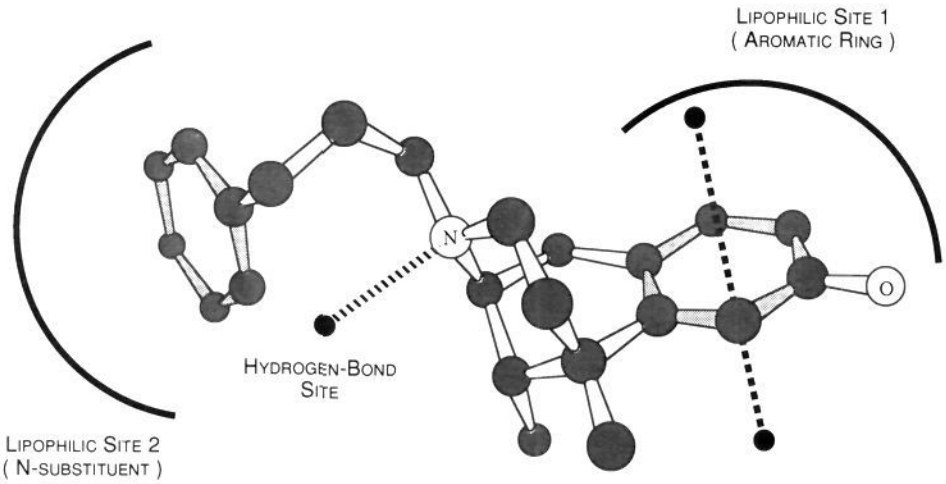


Figure 2. Hypothetical features of the PCP and σ receptors.

potent for the σ site. However, the affinity for the σ and PCP receptor was dependent upon the exact structure of the N-substituted analog. The *N*-benzyl and *N*-phenylpropyl analogs (+)-**10** and (+)-**11** were both more potent than the (+)-pentazocine [(+)-**4**], the most potent previously reported benzomorphan analog. The *N*-benzyl analog (+)-**10** is also more selective for the σ receptor than (+)-**4**. Glennon et al.[25] have also found that the incorporation of an *N*-phenylalkyl group into the amphetamine structure (see structure **26**) led to compounds with high affinity for the σ site. However, in contrast to (+)-**10**, (+)-**11**, (+)-**12**, and (+)-**13**, which showed 15–55-fold enantioselectivity, the *N*-(phenylalkyl)amphetamine analogs showed less than twofold enantioselectivity.[25]

It is interesting to note that the analogs (+)-**14** and (+)-**15**, which differ from (+)-pentazocine [(+)-**4**] by the absence of one methyl group, are both less potent than (+)-**4** with the trans isomer (+)-**14** being more potent than the cis isomer (+)-**15**. Reduction of the double bond in (+)-pentazocine to give the *N*-alkyl analog (+)-**8** resulted in a 2-fold reduction in affinity for the σ site.

The binding of benzomorphans to the PCP receptor can be understood in the context of a previously published model of the PCP pharmacophore.[27,28] This pharmacophore model (Figure 2) consists of the three points defined by (1) the two endpoints of a line extending 3.5 Å above and below the phenyl ring and passing through the centroid of and normal to the plane of the ring and (2) a hydrogen-bonding site defined by the endpoint of a 2.8-Å line directed along the nitrogen lone-pair vector. The structural requirements for PCP-receptor binding specified by this pharmacophore model are both geometric (alignment to the lipophilic site and hydrogen-bonding site) and steric (no part of the ligand should intrude into the vicinity of the hydrogen-bonding site). The finding that only compounds (+)-**1** and (+)-**5** where the N-substituent is a proton or methyl group show good affinity for the PCP receptors (K_i = 30 and 41 nM, respectively) is consistent with this model. The lower affinity of all the other analogs can be explained by the fact that these compounds exhibit a close approach (on average) of a side-chain atom and the model hydrogen-bond site. Moreover, the overall side-chain conformational behavior of these compounds is remarkably similar. This finding may indicate that a highly directional or specific hydrogen-bonding interaction may be necessary for the binding of benzomorphans to the PCP receptor.

