A correlation between the IE_1 and log $(1/ED_{30})$ does not exist, but this does not necessarily mean that formation of an EDA complex will not occur. The IE_1 and HOMO energy are only crude approximations of the electron-donating ability. Differences in the spatial extension of the HOMO's have to be taken into account, as Weinstein has shown for some tryptamine congeners.[45] More important, however, is the fact that log $(1/ED_{30})$ is obtained after intravenous administration of the drugs, whereas there is considerable evidence that the 2-(phenylimino)-imidazolidines react with central receptor sites.[46] These compounds have to cross the blood-brain barrier before reaching the receptor sites, and it is therefore likely that for these molecules the lipid solubility will determine their potency to a great extent. A parameter reflecting the direct attachment of drugs with a receptor without interference of differences in pharmacokinetic properties will be of great value for the assessment of the role of the first IE in receptor complex formation. Such a parameter for the α-adrenergic receptor might be the ability to displace specifically bound [^{3}H]clonidine from high-affinity binding sites in rat brain homogenates.[40] In this laboratory, receptor binding studies on a large variety of imidazolines are now in progress.

Experimental Section

Ultraviolet photoelectron spectra were recorded on a Perkin-Elmer PS-18 photoelectron spectrometer modified with a Helectros He(I)-He(II) source. The spectra were calibrated with respect to Ar and Xe lines as internal calibrant. Vertical ionization energies were taken from band maxima. Resolution as measured on the argon doublet was 25-30 meV.

Compound 8 was synthesized via the dichloroimino method described in the literature.[4,6] Compound 10 was synthesized according to the method of Jen et al.[21] Both compounds were purified by recrystallization from methanol and ethanol, respectively. Identity and purity were verified by NMR, IR, mass spectroscopy, and TLC. Compounds **5**, **6**, and **9** were a gift from Boehringer, Ingelheim. The other compounds were kindly provided by Dr. P. B. M. W. M. Timmermans, University of Amsterdam.

Molecular orbital calculations were performed using the CNDO/s method of Del Bene and Jaffé[22] with the parametrization of Kuehnlenz and Jaffé.[23] The two-electron two center integrals were approximated with the aid of the Nishimoto-Mataga formula.[24] Input to the CNDO/s program consisted of the Cartesian coordinates of the atoms, which were calculated from standard bond lengths and angles.[25]

Acknowledgment. We thank A. Terpstra for running most of the spectra. We gratefully acknowledge Drs. W. Soudijn, A. Oskam, and E. Tomlinson for helpful discussions and suggestions, J. Bley for drawing the structures and figures, and Mrs. B. Onderweegs for typing the manuscript.

(45) Weinstein, H.; Chou, D.; Kang, S.; Johnson, C. L.; Green, J. P. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1976**, *no. 3*, 135.
(46) Kobinger, W. *Rev. Physiol. Biochem. Pharmacol.* **1978**, *81*, 40.
(47) Timmermans, P. B. M. W. M.; Fluitman, P. H. M.; Mackaay, J. C. J.; Van Zwieten, P. A. *Arch. Int. Pharmacodyn. Ther.* **1978**, *231*, 98.

4-Anilidopiperidine Analgesics. 3. 1-Substituted 4-(Propananilido)perhydroazepines as Ring-Expanded Analogues

Z. Gail Finney and Thomas N. Riley*

School of Pharmacy, Department of Medicinal Chemistry, University of Mississippi, University, Mississippi 38677.
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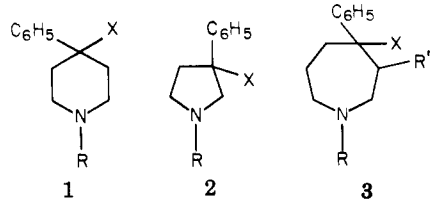
A study of ring-expanded analogues of the 4-(propananilido)piperidine analgesics has been undertaken in order to evaluate the influence of this structural modification on both analgesic activity and physical-dependence capacity. Thus, a series of 1-substituted 4-(propananilido)perhydroazepine derivatives was synthesized and pharmacologically evaluated in mice for analgesic activity and physical-dependence capacity. The results of this study indicate that the ring-expanded analogues of the 4-(propananilido)piperidines retain a relatively high degree of analgesic potency, except in the case of the 1-phenylethylated analogue which is approximately 150-fold less potent than the correspondingly 1-substituted piperidine analgesic. Evaluation of physical-dependence capacity of the most potent 1-substituted 4-(propananilido)perhydroazepines reveals no significant difference for these compounds as compared with morphine. The 4-(propananilido)perhydroazepines having 1-substitutents in common with known opiate antagonists failed to exhibit antagonism of morphine analgesia.

