Analgetic Activity and in Vitro Binding Constants of Some *N*-Alkyl-3-benzazocines

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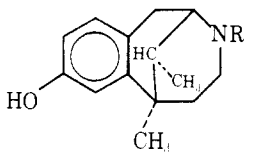
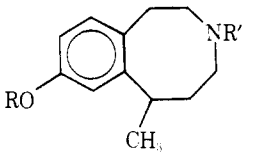
A homologous series of 3-alkyl-1,2,3,4,5,6-hexahydro-8-hydroxy-6-methyl-3-benzazocines (**2**) has been synthesized. Analgetic activity and binding constants for the opiate receptor for **2** and for an analogous series of benzomorphans (**1** and **3**) are reported. In **1**, hot-plate analgesic activity is lost on increase of the *N*-alkyl chain length from ethyl through butyl (**1c–e**) and regained with amyl (**1f**) and hexyl (**1g**). Compounds **1c–e** show that antagonist properties and binding constants are similar throughout the series. With **2**, where there has been loss of steric constraints through removal of the 2,6-methano bridge of **1** and **3**, greatly diminished analgetic activity and receptor affinity and no antagonist properties were observed. Like **1**, however, greatest agonist activity was shown by the *N*-methyl (**2c**), amyl (**2g**), hexyl (**2h**), and heptyl (**2i**) homologs and there is a parallel of in vitro binding strength and analgetic activity.

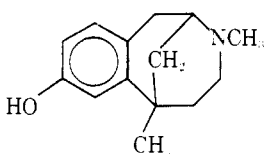
3,6-Dimethyl-1,2,3,4,5,6-hexahydro-8-hydroxy-3-benzazocine (**2c**) was recently synthesized[2a] for biologic comparison with its 2,6-methano analog **3**.[2b] In addition, higher N-homologs of **2c** were of interest because of the analgetic and/or antagonist properties conferred on **1a**[3] and on the ketobemidone molecule[4] by lengthening the *N*-alkyl chain. Finally, for the *N*-alkylnorketobemidones, a significant correlation between analgetic potencies and binding strengths to narcotic binding sites in brain homogenates has been observed.[5] We now wish to report analgetic activities and in vitro binding constants for **1** (C_1–C_6), **2** (R′ = C_7), and **3** along with some of their pharmacologic properties seen in morphine-dependent monkeys.



1a, R = H
b, R = Me
c, R = Et
d, R = Pr
e, R = Bu
f, R = Am
g, R = Hex



2a, R = Me; R′ = H
b, R = H; R′ = H
c, R = H; R′ = Me
d, R = H; R′ = Et
e, R = H; R′ = Pr
f, R = H; R′ = Bu
g, R = H; R′ = Am
h, R = H; R′ = Hex
i, R = H; R′ = Hep



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Chemistry. The synthesis of **1a–g**,[3] **2a,b**,[2a,6] and **3**[3] was previously reported. Compounds **2c–i** were obtained in overall yields of 5–30% by alkylation of **2a**[7] with the appropriate halide followed by O-demethylation with 48% HBr. They were characterized as the free bases after purification by either column or thin-layer chromatography (silica gel).

Pharmacological Methods. Analgetic potencies were determined as previously described (Table I).[3d,8]

Interaction of the compounds with the opiate receptor of rat brain homogenates was measured (Table I) by assaying the displacement of [^{3}H]dihydromorphine (New England Nuclear Corp. and Amersham-Searle) from specifically bound sites using the centrifugation assay described before.[9] Briefly, identical portions of the P_2 fraction[10] (0.5 mg of protein in 1 ml of 0.29 *M* sucrose, 0.009 *M* tris, pH 8) are incubated at 37° with [^{3}H]dihydromorphine (10^{-9} *M*, 30,000 cpm) and multiple concentrations of opiate in the range of 10^{-10}–10^{-4} *M*. After 10 min of incubation, the samples are centrifuged, and the washed pellets are transferred to scintillation vials and assayed for radioactivity. The concentration of narcotic which reduces by half the amount of specifically bound (displaceable by *l* (−) but not by *d* (+) opiates) [^{3}H]dihydromorphine is, in this assay, a close approximation to the dissociation constant of the narcotic and is so reported. The measured values of affinity are 1.3 times the true dissociation constants since the concentration of [^{3}H]dihydromorphine is one-third of its K_d. We, therefore, have not corrected our data for this very small (and constant) factor. The interpretation of results is not affected one way or the other. Log probit data do give parallel straight lines. Benzomorphans and **2c** were used as the hydrochlorides or hydrobromides dissolved in water. Benzazocines (except **2c**) were in the free base form and were prepared as 10^{-2} *M* solutions in ethanol and then treated with 1 equiv of HCl. These solutions were diluted with water to achieve the appropriate concentrations for assay. Benzazocines of concentrations above 10^{-5} *M* decreased nonspecific as well as specific binding of [^{3}H]dihydromorphine. These compounds were therefore routinely assayed in the presence and absence of a saturating amount (10^{-5} *M*) of morphine.