A model of σ-site pharmacophore can be constructed by adding a second lipophilic site to the three-point PCP pharmacophore (lipophilic site 2, Figure 2). This subsite would accommodate benzomorphan *N*-alkyl side chains and account for the observed structure–activity relationship of these substituents. (With the N-substituent in the equatorial conformation, benzomorphans satisfy the geometric requirements of the PCP pharmacophore.) Both of these PCP and σ pharmacophore models are different from those proposed by other workers[29] in that the location of the nitrogen atom itself is not constrained to any single location by either model. The only requirement is that the nitrogen lone pair point in the direction of the hydrogen-bonding site and that the nitrogen remain within appropriate hydrogen-bonding range.

There is evidence from several laboratories supporting heterogeneity of σ sites.[18,20] We have proposed the existence of σ-1 and σ-2 sites.[19] Using enantiomers of pentazocine and SKF 10,047, we showed that a salient feature differentiating these two sites is affinity for the (+)-enantiomers: σ-2 sites exhibited markedly reduced affinity compared to σ-1 sites. Interestingly, the (−)-enantiomers failed to differentiate the two sites, resulting in reversed enantioselectivity [(−) > (+)] at σ-2. While [^{3}H]-(+)-pentazocine is highly selective for sites with the σ-1 profile,[30,31] [^{3}H](+)-3-PPP is only moderately selective for σ-1

(25) Glennon, R. A.; Smith, J. D.; Ismaiel, A. M.; El-Ashmawy, M.; Battaglia, G.; Fischer, J. B. Identification and Exploitation of the σ-Opiate Pharmacophore. *J. Med. Chem.* **1991**, *34*, 1094–1098.

(26) Glennon, R. A.; Ismaiel, A. M.; Smith, J. D.; Yousif, M.; El-Ashmawy, M.; Herndon, J. L.; Fischer, J. B.; Burke-Howie, K. J.; Server, A. C. Binding of Subsituted and Conformationally Restricted Derivatives of N-(3-Phenyl-n-propyl)-1-phenyl-2-aminopropane at σ-Receptors. *J. Med. Chem.* **1991**, *34*, 1855–1859.

(27) Carroll, F. I.; Brine, G. A.; Mascarella, S. W. Phencyclidine (PCP) and Phencyclidine-like Compounds: A Molecular Graphics-molecular Mechanics Study. *NIDA Res. Monogr.* **1988**, *90*, 122–128.

(28) Thurkauf, A.; Zenk, P. C.; Balster, R. L.; May, E. L.; George, C.; Carroll, F. I.; Mascarella, S. W.; Rice, K. C.; Jacobson, A. E.; Mattson, M. V. Synthesis, Absolute Configuration, and *Molecular Modeling Study of etoxadrol, A potent Phencyclidine-like Agonist.* *J. Med. Chem.* **1988**, *31*, 2257–2263.

(29) Manallack, D. T.; Wong, M. G.; Costa, M.; Andrews, P. R.; Beart, P. M. Receptor Site Topographies for Phencyclidine-like and σ Drugs: Predictions from Quantitative Conformational, Electrostatic Potential, and Radioreceptor Analyses. *Mol. Pharmacol.* **1988**, *34*, 863–879.

over σ-2 sites.[19] Since guinea pig brain (minus cerebellum) contains predominantly σ-1 sites,[19] the values reported here determined with either radioligand represent σ-1 affinities. It should be noted that some (but not all) of the (+)-enantiomers exhibited biphasic curves when [^{3}H]-(+)-3-PPP was used as radioligand, displacing 70–80% of the binding from high-affinity sites and 20–30% of binding from low-affinity sites (not shown). These high- and low-affinity sites presumably represent the σ-1 and σ-2 labeling by [^{3}H]-(+)-3-PPP, respectively. When these same test ligands were assayed vs [^{3}H]-(+)-pentazocine, the K_i determined with [^{3}H]-(+)-pentazocine was nearly identical to that of the high-affinity site from which [^{3}H]-(+)-3-PPP was displaced, supporting the notion that the high-affinity site is σ-1. The σ-2 binding properties of these enantiomeric N-substituted normetazocine analogs is currently being investigated, and preliminary results have thus far supported the proposed subtype classification scheme.[32] Further studies with these compounds should allow a more detailed comparison of the putative σ-1 and σ-2 binding sites.

