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INHIBITION OF MICROSOMAL BIOTRANSFORMATION BY A SERIES OF NITROGEN AND OXYGEN HETEROCYCLIC HISTAMINE H_2-ANTAGONISTS

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Abstract—A homologous series of potent, long-lasting thiazolo-pyrimidone-pyridine histamine H_2-antagonists were examined for their inhibitory effects on rat hepatic ethylmorphine N-demethylation. Inhibitory potency increased in the order: 2-pyridinyl < 3-pyridinyl < 4-pyridinyl histamine H_2-antagonist. Substitution ortho to the pyridine nitrogen decreased inhibitory potency. Hydroxylation of the pyridine heterocycle decreased inhibitory potency, whereas substituent electronic effects did not appreciably alter the inhibitory potency of these compounds. Antagonists containing oxygen heterocycles were moderately potent inhibitors compared to those containing unsubstituted pyridine as the heterocycle. A 3-(6-methylpyridine) histamine H_2-antagonist was shown to be a slightly more potent inhibitor of ethinamate metabolism than cimetidine in rats. However, unlike cimetidine, it did not inhibit the plasma half-life of antipyrine in dogs at doses that were equally efficacious in inhibiting gastric acid secretion.

Several investigators have reported on the inhibition of hepatic microsomal cytochrome P-450 biotransformation by heterocyclic compounds, especially nitrogenous aromatic heterocycles. Foremost among this class of compounds in inhibitory potency are imidazole and imidazole derivatives [1]. The inhibitory effects of several simple alkylimidazoles [2, 3], phenylimidazoles [1], benzimidazoles [4, 5], benzoxazoles, benzothiazoles [4], pyridines [6, 7], phenylpyridines, imidazopyridines [1], phenyloxazoles and phenylthiazoles [8] have also been studied. Imidazole derivatives are generally more potent inhibitors than pyridine derivatives with potency of inhibition being inversely correlated with steric hinderance of the heterocyclic nitrogen atom [1]. In addition, heterocycle lipophilicity is correlated with inhibitory potency [1, 3–7].

Histamine H_2-antagonists such as cimetidine and ranitidine contain nitrogen and oxygen heterocycles, respectively, and are inhibitors of microsomal biotransformation to various degrees [9, 10], whereas the thiazole heterocycle of nizatidine [11] and famotidine [12] does not appear to be associated with inhibition. In this paper, we present data on the inhibition of hepatic microsomal N-demethylation by nine thiazolo-pyrimidone-pyridine histamine H_2-antagonists of a homologous series and four additional heterocyclic histamine H_2-antagonists of a thiazolo-pyrimidone series.

MATERIALS AND METHODS

Histamine H_2-antagonists were synthesized at the Lilly Research Laboratories by R. P. Pioch, J. A. Nixon and L. Merritt. Ethylmorphine N-demethylation was assayed in homogenates of livers obtained from 200 g male Sprague–Dawley rats (Harlan Industries, Indianapolis). Livers were perfused with 1.15% KCl (4°), a 20% homogenate was prepared in 0.01 M phosphate buffer (pH 7.4) containing 1.15% KCl, and the homogenate was centrifuged for 20 min at 9000 *g* (4°). Ethylmorphine N-demethylation activity was quantitated by measuring formaldehyde [13] formed in 3-ml reaction mixtures that contained hepatic 9000 *g* fraction equivalent to 3 nmol of cytochrome P-450, 0.6 mmol ethylmorphine·HCl, 3 mg $MgCl_2 \cdot 6H_2O$, 3 mg sodium isocitrate and 0.8 mg $NADP^+$ in 0.1 M phosphate buffer, pH 7.4. The mixtures were incubated for 20 min at 37° in the presence of 10–2000 μM histamine H_2-antagonists. Cytochrome P-450 was measured by the method of Omura and Sato [14]. Difference spectra were recorded on a Varian Cary 210 spectrophotometer in hepatic microsomes prepared from male Sprague–Dawley rats; microsomes were suspended in 0.1 M phosphate buffer, pH 7.4, and contained 1.8 nmol cytochrome P-450/ml. Histamine H_2-antagonists were added in microliter amounts in 0.1 M phosphate buffer, pH 7.4, containing 20% ethanol to give final concentrations of 10–100 μM antagonist. A corresponding volume of solvent was added to the reference cuvette. Plasma antipyrine was assayed by HPLC according to the method of Shargel *et al.* [15] with aminopyrine as internal standard. Ethinamate (100 mg/kg, i.p.) was administered to male Sprague–Dawley rats 2 hr subsequent to oral administration of histamine H_2-antagonist. The sleeping time was taken to be the time interval from loss to regaining of the righting reflex. Antipyrine was administered intravenously at a dose of 100 mg/kg 1 hr after oral administration of histamine H_2-antagonist at multiples of the ED_{70} dose (see Table 4).