Discussion and Results

As shown in Table I, maximum (hot-plate) analgesic activity is shown by *N*-methyl, amyl, hexyl, and heptyl in both **1** and **2**. This is roughly parallel to the ketobemidone series in which it was also found that extension of the carbon chain beyond seven gave compounds of little or no activity. The binding constants for nor analgetics, which are usually analgetically inactive in vivo, presumably due to their inability to cross the "blood-brain barrier", can provide an indication of potency to be expected in the *N*-alkyl derivatives as can be seen from the binding constants of **1a** and **2b**.

The *N*-alkylnormetazocines, **1b–g**, all interact with the opiate receptor in vitro with comparable affinity, there being only a fourfold difference between the strongest (*N*-propyl, **1d**) and the weakest (*N*-ethyl, **1c**) binders of this series even though analgetic potencies are greatly different. This can be explained by the fact that **1c–e**, inactive in the hot-plate test for analgesia, are antagonists in the morphine-dependent monkey with 0.2 (for 1c and 1e)[11,12] and

Table I. Analgetic and Binding Data

No.	N–R	Analgesic activity, hot-plate ED_{50} sc[a,b] mg/kg	Analgesic activity, hot-plate ED_{50} sc[a,b] μmol/kg	Binding constant, nM[c]
1a	H	I[d,e]	I[d,e]	50
1b	Me	1.2 (1.0–1.3)[e]	4.4	9
1c	Et	I[d,e]	I[d,e]	20
1d	Pr	I[d,e]	I[d,e]	5
1e	Bu	I	I[d,e]	8
1f	Am	1.1 (0.9–1.3)[e]	4.1	10
1g	Hex	1.4 (1.3–1.6)[e]	4.1	10
2b	H			10,000
2c	Me	12.5 (10.2–15.4)	43.7	800
2d	Et	16.5 (11.0–24.8)	75.2	1,500
2e	Pr	~30[f]	128.5[f]	4,000
2f	Bu	24.0 (15.9–36.4)	97.0	3,000
2g	Am	15.4 (11.4–20.8)	58.9	2,000
2h	Hex	11.5 (8.9–14.9)	41.8	1,500
2i	Hep	9.8 (6.4–14.9)	33.9	800
3	Me	3.3 (2.6–4.2)	13.0	40
Nalorphine hydrochloride		36.3 (27.1–48.7)[g]	104.4[g]	2
Morphine hydrochloride		1.2 (0.9–1.3)	4.1	3

[a] Administered as HCl or HBr salts were 1a–g, 2c, and 3; titrated with 2% HCl were 2d–i. [b] See ref 8. [c] Concentration of drug in nanomoles per liter required to inhibit stereospecific binding of [^{3}H]dihydromorphine (1 nmol) by 50% in brain homogenates. [d] Inactive. [e] See ref 3. [f] Estimated from dose range finding studies. [g] In Nilsen test 4.8 (2.9–8.5); see ref 8 and 3a,b,d.

1.5 (for 1d)[11,12] times the potency of nalorphine. Furthermore, 1c and 1e show some analgetic activity in the Nilsen test,[13] an indication that they are agonist–antagonists like many other members of the benzomorphan series.[8,13] Compound 1d is inactive in the Nilsen test.[13]

Removal of the 9-methyl group from 1b gives 3 which is somewhat less potent in vivo and as an in vitro binder. There is an even more dramatic decrease in potency, however, when the 2,6-methano bridge of 3 is removed to give 2c.