Conclusions

As expected, the opiate receptors show enantioselectivity for the (1*R*,5*R*,9*R*)-(−)-isomers of the N-substituted *N*-normetazocines, whereas the σ sites (σ-1) are more enantioselective for the (1*S*,5*S*,9*S*)-(+)-isomers. Analogs with larger substituents showed the highest affinity for the σ site with the (+)-*N*-benzyl-*N*-normetazocine [(+)-**10**] being the most potent and selective analog. The PCP receptor is not enantiospecific but does show some degree of enantioselectivity. (+)-*N*-Normetazocine [(+)-**1**] showed the highest affinity and selectivity for the PCP site relative to the σ receptor. (+)-Metazocine [(+)-**5**], which was slightly less potent than (+)-**1** at PCP receptors and slightly more potent than (+)-**1** at σ receptors, showed high selectivity for the PCP site relative to both the σ and μ opioid receptor. Molecular modeling studies have provided some new information concerning the PCP and σ pharmacophore.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1-dm cell). NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. Visualization was accomplished under UV or in an iodine chamber. Microanalyses were carried out by Atlantic Microlab, Inc.

General Procedures for the Synthesis for (+) and (−)-N-Substituted *N*-Normetazocine Analogs. Method A. A stirred mixture of the appropriate *N*-normetazocine isomer (15 mmol), the alkyl chloride (20 mmol), and sodium bicarbonate (23 mmol) in 50 mL of dry DMF was heated at 60 °C under argon atmosphere for 24 h. The insoluble material was separated by filtration and washed with EtOH. The filtrate was evaporated and the residue taken up in Et_2O. Any insoluble material was separated by filtration. The Et_2O filtrate was extracted with 3 N HCl (25 mL). Dilute NH_4OH was added dropwise to precipitate the product. The solid was collected, washed with H_2O, dried under vacuum, and then crystallized from MeOH to give a white solid.

Method B. A stirred mixture of 300 mg (1.4 mmol) of the appropriate *N*-normetazocine (**1**), the alkyl halide or tosylate (1.54 mmol), and 360 mg (4.3 mmol) of sodium bicarbonate in 15 mL of EtOH was refluxed for 5 h. The mixture was concentrated and the residue was extracted with Et_2O. The etheral solution was concentrated under vacuum to give the crude product which was chromatographed on silica gel (hexane–EtAc–MeOH, 8:1:1) to give a white solid. Recrystallization from ethanol–water gave a colorless product.

In some cases the hydrochloride salts were formed by adding a 1 N hydrogen chloride in Et_2O solution to an etheral solution of the N-substituted *N*-normetazocine analog. The precipitated product was thoroughly washed with Et_2O.

Method C. To a suspension of 150 mg of 5% Pd/$BaSO_4$ in 150 mL of MeOH was added four drops of quinoline. The appropriate (+)- or (−)-isomer of compound **9** (5 mmol) was added to the suspension, and the mixture was stirred under a hydrogen atmosphere for 0.5 h. The solid was separated by filtration, and the filtrate was concentrated under vacuum. The residue was applied to a silica gel column and eluted with CH_2Cl_2–$CHCl_3$–MeOH–concentrated NH_4OH (50:40:9:1). The product from the column was crystallized from MeOH–H_2O to give a white solid.

