also points to the presence (in the active site) of other centers for acid–base (or hydrogen bond) interaction but the evidence is not comparable to that accumulated for the AH–B entity.

Another very important unsolved problem is the actual mechanism of nerve impulse triggering. Whether it is due to molecular motions of the tastants in the receptor site or by a conformational modification of the protein (that, in turn, induces a modification of the lipidic part of the membrane) it cannot be said on the basis of our data alone. The only thing we can say on the basis of our models (of the receptor sites) is that the intensity of the taste sensation may be correlated in part with the value of the association constants compatible with a critical geometrical fitting and with the apolar nature of the upper part of the site. This finding can prove to be quite relevant for future studies on the mechanism of the triggering of the nerve impulse.

At any rate, it seems fair to conclude that the model presented in this paper can be helpful in the choice of the first synthetic targets of prospective analogues of known sweeteners or even in the design of new tastants.

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Notes

2-(Substituted phenyl)oxazolo[4,5-*b*]pyridines and 2-(Substituted phenyl)oxazolo[5,4-*b*]pyridines as Nonacidic Antiinflammatory Agents

Robert L. Clark, Arsenio A. Pessolano, Bruce Witzel, Thomas Lanza, T. Y. Shen,*

Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, New Jersey 07065

C. Gordon Van Arman,[1] and Edwin A. Risley

Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., West Point, Pennsylvania 19486.

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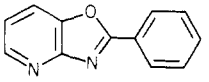
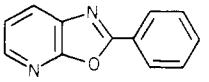
Some 2-(substituted phenyl)oxazolo[4,5-*b*]pyridines and 2-(substituted phenyl)oxazolo[5,4-*b*]pyridines have good antiinflammatory and analgesic activity. A few possess activity comparable to phenylbutazone or indomethacin without producing the irritation in the gastrointestinal tract that acidic antiinflammatory compounds cause.

In our continuing study of nonacidic antiinflammatory compounds[2] it was found that thiabendazole (4-thiazolyl-2-benzimidazole) has moderate antiinflammatory and analgesic activity.[3] In the course of testing compounds with similar ring systems (aryl-fused heterocycles) 2-phenyloxazolo[4,5-*b*]pyridine (I) and 2-phenyloxazolo-



I



II

[5,4-*b*]pyridine (II) were found to have interesting activity in the carrageenan rat foot edema assay.[4] Both of the parent compounds are known in the literature;[5,6] however, no biological activity is recorded for them. Thus, a number of substituted phenyloxazolopyridines in both series were prepared and compared, in several standard antiinflammatory assays, with known antiinflammatory compounds.

Chemistry. Fraser and Tittensor[5] prepared 2-phenyloxazolo[4,5-*b*]pyridine by heating 2-amino-3-hydroxypyridine with benzoic anhydride. We have found this procedure can be simplified by heating a mixture of the benzoic acid and the aminohydroxypyridine in polyphosphoric acid. After quenching in water, the desired compound is obtained.

It is necessary to use a stepwise procedure to prepare the oxazolo[5,4-*b*]pyridine derivatives. The amine is acylated first and ring closure of the product is accom-

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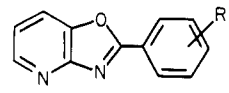
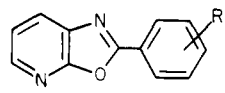
Table I. 2-(Substituted phenyl)oxazolo[4,5-*b*]pyridines



compd	R	formula	mp, °C	method of prepn	yield, %	inhibn of edema at 30 mg/kg, %
I-1	H			*a*		41
I-2	4-F	$C_{12}H_7FN_2O$	146	*b*	20	28
I-3	4-MeO	$C_{13}H_{10}N_2O_2$	175-176	A	45	14
I-4	4-Cl	$C_{12}H_7ClN_2O$	165-166	*b*	22	24
I-5	4-CF_3O	$C_{13}H_7F_3N_2O_2$	139-141	A	20	0
I-6	4-Me	$C_{13}H_{10}N_2O$	135-136	*c*	15	5
I-7	3-NO_2	$C_{12}H_7N_3O_3$	199-201	A	22	21
I-8	3-MeO	$C_{13}H_{10}N_2O_2$	112-113	A	40	14
I-9	3-Cl	$C_{12}H_7ClN_2O$	142-143	A	55	32
I-10	3-CN	$C_{13}H_7N_3O$	151-154	C	60	21
I-11	3-Br	$C_{12}H_7BrN_2O$	153-156	A	60	23
I-12	2-Cl	$C_{12}H_7ClN_2O$	92-93	A	50	38
I-13	2-F	$C_{12}H_7FN_2O$	126-127	A	50	64
I-14	2-MeO	$C_{13}H_{10}N_2O_2$	108-109	A	5	9
I-15	2-Me	$C_{13}H_{10}N_2O$	64-66	A	15	0
I-16	2-Br	$C_{12}H_7BrN_2O$	60-61	A	13	24
I-17	2-CF_3	$C_{13}H_7F_3N_2O$	63-64	A	3	19
I-18	2-MeS	$C_{13}H_{10}N_2OS$	132-136	A	50	21
I-19	2-CN	$C_{13}H_7N_3O$	166-167	C	55	62
I-20	2-F,4-Cl	$C_{12}H_6ClFN_2O$	154-156	A	48	19
I-21	2,5-F_2	$C_{12}H_6F_2N_2O$	130-132	A	16	18
I-22	2,6-F_2	$C_{12}H_6F_2N_2O$	113-114	A	38	66
I-23	2,4-F_2	$C_{12}H_6F_2N_2O$	140-142	A	24	23
I-24	2-CN,5-MeO	$C_{14}H_9N_3O_2$	196-198	C	22	3
I-25	2,6-$(CN)_2$	$C_{14}H_6N_4O$	244-246	C	11	19
I-26	2,6-Cl_2	$C_{12}H_6Cl_2N_2O$	139-140	A	16	14
I-27	2-CN,6-F	$C_{13}H_6FN_3O$	149-150	C	30	50
I-28	2-Br,5-MeO	$C_{13}H_9BrN_2O_2$	110-111	A	25	8