The influence of ring contraction and ring expansion on the analgesic activity of 4-phenylpiperidines (1) related to



1 2 3

a, X = $COOCH_2CH_3$, R' = H
b, X = $OCOCH_2CH_3$, R' = CH_3

meperidine is reported to involve a substantial or complete loss of activity, as seen in the 3-phenylpyrrolidine (2) analogues, and a retention of varying degrees of analgesic activity, as reported for 4-phenylperhydroazepine (3) analogues.[1] Pharmacological studies of ethoheptazine (**3a**) and proheptazine (**3b**) indicate that these ring-expanded analogues of meperidine and the prodines, respectively, possess clinically useful levels of analgesia associated with a favorable separation from certain opiate side effects, including physical-dependence capacity.[2,3]

Similar studies of the influence of ring size of the highly potent 4-anilidopiperidines (5) on analgesic activity have been confined to the synthesis and evaluation of a series

(1) Casy, A. F.; Birnbaum, H; Hall, G. H.; Everitt, B. J. *J. Pharm. Pharmacol.* **1965**, *17*, 157.
(2) Cass, L. J.; Frederik, W. S.; Bartholomay, A. F. *J. Am. Med. Assoc.* **1958**, *166*, 1838.
(3) Eddy, N. B.; Halbach, H.; Braenden, O. J. *Bull. W.H.O* **1956**, *14*, 353.

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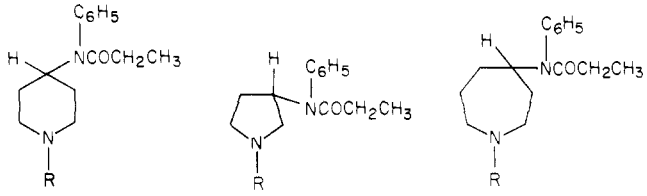


4 5 6

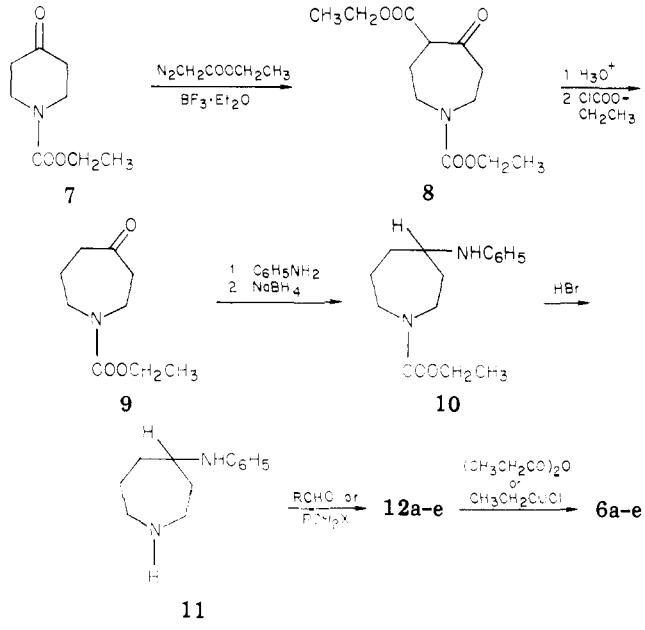
a, R = CH_3; b, R = $CH_2C_6H_5$; c, R = $CH_2CH_2C_6H_5$; d, R = $CH_2CH{=}CH_2$; e, R = CH_2-c-C_3H_5

of 1-substituted 3-(propananilido)pyrrolidines (**4**).[4] Certain of these ring-contracted analogues of **5** exhibited analgesic activity in mice at dose levels similar to the ED_{50} of morphine. The analgesically active members of this series were noted to produce neither sedation nor excitement at effective doses.

As a part of our continuing study of certain structural[5] and stereochemical[6] aspects of the 4-anilidopiperidine analgesics, we have extended the study of piperidine ring analogues of **5** to include the ring-expanded 4-(propananilido)perhydroazepines (**6**). On the basis of studies of the 4-phenylperhydroazepine analogues, it is reasonable to assume that the ring-expanded analogues of **5** should retain a substantial degree of analgesic activity having a potency greater than **3** but less than **5** and, in addition, these analogues may exhibit a favorable separation of analgesia and certain opiate side effects. Therefore, the 1-methyl (**6a**), 1-benzyl (**6b**), and 1-(phenylethyl) (**6c**) derivatives of 4-(propananilido)perhydroazepine were prepared in view of the agonistic activities of similarly 1-substituted 4-(propananilido)piperidines.[6,7] Further, the 1-allyl (**6d**) and 1-(cyclopropylmethyl) (**6e**) derivatives of **6** were also prepared in order to determine if ring expansion of **5** might result in narcotic antagonist agents.