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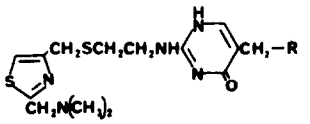
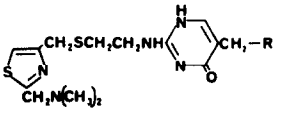
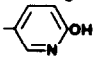
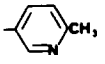
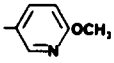
Table 1. Inhibition of ethylmorphine N-demethylation by a series of histamine H_2-antagonists



Compound	R Group	IC_{50} (μM)
I	3-(1-oxidopyridine)	>2000
II	3-(6-hydroxypyridine)	>2000
III	4-(6-hydroxypyridine)	>2000
IV	2-pyridine	1070
V	4-(6-methoxypyridine)	1050
VI	3-(6-methylpyridine)	700
VII	3-(6-methoxypyridine)	650
VIII	3-pyridine	80
IX	4-pyridine	19
X	2-tetrahydrofuran	360
XI	2-thiazole	350
XII	5-(1,3-benzodioxole)	280
XIII	2-furan	150
Ranitidine		>2000
Cimetidine		920

Table 2. Effects of substitution of 3- and 4-pyridine histamine H_2-antagonists on inhibition of ethylmorphine N-demethylation



Compound	R Group	IC_{50} (μM)
I		>2000
II		>2000
VI		700
VII		650
VIII		80
III		>2000
V		1050
IX		19

RESULTS

A series of orally active thiazolo-pyrimidone-pyridine histamine H_2-antagonists that differ only in substitution on the pyridine moiety has been synthesized. These compounds inhibit gastric acid secretion in the Heidenhain pouch dog model and have antisecretory potencies that range from 2.3 (**II**) to 19.4 (**VII**) times that of cimetidine. Since it is known that pyridine derivatives can be inhibitors of microsomal cytochrome P-450 biotransformation, it was of interest to test this homologous series for the effects upon cytochrome P-450-dependent ethylmorphine N-demethylation. The IC_{50} values shown in Table 1 indicate a wide range of effects upon N-demethylation which is dependent upon the substituents on the pyridine moiety and the location of attachment of pyridine to the base molecule. When unsubstituted pyridine was the R-group, inhibition was greatest with the 4-pyridine compound (**IX**) and least with the 2-pyridine compound (**IV**); the 3-pyridine compound (**VIII**) exhibited intermediate inhibition.

Within the substituted 3-pyridine class of compounds (Table 2), the *N*-oxide (**I**) and 6-hydroxypyridine (**II**) compounds were very poor inhibitors with the *N*-oxide exhibiting lower inhibition than the 6-hydroxypyridine compound throughout the entire 10–2000 μM concentration range. The 6-methylpyridine (**VI**) and 6-methoxypyridine (**VII**) compounds were about equipotent but substantially more inhibitory than the 6-hydroxypyridine or *N*-oxide compounds. The unsubstituted 3-pyridine compound (**VIII**) exhibited the greatest inhibitory activity in this class. The substituted 4-pyridine class of compounds exhibited a similar pattern of inhibition; the 6-hydroxypyridine compound (**III**) was the weakest inhibitor and the unsubstituted 4-pyridine compound (**IX**) was the most potent inhibitor, with the 6-methoxypyridine compound (**V**) being intermediate.