Method D. A mixture of (+)- or (−)-*N*-normetazocine (2.0 g, 0.009 mol), lactonitrile (1.5 g, 0.02 mol), and 3 mL of toluene were placed in a flask and heated at 60 °C for 2 h. The solvent was evaporated to leave **18** as a crude yellow oil. This material was dissolved in dry THF (30 mL) and added dropwise to an excess of vinylmagnesium bromide (30 mL of 1 M solution in THF) under argon at room temperature. After 0.5 h, the reaction was quenched with H_2O and extracted with Et_2O. The organic extract was dried (Na_2SO_4), filtered, and evaporated to give a product as a pale yellow semisolid foam. Flash chromatography on a silica gel (Merck 60) column and eluted with CH_2Cl_2–$CHCl_3$–MeOH–concentrated NH_4OH (50:40:9:1) gave a pure mixture of two isomers as semisolid foam.

Pure (+)-**16B** and (+)-**16A** were prepared by converting small portions of the mixture to their di-*p*-toluoyl-L-tartrate and di-*p*-toluoyl-D-tartrate salts, respectively. Thus, to a 358-mg (1.32-mmol) mixture of **16A** and **16B** in 2.6 mL of methanol was added 459 mg (1.19 mmol) of di-*p*-toluoyl-L-tartaric acid followed by 0.26 mL of water. The resulting crystals were recrystallized two times from a 4:1 methanol–water mixture to give 160 mg (18%) of **16B** di-*p*-toluoyl-L-tartrate: mp 168–170°C. Anal. ($C_{38}H_{43}NO_9$) C, H, N.

The above crystal (140 mg) and 10% sodium carbonate aqueous solution (38 mL) in chloroform (55 mL) were warmed and stirred for 20 min. The organic layer was separated, washed with water, and dried (Na_2SO_4). The solid obtained after removal of solvent was chromatographed on silica gel 60 using $CHCl_3$–CH_3OH–NH_4OH (90:9:1) as eluent. Removal of the solvent from the product fractions gave 32 mg (8.9%) of **16B**: ^{1}H NMR ($CDCl_3$) δ 0.81 (d, 3, CH_3), 1.18 (d, 3, CH_3), 1.30 (s, 3, CH_3), 5.02–5.21 (dd, 2, =CH_2), 5.74–5.88 (q, 1, CH=).

Another sample (72 mg, 0.26 mmol) of the **16A** and **16B** mixture was dissolved in 0.6 mL of methanol and 0.1 mL of water, and 116 mg of di-*p*-toluoyl-D-tartrate was added. The crystals obtained were recrystallized from a 4:1 methanol–water mixture to give 15.6 mg (22%) **16A** di-*p*-toluoyl-D-tartrate. Anal. ($C_{38}H_{43}NO_9$) C, H, N.

The salt was converted to **16A** as described for the **16B** salt to give 6.8 mg (9.4%) of **16A**: ^{1}H NMR ($CDCl_3$) δ 0.81 (d, 3, CH_3), 1.18 (d, 3, CH_3), 1.30 (s, 3, CH_3), 5.02–5.21 (q, 2, =CH_2), 5.74–5.88 (p, 1, =CH).

(30) de Costa, B. R.; Bowen, W. D.; Hellewell, S. B.; Walker, J. M.; Thurkauf, A.; Jacobson, A. E.; Rice, K. C. Synthesis and Evaluation of Optically Pure [^{3}H](+)-Pentazocine, a Highly Potent and Selective Radioligand for Sigma Receptors. *FEBS Lett.* **1989**, *251*, 53–58.

(31) Bowen, W. D.; de Costa, B.; Hellewell, S.; Thurkauf, A.; Walker, J. M.; Rice, K. C. Characterization of [^{3}H](+)-Pentazocine, a Highly Selective Sigma Ligand. In *Progress in Clinical and Biological Research, vol. 328: The International Narcotics Research Conference (INRC) '89*; Quirion, R., Jhamandas, K., Gianoulakis, C., Eds.; Alan R. Liss, Inc.: New York, 1990; pp 117–120.

(32) Di Paolo, L.; Carroll, F. I.; Abraham, P.; Bai, X.; Parham, K.; Mascarella, S. W.; Zhang, X.; Wallace, P.; Walker, J. M.; Bowen, W. D. N-Substituted Derivatives of Normetazocine: Differentiation of Sigma-1 and Sigma-2 Receptors. *Soc. Neurosci. Abstr.* **1991**, *17*, 814, #322.15.