Chemistry. The desired 4-(propananilido)perhydroazepines (**6a–e**) were prepared as illustrated in Scheme I. The key intermediate in the synthesis, 1-carbethoxyperhydroazepin-4-one (**9**), was prepared in 50% yields via ring homologation of 1-carbethoxy-4-piperidinone (**7**) following a modification of the procedure of Krogsgaard-Larsen and Hjeds.[8] The piperidinone was treated with ethyl diazoacetate and boron trifluoride at −25 to −30 °C to provide the β-keto ester intermediate, **8**, which was hydrolyzed and decarboxylated in refluxing 4 N HCl, followed by carbamylation with ethyl chloroformate, to yield **9**. Treatment of **9** with aniline, followed by $NaBH_4$ reduction, provided 1-carbethoxy-4-anilinoperhydroazepine (**10**), which was decarbamylated to **11** in refluxing 48% HBr. The appropriate 1-substituent was incorporated by treating **11** with either an alkyl (**12d** and **12e**) or aralkyl (**12b**) halide or by reductive alkylation using the appropriate aldehyde (**12a** and **12c**). The target compounds were then obtained by propionylation of the respective 1-substituted 4-anilinoperhydroazepine derivative (**12**) with either propionic anhydride or propionyl chloride (Table I). Relatively low yields of **6e** were obtained upon treatment of **12e** with propionic anhydride, apparently as a result of cyclopropane ring cleavage (NMR evidence) induced by

Scheme I



the heat and acid generated during the acylation reaction. The yield of **6e** was significantly improved using milder conditions of propionyl chloride in ether containing triethylamine.

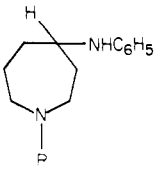
Pharmacological Studies. The 1-substituted 4-(propananilido)perhydroazepine derivatives (**6a–e**) prepared for this study were evaluated for analgesic activity in mice using the tail-flick procedure of D'Amour and Smith (Table II).[9] The dose–response curves obtained for the analgesically active compounds were parallel to the curve generated for morphine.[10] Further, naloxone pretreatment of mice prevented analgesia by **6a–c** when these compounds were administered in ED_{100} doses. These data suggest that the analgesic derivatives of **6** and morphine are exerting their activity by similar mechanisms at the opiate receptor. Ethoheptazine (**3a**), employed as a standard in this study, exhibited an ED_{50} somewhat higher than that reported in other studies[2] and produced excitement and Straub tail effect in mice at a dose of 195 mg/kg. Ethoheptazine analgesia (134.2 mg/kg) was not observed following naloxone pretreatment. Compounds **6a–c** produced signs of hyperactivity, excitement, and Straub tail effect in mice treated with doses in the ED_{50} range.

The 1-benzyl (**6b**) and 1-phenylethyl (**6c**) derivatives were evaluated for their ability to produce physical dependence in mice by examining naloxone-precipitated jumping in mice.[11] Naloxone-induced jumping in mice pretreated with **6b** (25 and 50 mg/kg free base) and **6c** (40 and 30 mg/kg free base) was significantly greater than that produced by saline control ($p < 0.01$) and comparable to that induced in mice pretreated with morphine (5 mg/kg free base).

The derivatives of **6** were also evaluated for antagonism of the analgesic actions of morphine. All of the test compounds were found to lack significant antagonsim of morphine analgesia, except for the 1-methyl derivative (**6a**) which shortened the response time to pain stimulus in three or six morphine-treated mice.