Four additional heterocycles in a thiazolo-pyrimidone series of histamine H_2-antagonists were tested for inhibition of *N*-demethylation activity (Table 1). The 2-tetrahydrofuran compound (**X**) was about 2.5 times less inhibitory than the 2-furan compound (**XIII**) but equipotent with the 2-thiazole compound (**XI**). The 1,3-benzodioxole substituent (**XII**) was more inhibitory than 2-tetrahydrofuran but less than 2-furan.

Compound **VI** was investigated further for its ability to inhibit cytochrome P-450-dependent biotransformation *in vivo*. Table 3 shows the effects of compound **VI** and cimetidine upon ethinamate sleeping times in rats. At a dose of 1 mg/kg, neither compound **VI** nor cimetidine significantly prolonged ethinamate sleeping time; however, both compounds significantly increased ethinamate sleeping time at a dose of 10 mg/kg. Although cimetidine appeared to be a more potent inhibitor on a mg/kg basis, the ratio of the molecular weight of compound **VI** to cimetidine is 1.7 which suggests that, on a molar basis, compound **VI** is a more potent inhibitor of ethinamate metabolism than cimetidine. Both compounds exhibited type II difference spectra when added to rat hepatic microsomes. Scatchard analysis of the binding data indicated biphasic binding of cimetidine to cytochrome P-450 characterized by apparent dissociation constants of 5 and 80 μM. In contrast, compound **VI** was characterized by only

Table 3. Effects of compound VI and cimetidine upon ethinamate sleeping times in male rats

Treatment	Sleeping time (min)
Control	45.9 ± 7.1 (10)
Compound VI	
1 mg/kg	58.8 ± 11.2 (8)
10 mg/kg	71.1 ± 8.2* (10)
Cimetidine	
1 mg/kg	50.7 ± 6.6 (9)
10 mg/kg	84.1 ± 9.3* (9)

Histamine H_2-antagonists were administered orally in aqueous solution 2 hr prior to i.p. administration of 100 mg/kg of ethinamate. Values are the mean ± SE of the sleeping times of the number of rats shown in parentheses.

* Significantly different from control by Student's *t*-test ($P < 0.05$).

with simple substituted imidazole and pyridine compounds has revealed that the presence of a nitrogen atom in which sp^2 or sp^3 nonbonded electrons are accessible is highly correlated with the intensity of physical interaction with cytochrome P-450 (binding spectrum) and potency of inhibition of cytochrome P-450-dependent activities [6, 8, 16, 17]. It has been suggested that the type II binding spectrum observed with a number of simple heterocyclic nucleophiles results from the interaction of heteroatom nonbonded electrons with the fifth or sixth ligand of the heme iron of cytochrome P-450. The histamine H_2-antagonists cimetidine [9] and ranitidine [10] yield type II binding spectra with hepatic microsomes, although the substituted furan heterocycle of ranitidine results in a weaker interaction than the

Table 4. Effects of compound VI and cimetidine upon plasma antipyrine half-life in dogs

Treatment	Histamine H_2-antagonist dose (mg/kg)	Antipyrine $T_{\frac{1}{2}}$ (hr)	Corr. Coef.	% Change
Control		1.79 ± 0.11	0.99	
Compound VI				
ED_{70} dose	0.7	2.06 ± 0.14	0.99	15
5 × ED_{70} dose	3.5	1.88 ± 0.23	0.98	5
10 × ED_{70} dose	7.0	1.81 ± 0.11	0.99	1
Control		2.39 ± 0.14	0.99	
Cimetidine				
ED_{70} dose	4.7	2.22 ± 0.27	0.99	7
5 × ED_{70} dose	23.5	3.29 ± 0.57	0.98	38
10 × ED_{70} dose	47.0	5.50 ± 0.50*	0.99	130

Half-life values are the mean ± SE of three female mongrel dogs per drug group. Antipyrine was administered intravenously at a dose of 100 mg/kg 1 hr after oral administration of histamine H_2-antagonist. Dogs were allowed a 3-day washout period between control and drug treatment and between histamine H_2-antagonist doses.