(33) Lowry, O.H.; Roseebrough, J. M.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

(34) Cheng, Y.-C.; Prusoff, W. H. Relationship between the Inhibition Constant (K_i) and the Concentration of Inhibitor which Causes 50 Percent Inhibition (I50) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

Pure samples of 17A and 17B were prepared by a procedure analogous to that described for 16A and 16B. Thus, 17B di-*p*-toluoyl-D-tartrate and 17A di-*p*-toluoyl-L-tartrate were prepared and recrystallized to purity. Anal. ($C_{38}H_{43}NO_9$) C, H, N for both samples.

The salts were converted to their free bases 17B and 17A as described for 16B and 16A. The ^1H NMR spectra of 17B and 17A were identical to the spectra of 16B and 16A, respectively.

Biochemical. σ Receptor Assay. Crude P_2 membrane fractions were prepared from frozen (-80 °C) guinea pig brains (Pel-Freeze, Rogers, AK), minus cerebellum. After removal of cerebella, the brains were allowed to thaw slowly on ice and placed in ice-cold 10 mM Tris-HCl, pH 7.4, containing 320 mM sucrose (Tris-sucrose buffer). The brains were then homogenized in a Potter-Elvehjem homogenizer by 10 strokes of a motor driven Teflon pestle in a volume of 10 mL/g tissue weight. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C, and the supernatants were saved. The pellets were resuspended by vortexing in 2 mL/g of ice-cold Tris-sucrose and centrifuged again at 1000*g* for 10 min. The combined 1000*g* supernatants were centrifuged at 31000*g* for 15 min at 4 °C. The pellets were resuspended by vortexing in 3 mL/g of 10 mM Tris-HCl, pH 7.4, and the suspension allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the pellets were resuspended by gentle Potter-Elvehjem homogenization to a final volume of 1.53 mL/g in 10 mM Tris-HCl, pH 7.4. Aliquots were stored at -80 °C until use. Protein concentration was determined by the method of Lowry et al.[33] using bovine serum albumin as standard.

σ sites were labeled with [^{3}H]-(+)-pnetazocine (52 Ci/mmol) or [^{3}H]-(+)-3-PPP (98.9 Ci/mmol). Incubations were carried out in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a volume of 0.5 mL with 500 μg of membrane protein and 3 nM radioligand. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine ([^{3}H]-(+)-pentazocine) or 1 μM haloperidol ([^{3}H]-(+)-3-PPP). Assays were terminated by the addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and filtration through glass fiber filters (Schleicher and Schuell), which were soaked in 0.5% polyethylenimine for at least 30 min at 25 °C prior to use. Filters were then washed twice with 5 mL of ice-cold Tris-HCl buffer. All filtration was carried out using a Brandel cell harvester (Gaithersburg, MD).

PCP Receptor Assay. Adult male Sprague-Dawley rats (Dominion Laboratories, Dublin, VA) were decapitated, and the whole brain minus cerebellum was homogenized in 10 volumes of 5 mM Tris-HCl buffer (pH 7.7) and centrifuged at 45000*g* for 15 min at 4 °C. The pellet was resuspended and recentrifuged as before. The final pellet was resuspended in 10 volumes of buffer. Aliquots (200 μL) of freshly prepared homogenate were incubated in triplicate (25 °C, 20 min) with the appropriate concentration of [^{3}H]TCP. Nonspecific binding was determined in the presence of 1 μM unlabeled TCP. The reaction was terminated by rapid filtration on a Brandel cell harvester (Gaithersburg, MD). Schleicher and Schuell (Keene, NH) #32 glass filters were soaked for 5 min in a 0.05% polyethylenimine solution prior to filtration. Filters were washed two times with 5 mL of cold buffer, suspended in 10 mL of Budgetsolve (Research Products International, Mount Prospect, IL) and shaken for 1 h. Radioactivity was determined by liquid scintillation spectrometry at a counting efficiency of approximately 50% corrected by external standardization. Specific [^{3}H]TCP binding was defined as total binding minus nonspecific binding. Protein concentrations were determined by the method of Bradford.[35] Displacement studies were conducted by incubating the drugs with 1 nM [^{3}H]TCP as described above. K_i values were calculated using the Cheng-Prusoff equation.[34]