(4) Helsley, G. C.; Lunsford, C. D.; Welstead, W. J., Jr.; Boswell, R. F., Jr.; Funderburk, W. H.; Johnson, D. N. *J. Med. Chem.* **1969**, *12*, 583.
(5) Riley, T. N.; Hale, D. B.; Wilson, M. C. *J. Pharm. Sci.* **1973**, *62*, 983.
(6) Riley, T. N.; Bagley, J. R. *J. Med. Chem.* **1979**, *22*, 1167.
(7) Casy, A. F.; Hassan, M. M. A.; Simmonds, A. B.; Staniforth, D. *J. Pharm. Pharmacol.* **1969**, *21*, 434.
(8) Krogsgaard-Larsen, P.; Hjeds, H. *Acta Chem. Scand., Sect. B* **1976**, *30*, 884.
(9) D'Amour, F. E.; Smith, D. L. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74.
(10) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.
(11) Wiley, J. N.; Downs, D. A. *Life Sci.* **1979**, *25*, 797.

Table I. Structures, Physical Properties, and Yields of 1-Substituted 4-Anilino- and 4-(Propananilido)perhydroazepines



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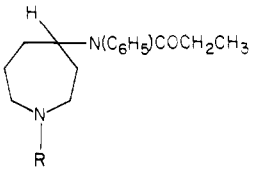
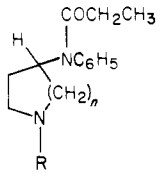


6

no.	R	% yield	bp (mm), °C	formula[a]	no.	R	% yield	crystn solv[b]	mp, °C	formula[a]
12a	CH_3	75	108-115 (0.1)	$C_{13}H_{20}N_2$[c]	6a	CH_3	68	EtOAc/ Et_2O	137-139	$C_{16}H_{24}N_2O\cdot HCl$
12b	$CH_2C_6H_5$	70	165-168 (0.1)	$C_{19}H_{24}N_2$	6b	$CH_2C_6H_5$	64	Me_2CO/ Et_2O	169-171	$C_{22}H_{28}N_2O\cdot HCl$
12c	$CH_2CH_2C_6H_5$	53	155-160 (0.03)	$C_{20}H_{26}N_2$	6c	$CH_2CH_2C_6H_5$	84	EtOAc	157-159	$C_{23}H_{30}N_2O\cdot HCl$
12d	$CH_2CH{=}CH_2$	57	122-125 (0.5)	$C_{15}H_{22}N_2$[d]	6d	$CH_2CH{=}CH_2$	66		e	$C_{18}H_{26}N_2O$[f]
12e	CH_2-c-C_3H_5	57	121-123 (0.5)	$C_{16}H_{24}N_2$	6e	CH_2-c-C_3H_5	70	EtOH	84-86	$C_{19}H_{28}N_2O\cdot C_6H_8O_6\cdot H_2O$

[a] All compounds were analyzed for C, H, and N with results within ±0.4% of theoretical values except where noted. [b] Solvent used to crystallize indicated salt. [c] C: calcd, 76.42; found, 75.88. [d] C: calcd, 78.21; found, 77.76. [e] bp 180-185 °C (0.1 mm). [f] C: calcd, 75.48; found, 74.73; N: calcd, 9.78; found, 10.63.

Table II. Analgesic Activities of Cyclic Basic Anilides



4, n = 1
5, n = 2
6, n = 3

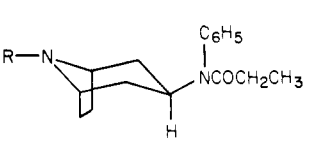
compd	R	4: ED_{50}, mg/kg, ip (95% CL)[a]	5: ED_{50}, mg/kg, sc[b]	6: ED_{50}, mg/kg, sc (95% CL)[c]
a	CH_3	d	>100	11.6 (9.2-14.6)
b	$CH_2C_6H_5$	5.7 (4.0-8.2)	10.45	3.6 (2.5-5.3)
c	$CH_2CH_2C_6H_5$	2.0 (1.0-4.0)	0.01	1.5 (0.9-2.6)
d	$CH_2CH{=}CH_2$	8.0 (3.8-16.8)	12.10	81.0 (66.1-99.2)
e	CH_2-c-C_3H_5	e	e	>200
ethoheptazine citrate				128.0 (73.1-224.0)
morphine sulfate				1.9 (1.3-2.8)

[a] 95% confidence limits; data obtained from ref 4. [b] Data obtained from ref 7; confidence limits not cited. [c] See Experimental Section for details. [d] Analgesia noted in 60% of test animals at 20 mg/kg. [e] Not reported.