The *N*-alkylbenzazocines (2c–i) interact with receptors approximately 100-fold more weakly than do the corresponding benzomorphans. This remarkable reduction in receptor affinity is reflected somewhat in the much reduced pharmacological potency of 2c–i. That differences in affinity (100-fold) are not the same as differences in analgetic potency (tenfold) may be a reflection of differences in metabolism and distribution. There is a good correlation between the receptor affinity and the analgesic potency of the benzazocines (see Figure 1) in marked contrast to the benzomorphans which show no such correlation. Since, with the benzomorphans, the lack of a demonstrable relationship between analgetic activity and receptor binding is ascribable to the antagonist activity of some members, it would seem that the benzazocines are either devoid of antagonist activity or that the ratio of agonist to antagonist activity in this series of analgetics is constant. In fact, the benzazocines have not been found to display typical antagonist activity in morphine-dependent monkeys and none will support morphine dependence in this species.[11] In general, they cause CNS depression and malaise.

The benzomorphans are benzazocines which are bridged by a methylene group to form a tricyclic structure from a bicyclic one. The extra ring of the benzomorphans imposes a degree of conformational rigidity not found in the more flexible benzazocines. This decreased conformational flexibility may be primarily responsible for the large increase in binding energy between benzomorphans and the opiate receptor when compared with the benzazocines. The extra binding energy ascribable to the methylene bridge directly accounts for only a small part of this difference. Thus, the benzomorphan structure favors conformations which are similar to those needed for interaction with the opiate receptor whereas the benzazocine structure does not do so.

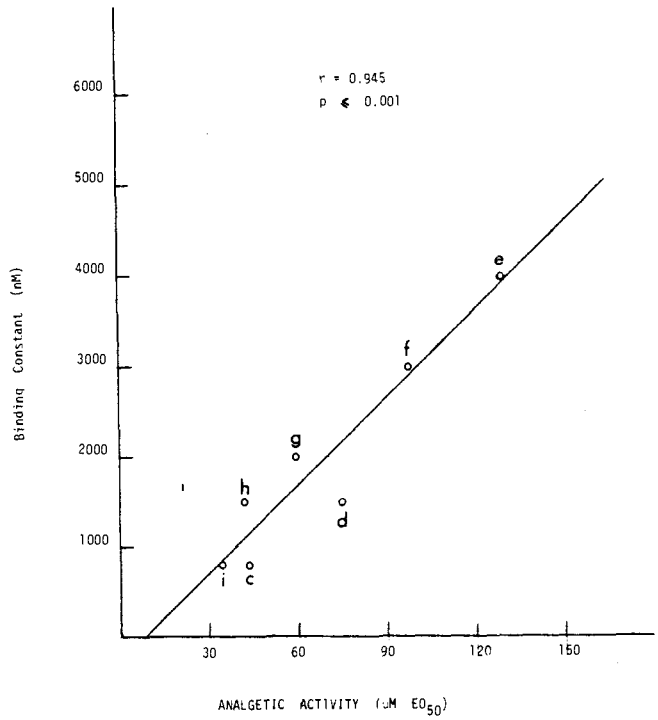

Figure 1. The relationship between analgetic activity and opiate receptor binding strength for *N*-alkylnorbenzazocines (2c–i). The correlation coefficient (see ref 14) calculated for these data is 0.945.

Experimental Section

Melting points (capillary, uncorrected) were determined with a Thomas-Hoover apparatus. Spectral data are consistent with assigned structures. Elemental analyses (indicated by C, H, and N when within ±0.4% of calculated values) were performed by the

Section on Microanalytical Services and Instrumentation of this laboratory.

3-Ethyl-1,2,3,4,5,6-hexahydro-8-hydroxy-6-methyl-3-benzazocine (2d). To 20 ml of 2-butanone was added 1.6 g (6.6 mmol) of **2a**·HCl, 1.1 g (7.0 mmol) of EtI, and 1.5 g of K_2CO_3 (DMF as solvent and the appropriate alkyl bromide were used for the remainder of the series). The mixture was stirred overnight at 90–95°, filtered, and evaporated to dryness in vacuo. The residue was dissolved in 50 ml of $CHCl_3$ and washed with H_2O. Drying and evaporation of the $CHCl_3$ gave a residue which was refluxed (for O-demethylation) with 48% HBr (10 ml, 30 min) and evaporated to dryness in vacuo. The residue, in 50 ml of $CHCl_3$, was washed with saturated $NaHCO_3$. The $CHCl_3$ layer was dried ($MgSO_4$) and evaporated to give, after purification by preparative TLC (silica gel), white crystals, mp 171–172°. Anal. ($C_{14}H_{21}NO$) C, H, N.