* Significantly different from control by Student's *t*-test ($P < 0.05$).

one apparent dissociation constant (20 μM) which was about 4-fold greater than the high-affinity dissociation constant for cimetidine. The maximal absorbance change produced by compound VI ($A_{max} = 0.011$) was about 3-fold less than that produced by cimetidine ($A_{max} = 0.034$).

Compound VI and cimetidine were also tested *in vivo* in dogs for effects upon plasma antipyrine half-life at multiples of the ED_{70} dose (the dose which caused a 70% reduction of maximal gastric acid secretion stimulated by histamine in dogs). As shown in Table 4, there was no significant effect on antipyrine half-life in dogs by compound VI at up to ten times the ED_{70} dose. While there was not a statistically significant effect of cimetidime on antipyrine half-life at up to five times the ED_{70} dose, there was a trend towards prolongation of the antipyrine half-life with cimetidine which attained significance at ten times the ED_{70} dose.

DISCUSSION

A homologous series of potent, orally active pyridine and substituted pyridine containing histamine H_2-antagonists have been evaluated for inhibition of hepatic microsomal N-demethylation. Previous work

substituted imidazole heterocycle of cimetidine. Similarly, compound VI from this series of pyridine containing histamine H_2-antagonists induced a type II binding spectrum with rat hepatic microsomes. Within this series, the greatest inhibition of cytochrome P-450-dependent ethylmorphine N-demethylation was associated with the accessibility of the pyridine nitrogen. Compound IX contained the least sterically hindered pyridine nitrogen and was the most potent inhibitor, whereas compound I, an *N*-oxide, was nearly devoid of inhibitory influence. Within the unsubstituted pyridine series of compounds (IV, VIII, IX), inhibitory potency decreased with increased steric hindrance of the pyridine nitrogen and, within the 3- and 4-pyridinyl series, substitution of the position ortho to the nitrogen decreased inhibitory activity relative to the corresponding unsubstituted compound. These steric effects are consistent with previous observations on simple substituted imidazoles [6, 17].

Inductive effects within the pyridinyl moiety do not appear to be significant in this series relative to steric effects. Those substituents which release electrons into the ring would be expected to facilitate the interaction of nitrogen nonbonded electrons with

cytochrome P-450 and thus increase the inhibitory effect. Whereas the order of electron releasing capacity generally follows the order hydroxy > methoxy > methyl, substitution with hydroxyl adjacent to the pyridine nitrogen (**II, III**) greatly reduced rather than increased the inhibitory potencies of the corresponding unsubstituted compounds (**VIII, IX**). However, addition of the hydroxyl moiety would also increase the polarity of the pyridinyl moiety which has been shown to result in a decrease of inhibition [6]. Alternatively, hydroxylation may yield the corresponding lactam which would have a very low capacity for heme–ligand interaction. There was no appreciable difference in the inhibition caused by methyl versus methoxy substitution adjacent to the pyridinyl nitrogen even though the methoxy function is more electron releasing and presumably would result in a stronger heme–ligand interaction.

The three oxygen heterocycle histamine H_2-antagonists (**X, XII, XIII**) were more potent inhibitors of N-demethylation than the 2-pyridine and substituted pyridine antagonists, indicating that nucleophilic oxygen heterocycles can also cause appreciable inhibition of cytochrome P-450 activity. The 1,3-benzodioxole structure in compound **XII** is a well-known inhibitor of cytochrome P-450 activities [18]. That the 2-furan compound (**XIII**) was twice as potent an inhibitor of N-demethylation as the 2-tetrahydrofuran compound (**X**) cannot be explained solely upon the availability of heteroatom electrons but may be influenced by π–π interactions between the heterocycle and the tetrapyrrole structure of the cytochrome P-450 heme moiety.