Discussion of Results

The analgesic activities of a series of identically N-substituted homologues of cyclic basic anilides are compared in Table II. Examination of these data indicate that either ring expansion (**6**) or ring contraction (**4**) of the 4-(propananilido)piperidines (**5**) does not significantly alter analgesic activity, except in the case of the 1-phenylethylated derivatives where **4c** and **6c** are 150- to 200-fold less potent than the corresponding piperidine homologue (**5c**). It is also of interest to note the similarity in rank-order potencies among the ring homologues of the cyclic basic anilides (phenylethyl > benzyl > methyl), suggesting a similar mode of interaction of these homologues with the opiate receptor.[12] The significantly higher potency of the *N*-methyl derivative of **6** as compared to **4a** and **5a** is of interest and may be due, in part, to greater lipophilicity, as suggested by the observation that **6a** possesses potency comparable to that of the 1,3-dimethyl derivative of **5**.[5]

The marked difference in analgesic potencies found for the 1-phenylethylated derivaties of the ring homologues of the cyclic basic anilides suggests that structural and/or stereochemical differences may account for these differences. Previous studies in our laboratories[6] suggest that a chair conformation of the piperidine ring of **5c** is optimal for the production of an analgesic response and that such stereochemical considerations are less important in those derivatives of **5** having other N-substitutents. These conclusions are based upon the observation of a 50-fold greater analgesic potency of 1-phenylethyl-3β-(propananilido)nortropane (**13**, R = $C_6H_5CH_2CH_2$) as compared



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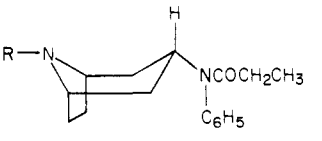

14

to the 3α isomer (**14**, R = $C_3H_5CH_2CH_2$), which was shown to exist in a piperidine boat conformation. A significant reduction in the 3β/3α isomer potency ratio was noted when less efficacious N-substituents, such as $C_6H_5CH_2$, were incorporated into **13** and **14**. Hence, it is conceivable

(12) Portoghese, P. S. *J. Med. Chem.* **1965**, *8*, 609.

that the substantially decreased analgesic potencies of **4c** and **6c** as compared to **5c** may be related to less than optimal conformational features of the relatively planar pyrrolidine ring (**4**) and the preferred twist-chain conformation[13] of the perhydroazepine ring system (**6**). The similar level of analgesic potency of the *N*-benzyl derivatives of **4**–**6** may reflect the lesser contribution of ring conformation to the production of an analgesic response by these compounds at the opiate receptor. Factors other than or in addition to conformational factors may also be involved in accounting for the lesser analgesic potencies of **4c** and **6c**. It is plausible that the affinity of these homologues of **5c** for the opiate receptor might be reduced as a result of differences in intramolecular relationships among important opiate receptor binding moieties brought about by differences in ring size.

Hence, the results of this study indicate that ring-expanded homologues of the highly potent 4-anilidopiperidine analgesics retain a substantial level of analgesic activity comparable to that of morphine. However, the observation that **6b** and **6c** possess physical-dependence capacity in mice similar to that of morphine indicates that a favorable separation of this action from analgesic activity is not achieved. These results are in contrast to the achievement of such a separation as a result of an identical structural modification of the 4-phenylpiperidine analgesics (**1**).

Ring homologation of **5** results in the incorporation of a center of asymmetry into these compounds at the 4 position. Studies are continuing in our laboratories with regard to an investigation of the influence of configurational isomerism on the analgesic activity of **6**.

Experimental Section

Chemistry. All melting points are uncorrected and were determined with a Mel-Temp apparatus. IR spectra were determined with a Beckman IR-33 spectrophotometer. NMR spectra were taken on a Jeolco C-60HL spectrometer using $CDCl_3$ as solvent and Me_4Si as internal standard. Spectral data obtained for synthetic intermediates and target compounds were consistent with the structures. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