μ Opiate Receptor Assay. In the μ-opiate assay, the homogenate was prepared as described above for the PCP receptor assay in a 50 mM Tris-HCl buffer (pH 7.4) and incubated (30 °C, 2.5 h) with 1 nM [^{3}H]DAMGO for displacement studies. Nonspecific binding was determined in the presence of 1 μM levorphanol. The reactions were filtered and counted for radioactivity as described for the PCP assay. K_i values were calculated using the Cheng-Prusoff equation.[34] A K_d of 1.9 ± 0.36 nM for [^{3}H]DAMGO was used.

Molecular Modeling. Molecular modeling studies were performed with the Tripos Associates SYBYL software package (version 5.41) installed on a Silicon Graphics 4D/310VGX graphics workstation. An Apple Macintosh IIcx running the Tripos Associates NITRO terminal emulator was used as the graphics terminal.

Acknowledgment. This work was supported by the National Institute on Drug Abuse under Grant No. DA05721, DA02396, and DA00490.

(35) Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding. *Anal. Biochem.* **1976**, *72*, 248-254.

Stereoisomers of Ketoconazole: Preparation and Biological Activity[1]

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The four stereoisomers of the antifungal agent ketoconazole (1) were prepared and evaluated for their selectivity in inhibiting a number of cytochrome P-450 enzymes. Large differences in selectivity among the isomers were observed for inhibition of the cytochromes P-450 involved in steroid biosynthesis, whereas little difference was observed for inhibition of those associated with hepatic drug metabolism. The cis-(2*S*,4*R*) isomer **2** was the most effective against rat lanosterol 14α-demethylase, (2*S*,4*R*)-**2** > (2*R*,4*S*)-**4** ≫ (2*R*,4*R*)-**3** = (2*S*,4*S*)-**5**, and progesterone 17α,20-lyase, (2*S*,4*R*)-**2** ≫ (2*S*,4*S*)-**5** > (2*R*,4*R*)-**3** = (2*R*,4*S*)-**4**, whereas the cis-(2*R*,4*S*) isomer **4** was more effective against cholesterol 7α-hydroxylase, (2*R*,4*S*)-**4** > (2*S*,4*S*)-**5** > (2*R*,4*R*)-**3** > (2*S*,4*R*)-**2**, and the trans-(2*S*,4*S*) isomer **5** was the most effective against aromatase, (2*S*,4*R*)-**5** ≫ (2*R*,4*R*)-**3** = (2*R*,4*S*)-**4** > (2*S*,4*R*)-**2**. The cis-(2*S*,4*R*) and trans-(2*R*,4*R*) isomers **2** and **3** are equipotent in inhibiting corticoid 11β-hydroxylase and much more effective than their antipodes. Little selectivity was observed for inhibition of cholesterol side chain cleavage or xenobiotic hydroxylases. These data indicate that the affinity of azoles for cytochrome P-450 enzymes involved in steroid synthesis is highly dependent on the stereochemistry of the entire molecule, whereas binding to drug metabolizing enzymes is a less selective process.

Introduction

Ketoconazole (1) is a potent, orally active, broad-spectrum antifungal agent.[2,3] The basis of the antifungal activity of ketoconazole and related azoles is blockade of the conversion of lanosterol to ergosterol, which is necessary for maintaining the integrity of the organism's cell

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(1) Contribution No. 839 from the Institute of Organic Chemistry.
(2) Heeres, J.; Backx, L. J. J.; Mostmans, J. H.; Van Cutsem, J. Antimycotic Imidazoles. 4. Synthesis and antifungal activity of Ketoconazole, a new potent orally active broad spectrum antifungal agent. *J. Med. Chem.* **1979**, *22*, 1003-1005.

0022-2623/92/1835-2818$03.00/0