Compound **VI** and cimetidine were administered orally to rats, and the effects upon the ethinamate sleeping times were measured. Ethinamate is a nonbarbiturate sedative agent that is biotransformed to a nonsedative metabolite by hepatic hydroxylation [19]. Neither antagonist significantly altered ethinamate sleeping time at a 1 mg/kg dose; however, both compounds did at 10 mg/kg. The bioavailability of compound **VI** (90%, unpublished observation) is slightly greater than that of cimetidine (75%) in rats [20]; however, since the molecular weight ratio of compound **VI** to cimetidine is 1.7, the data indicate that, on a molar basis, compound **VI** is somewhat more potent than cimetidine in inhibiting hepatic hydroxylation of ethinamate in rats.

When compound **VI** and cimetidine were given to dogs at multiples of the ED_{70} doses, compound **VI** did not affect significantly the plasma half-life of antipyrine at doses of up to 10 times the ED_{70} dose, whereas cimetidine tended toward increasing the half-life of antipyrine at five times the ED_{70} dose and reached statistical significance at ten times the ED_{70} dose. In dogs, the bioavailability of compound **VI** (70%, unpublished observation) is essentially the same as that of cimetidine [20]. Furthermore, clearance of the histamine H_2-antagonists from the plasma is not a factor in the relative effects of these two compounds upon plasma antipyrine half-life since the plasma half-life of compound **VI** (4 hr) is actually longer than the 1.5 hr plasma half-life of cimetidine in dogs [21]. Thus, the margin between the dose of compound which produces effective inhibition of gastric acid secretion and the dose which produced inhibition of hepatic biotransformation (antipyrine N-demethylation) is greater for compound **VI** than for cimetidine in dogs.

REFERENCES

1. M. Murray and C. F. Wilkinson, *Chem. Biol. Interact.* **50**, 267 (1984).
2. E. D. Palmer and M. A. Cawthorne, *Xenobiotica* **4**, 209 (1974).
3. C. F. Wilkinson, K. Hetnarski, G. P. Cantwell and F. J. DiCarlo, *Biochem. Pharmac.* **23**, 2377 (1974).
4. G. M. Holder, P. J. Little, A. J. Ryan and T. R. Watson, *Biochem. Pharmac.* **25**, 2747 (1976).
5. M. Murray and A. J. Ryan, *Xenobiotica* **13**, 707 (1983).
6. J. L. Born and W. M. Hadley, *J. pharm. Sci.* **69**, 465 (1980).
7. H. G. Jonen, B. Huthwohl, R. Kahl and G. F. Kahl, *Biochem. Pharmac.* **23**, 1319 (1974).
8. L. R. Smith and C. F. Wilkinson, *Biochem. Pharmac.* **27**, 2466 (1978).
9. R. G. Knodell, J. L. Holtzman, D. L. Crankshaw, N. M. Steele and L. N. Stanley, *Gastroenterology* **82**, 84 (1982).
10. K. V. Speeg, Jr., R. V. Patwardhan, G. R. Avant, M. C. Mitchell and S. Schenker, *Gastroenterology* **82**, 89 (1982).
11. C. G. Meredith, K. V. Speeg, Jr. and S. Schenker, *Toxic. appl. Pharmac.* **77**, 315 (1985).
12. U. Klotz, P. Arvela and B. Rosenkranz, *Eur. J. clin. Pharmac.* **28**, 671 (1985).
13. T. Nash, *Biochem. J.* **55**, 416 (1953).
14. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
15. L. Shargel, W. Cheung and A. B. C. Yu, *J. pharm. Sci.* **68**, 1052 (1979).
16. C. F. Wilkinson, K. Hetnarski and T. O. Yellin, *Biochem. Pharmac.* **21**, 3187 (1972).
17. T. D. Rogerson, C. F. Wilkinson and K. Hetnarski, *Biochem. Pharmac.* **26**, 1039 (1977).
18. B. Testa and P. Jenner, *Drug Metab. Rev.* **12**, 1 (1981).
19. C. J. Parli, N. Wang and R. E. McMahon, *J. Pharmac. exp. Ther.* **180**, 791 (1972).
20. D. C. Taylor and P. R. Cresswell, *Biochem. Soc. Trans.* **3**, 884 (1975).
21. P. Johnson, R. Griffiths, R. M. Lee and M. E. Parsons, *Scand. J. Gastroent.* **17**, (Suppl. 78), 77 (1982).