1,2,3,4,5,6-Hexahydro-8-hydroxy-6-methyl-3-propyl-3-benzazocine (2e). As described for **2d** above, **2e**, mp 147–148° (from *n*-hexane), was obtained. Anal. ($C_{15}H_{23}NO$) C, H, N.

3-Butyl-1,2,3,4,5,6-hexahydro-8-hydroxy-6-methyl-3-benzazocine (2f). This base, prepared as described for **2d**, crystallized from Me_2CO–H_2O: mp 147–149°. Anal. ($C_{16}H_{25}NO$) C, H, N.

3-Amyl-1,2,3,4,5,6-hexahydro-8-hydroxy-6-methyl-3-benzazocine (2g). This compound, prepared as described for **2d**, melted at 127–129° after recrystallization from *n*-hexane. Anal. ($C_{17}H_{27}NO$) C, H, N.

3-Hexyl-1,2,3,4,5,6-hexahydro-8-hydroxy-6-methyl-3-benzazocine (2h) crystallized from *n*-hexane: mp 89–90°. Anal. ($C_{18}H_{29}NO$) C, H, N.

3-Heptyl-1,2,3,4,5,6-hexahydro-8-hydroxy-6-methyl-3-benzazocine (2i) was recrystallized from *n*-hexane: mp 90–92°. Anal. ($C_{19}H_{31}NO$) C, H, N.

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Synthesis and Antibacterial Evaluation of 1,2,3,4-Tetrahydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid Esters, Carbonitriles, and Carboxamides

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A series of 1,2,3,4-tetrahydro-4-oxo-1,8-naphthyridine-3-carboxylic acid esters, carbonitriles, and carboxamides (**2a–k**) was synthesized and initially evaluated (dose range 50–400 mg/kg) in mice infected with *Escherichia coli*. Only two derivatives, the ethyl and butyl esters of 1-ethyl-1,2-dihydro-4-hydroxy-7-methyl-1,8-naphthyridine-3-carboxylic acid, protected the animals against *E. coli* and several other gram-negative bacterial pathogenic infections. A pro-drug type of mechanism appears to be operable since neither agent showed in vitro activity.

Several reports have appeared in the literature describing the preparation of 1,8-naphthyridine derivatives[1] that are structural variants of the antibacterial agent 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid (nalidixic acid), first described by Lesher and coworkers.[2] The 1,8-naphthyridines produced by various modifications of the Lesher synthesis invariably possess a double bond at the 2,3 position. Comparably substituted 1,8-naphthyridines having the 2,3 position fully saturated have not been accessible through syntheses thus far reported in the literature. Furthermore, it has been shown that catalytic hydrogenation of nalidixic acid type naphthyridines results in saturation of the 5,6,7,8 positions leaving the 2,3 double bond intact.[1c] We now report the synthesis and antibacterial screening results of several 1,2,3,4-tetrahydro-4-oxo-1,8-naphthyridine-3-carboxylic acid esters, carbonitriles, and carboxamides (**2a–k**, Scheme I and Table I) in which the 2,3 bond is fully saturated. The preparation of ethyl 1-ethyl-1,2-dihydro-4-hydroxy-7-methyl-1,8-naphthyridine-3-carboxylate (**2a**) illustrates the synthetic procedure used.

Chemistry. Treatment of methyl 2-chloro-6-methylnicotinate with ethyl 3-ethylaminopropionate in refluxing dichlorobenzene resulted in dechloroamination, giving compound **1a** (Scheme I, R_1 = Et; R_2 = H; R_3 = Me; Z = CO_2Et). Cyclization of this diester under Dieckmann conditions afforded ethyl 1-ethyl-1,2-dihydro-4-hydroxy-7-methyl-1,8-naphthyridine-3-carboxylate (**2a**). A number of other 1,2-dihydro-4-hydroxy-1,8-naphthyridine-3-carboxylic acid esters (**2b–g**) were prepared in this way, starting with the appropriately substituted esters of 3-aminopropionic acid and 2-chloronicotinic acid.[3] In these examples, however, the open-chain diesters **1b–g** were prepared using just 1 equiv of aminopropionate ester and sodium carbonate in dimethylformamide. The diesters were then converted directly to the corresponding 1,8-naphthyridines **2b–g** without purification.

The infrared spectrum of **2a** in KBr indicates the compound exists almost entirely in the enolic ester form, as indicated by the presence of a conjugated chelated ester C=O band at 6.0 μ with broad chelated OH absorption in the 3.5–4.3-μ region. In solution, the presence of both enol