1-Carbethoxyperhydroazepin-4-one (9). Solutions of 27.7 g (0.187 mol) of boron trifluoride etherate and 28.2 g (0.247 mol) of ethyl diazoacetate, each in 20 mL of anhydrous Et_2O, were simultaneously added over a 1.5- to 2-h period to a solution of 32.0 g (0.187 mol) of *N*-carbethoxy-4-piperidinone in 100 mL of anhydrous Et_2O maintained at −25 to −30 °C (dry ice–*i*-PrOH bath). After the additions were completed, the reaction was maintained at −25 to −30 °C for 1 h and then allowed to warm to room temperature. The solution was washed with 320 mL of 30% K_2CO_3, and the organic phase was separated, dried (anhydrous K_2CO_3), and concentrated in vacuo to give 52.5 g of **8** as an orange oil. The intermediate β-keto ester (**8**) was refluxed in 790 mL of 4 N HCl for 6 h; the solution was cooled, concentrated in vacuo, placed in a MeOH–ice bath (−5 °C), and 100 mL of H_2O, 225 mL of ice-cooled 30% K_2CO_6, and 60 mL of ethyl chloroformate were added. The mixture was stirred for 4 h at −5 °C, then warmed to room temperature, and extracted with Et_2O, and the organic phase was dried (anhydrous Na_2CO_3). The Et_2O extracts were filtered, concentrated in vacuo and distilled to give 17.6 g (50.8%) of **9** as a clear oil: bp 105–108 °C (0.25 mm); NMR ($CDCl_3$) δ 1.3 (t, 3 H, $CO_2CH_2CH_3$), 1.6–2.05 (m, 2 H, 6-CH_2), 2.55–3.0 (m, 4 H, 2- and 7-CH_2), 3.55–3.85 (m, 4 H, 3- and 5-CH_2), 4.25 (q, 2 H, $CO_2CH_2CH_3$).

4-Anilinoperhydroazepine (11). A mixture of 7.4 g (0.04 mol) of **9**, 7.5 g (0.081 mol) of aniline, a few crystals of $ZnCl_2$, and 180 mL of dry toluene was refluxed for 15 h and the H_2O produced in the reaction was collected in a Dean–Stark apparatus. The reaction mixture was cooled, filtered, and concentrated in vacuo, yielding an oily residue which was distilled in vacuo (0.5–1.0 mm) to remove unreacted aniline. The residue was treated with 2.0 g (0.053 mol) of $NaBH_4$ in 100 mL of MeOH, and the reaction was refluxed for 1 h, then treated with 50 mL of H_2O, concentrated in vacuo to a volume of approximately 100 mL, extracted with toluene, and dried (anhydrous $MgSO_4$). The toluene solution was filtered and concentrated in vacuo, and the residue was distilled to provide 4.3 g (41%) of **10** as a clear oil: bp 170–175 °C (0.75 mm); NMR ($CDCl_3$) δ 1.33 (t, 3 H, $CO_2CH_2CH_3$), 1.5–2.4 (m, 6 H, 3-, 5-, and 6-CH_2), 3.2–3.75 (br m, 5 H, 4-CH, 2- and 7-CH_2), 4.22 (q, 2 H, $CO_2CH_2CH_3$), 4.74 (s, 1 H, NH), 6.67, 7.20 (m, 5 H, N-C_6H_5).

A solution containing 4.3 g (0.0164 mol) of **10** in 50 mL of 48% HBr was refluxed for 3 h, cooled, alkalinized with NaOH, and extracted with Et_2O. The Et_2O extracts were combined, dried (anhydrous K_2CO_3), filtered, and concentrated in vacuo, and the residue was distilled to provide 2.6 g (85%) of **11** as a clear oil that solidified on standing: bp 130–133 °C (0.5 mm); mp 52–54 °C; NMR ($CDCl_3$) δ 1.3–2.3 (m, 6 H, 3-, 5-, and 6-CH_2), 2.7–3.1 (m, 4 H, 2- and 7-CH_2), 3.3–4.2 (br m, 3 H, NH and 4-CH), 6.62, 7.17 (m, 5 H, N-C_6H_5).

1-Substituted 4-Anilinoperhydroazepines (12a–e). A solution of **11** in absolute EtOH, 0.5 g of 10% Pd/C, and formaldehyde solution (37%) or phenylacetaldehyde was hydrogenated at 40–45 psi for 20 h to provide **12a** and **12c**, respectively. Following an acid–base workup procedure, the products were purified by vacuum distillation to yield clear, viscous oils. Similarly, a solution of **11** in 2-butanone containing K_2CO_3 and a few crystals of KI was treated with benzyl chloride, allyl bromide, or (chloromethyl)cyclopropane and refluxed for 15–20 h to provide **12b**, **12d**, and **12e**, respectively. Following an acid–base workup procedure, the products were purified by vacuum distillation and obtained as clear, viscous oils. The NMR spectra ($CDCl_3$) of **12a–e** were consistent with their structures and included the following characteristic signals for the perhydroazepine moiety: δ 1.3–2.15 (m, 6 H, 3-, 5-, and 6-CH_2), 2.4–3.3 (m, 4 H, 2- and 7-CH_2), 3.3–3.8 (br m, 2 H, NH and 4-CH).

1-Substituted 4-(Propananilido)perhydroazepines (6a–e). Stirred solutions of **12a–d** in propionic anhydride were refluxed for 16–20 h. The cooled solution was diluted with Et_2O and stirred with an equal volume of 20% KOH for 20–30 min. The organic phase was separated, stirred with anhydrous K_2CO for 24 h, filtered, and extracted with 6 N HCl. The acid extract was basified with NaOH (pH 11) and extracted with Et_2O, and the ethereal extracts were dried (anhydrous K_2CO_3) and concentrated in vacuo to give crude yields of **6a–d**, which were purified by silica gel column chromatography [EtOAc/C_6H_6 (1:1), EtOAc/C_6H_6 (3:1), then 100% EtOAc]. The HCl salts of **6a–d** were prepared in the usual manner (Table I). A solution of 6.6 g (0.027 mol) of **12e** in 150 mL of anhydrous Et_2O containing 65 mL of triethylamine was treated with a solution of 4.4 g (0.047 mol) of propionyl chloride in 20 mL of anhydrous Et_2O added dropwise. After the addition, the reaction mixture was refluxed for 2 h, stirred at room temperature for 1 h, filtered, concentrated in vacuo, cooled in an ice bath, and 100 mL of 10% HCl was added. The acid extract was washed with Et_2O, basified (NaOH, pH 11, 0 °C), and extracted with Et_2O, and the ethereal extracts were dried (anhydrous K_2CO_3) and concentrated in vacuo to give 5.6 g (70%) of **6e** as a yellow oil. The citrate salt of **6e** was prepared and recrystallized from absolute EtOH (Table I). The NMR spectra ($CDCl_3$) of **6a–e** were consistent with structural assignments and included the following characteristic signals: δ 1.03 (t, 3 H, $COCH_2CH_3$), 1.5–2.23 (m, 8 H, 3-, 5-, 6-CH_2 and $COCH_2CH_3$), 2.4–2.87 (m, 4 H, 2- and 7-CH_2), 4.57–5.17 (br m, 1 H, 4-CH), 7.13–7.67 (m, 5 H, N-C_6H_5).

Pharmacology. All of the test compunds were evaluated as saline solutions of their HCl salts, except **6e** which was used as the citrate salt. A modification of the D'Amour–Smith tail-flick method was employed in the evaluation of analgesic activity.[9] The analgesic activities of fentanyl citrate, morphine sulfate, and ethoheptazine citrate were determined as standards. Male albino ICR mice weighing 25–30 g were given a thermal stimulus challenge 20 min postadministration (sc) of the test compounds and standards. Positive analgesia was defined as a tail-flick

(13) Thomas, E. J. In "Alicyclic Chemistry"; McKervey, M. A., Ed.; The Chemical Society, Burlington House: London, 1978; Vol. 6, pp 181–182.

response time greater than or equal to the mean response time of the control group of 10 mice plus two standard deviations of their mean. Median analgesic doses (ED_{50}) and their 95% confidence limits were determined by the method of Litchfield and Wilcoxon.[10] The mice used in the analgesic assay were closely observed for physical and behavioral changes during the test period and were examined for lethality 24 h after testing. No deaths were noted to occur at any dose levels of the test compounds, although **6b** produced lethality in the smaller mice used during the naloxone-precipitated jumping assay (vp). Naloxone hydrochloride (4 mg/kg) was administered to groups of eight mice, followed by sc ED_{100} doses of **6a-c** and ethoheptazine after a 5-min period. Analgesic activity was then measured 15 min after administration of the test compounds using the tail-flick procedure. Naloxone pretreatment abolished the analgesic activity of **6a** in all animals tested, while analgesic activity was still noted after **6b** and **6c** in one and in two animals, respectively; however, the level of analgesia was deemed insignificant, since the criterion for analgesia was exceeded by an average of only 0.11 s. The procedure of Wiley and Downs was employed in order to obtain data relevant to the physical-dependence capacity of **6b** and **6c**.[11] Swiss-Webster male mice weighing 14-20 g in groups of 10 mice each were given sc injections of **6b** and **6c** 1 h prior to injection of 100 mg/kg naloxone. Saline was administered to a group of mice as a vehicle control, and morphine sulfate was similarly tested as a positive control. All compound doses were calculated as free base and were administered in volumes of 10 mL/kg. Immediately after naloxone injection, mice were individually confined under 4-L beakers on a table top. The proportion of animals jumping at least once and the total number of jumps per treatment group were recorded for 10 min following naloxone injection. Jumping was defined as all four feet simultaneously off the table top. In this assay, a dose of 50 mg/kg of morphine elicited an average of 34.1 jumps with 90% of the naloxone-treated mice jumping. Doses of 25 and 50 mg/kg of **6b** produced 42.4 and 79.5 jumps, respectively, with 100% of the naloxone-treated mice jumping. Doses of 40 and 80 mg/kg of **6c** produced an average of 33.9 and 42.0 jumps in naloxone-treated mice, respectively. These doses of **6c** elicited jumping in 80 and 100% of the test mice. The average number of jumps elicited by morphine, **6b** and **6c** were significantly greater ($p < 0.01$) than those produced by saline injection. Groups of six mice were pretreated with morphine sulfate (5 mg/kg, AD_{100}, sc), followed 10 min later by a sc dose of twice the AD_{50} of **6a-d** and a 200 mg/kg dose of **6e**. The mice were then evaluated for analgesia using the tail-flick procedure 20 min after administration of the test compounds. None of the test compounds produced a significant antagonism of morphine analgesia, except in the case of **6a** which produced a slight shortening of the tail-flick response time in three of the six treated mice.

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Folate Analogues Altered in the C^9-N^{10} Bridge Region. 16. Synthesis and Antifolate Activity of 11-Thiohomoaminopterin[1]

M. G. Nair,* Shiang-Yuan Chen,

Department of Biochemistry, College of Medicine, University of South Alabama, Mobile, Alabama 36688

Roy L. Kisliuk, Y. Gaumont, and D. Strumpf

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111.
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The synthesis of 11-thiohomoaminopterin (**1**), which is a close analogue of 11-thiohomofolic acid (**2**), has been carried out by modification of the Boon-Leigh procedure. Treatment of 1-chloro-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone (**5**) with sodium azide gave 1-azido-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone (**6**). After protection of the carbonyl group of **6**, the product **7** was catalytically hydrogenated to 1-amino-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone ketal (**3**). Reaction of **3** with 6-chloro-2,4-diamino-5-nitropyrimidine gave the desired pyrimidine intermediate, which was elaborated to 4-amino-4-deoxy-11-thiohomopteroic acid (**20**) by standard procedures. Alternately, 1-azido-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone ketal (**7**) was hydrolyzed to the corresponding acid (**8**) and coupled with diethyl L-glutamate to obtain diethyl *N*-[*p*-(1-azido-2-oxo-4-thiobutanoyl)benzoyl]-L-glutamate ketal (**10**), which was used for the large-scale preparation of 11-thiohomoaminopterin (**1**). Although 11-thiohomoaminopterin showed antifolate activity against two folate-requiring microorganisms and inhibited *Lactobacillus casei* dihydrofolate reductase, it did not exhibit any antitumor activity against L-1210 lymphoid leukemia in mice at a maximum dose of 48 mg/kg.

Folate antagonists are very widely used in the chemotherapy of neoplastic diseases, and methotrexate continues to be the most important drug belonging to this series.[2-4] The literature on the fate and functions of methotrexate has accumulated to the point that comprehensive review is impractical, if not impossible. Although several close analogues of methotrexate have been synthesized and evaluated, these compounds do not have therapeutic indices high enough to warrant their development as anticancer drugs.[5,6] However, quite recently 10-deazaaminopterin, which is a close analogue of aminopterin in which the 10-amino group was replaced with a methylene group,

(1) For the previous papers in this series, see Nair, M. G.; Colleen, S.; Chen, S. Y.; Kisliuk, R. L.; Gaumont, Y. *J. Med. Chem.* **1980**, *23* 59, and Hornbeak, H. R.; Nair, M. G. *Antimicrob. Agents Chemother.* **1979**, *15*, 730.
(2) Holland, J. F.; Glidewell, O. *N. Engl. J. Med.* **1972**, *287*, 769.
(3) Ross, G. T.; Goldstein, D. P.; Hertz, R.; Lipsett, M. B.; O'Dell, W. D. *Am. J. Obstet. Gynecol.* **1965**, *93*, 223.
(4) Weinstein, G. D. *Ann. N.Y. Acad. Sci.* **1971**, *186*, 467.
(5) Mead, J. A. R.; Wood, H. B.; Goldin, A. *Cancer Chemother. Rep.* **1968**, *2*, 273.
(6) Bertino, J. R., Ed. *Ann. N.Y. Acad. Sci.* **1971**, *186*, 1-519.

0022-2623/80/1823-0899$01.00/0