[a] See ref 5. [b] Prepared by method in ref 5. [c] Prepared stepwise from amide by use of $POCl_3$.

Table II. 2-(Substituted phenyl)oxazolo[5,4-*b*]pyridines



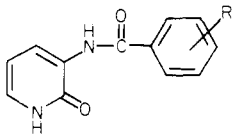
compd	R	formula	mp, °C	method of prepn	yield, %	inhibn of edema at 30 mg/kg, %
II-1	H			*a*		55
II-2	4-F	$C_{12}H_7FN_2O$	117-118	B	30	27
II-3	4-MeO	$C_{13}H_{10}N_2O_2$	146-148	B	48	29
II-4	4-Cl	$C_{12}H_7ClN_2O$	153-154	B	12	14
II-6	4-Me	$C_{13}H_{10}N_2O$	115-116	B	62	0
II-8	3-MeO	$C_{13}H_{10}N_2O_2$	107-109	B	95	0
II-9	3-Cl	$C_{12}H_7ClN_2O$	156-158	B	51	8
II-13	2-F	$C_{12}H_7FN_2O$	119-120	B	60	61
II-16	2-Br	$C_{12}H_7BrN_2O$	101-102	B	29	31
II-19	2-CN	$C_{13}H_7N_3O$	146-147	B	12	61
II-29	4-CN	$C_{13}H_7N_3O$	220-221	B	63	17
II-30	3-CF_3	$C_{13}H_7F_3N_2O$	147-149	B	50	7
II-31	3-CH_3	$C_{13}H_{10}N_2O$	101-102	B	55	14
II-32	2-NO_2	$C_{12}H_7N_3O_3$	123-125	B	25	57
II-33	2-NH_2	$C_{12}H_9N_3O$	142-144	*b*	40	13
II-34	2-Cl	$C_{12}H_7ClN_2O$	115-116	B	16	50
II-35	2-Me	$C_{13}H_{10}N_2O$	85-87	B	80	3
II-36	2,6-F_2	$C_{12}H_6F_2N_2O$	113-115	B	20	50
II-37	3-F	$C_{12}H_7FN_2O$	90-91	B	68	35
II-38	4-NO_2	$C_{12}H_7N_3O_3$	242-243	B	75	5

[a] See ref 6. [b] Prepared from II-32 by hydrogenation in 2 BA using 5% Pd/C as catalyst.

plished by the use of a dehydrating agent. Koshiro[6] distilled the compound with phosphorus pentoxide, but we found it more convenient to heat it in refluxing phosphorus oxychloride. The cyano group is unstable under these conditions, so nitriles are prepared from the bromo compounds after ring closure. The preparations can be depicted as shown in Scheme I.

Structure–Activity Relationships. All these compounds were evaluated in the carrageenan-induced foot edema assay in the rat.[4] The results are sited in Tables I–III. An attempt was made to correlate the biological activity with some parameter of the substituent on the phenyl ring. Several times equations were derived but, unfortunately, the predictive value of the correlations was

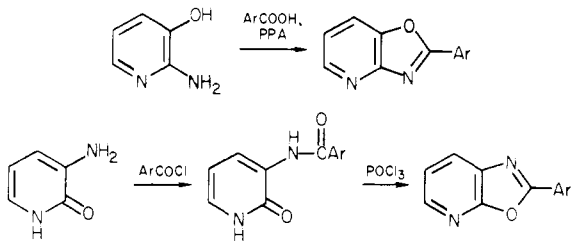
Table III. 3-(Substituted benzoyl)amino-2-pyridones



compd	R	formula	mp, °C	method of prepn	yield, %
III-2	4-F	$C_{12}H_9FN_2O_2$	200-202	B	65
III-3	4-MeO	$C_{13}H_{12}N_2O_3$	249-251	B	70
III-4	4-Cl	$C_{12}H_9ClN_2O_2$	215-217	B	75
III-6	4-Me	$C_{13}H_{12}N_2O_2$	210-211	*a*	45
III-8	3-MeO	$C_{13}H_{12}N_2O_3$	198-200	B	95
III-9	3-Cl	$C_{12}H_9ClN_2O_2$	206-207	B	45
III-13	2-F	$C_{12}H_9FN_2O_2$	223-224	B	90
III-16	2-Br[b]	$C_{12}H_9BrN_2O_2$	188	B	90
III-19	2-CN	$C_{13}H_9N_3O_2$	282	B	48
III-29	4-CN	$C_{13}H_9N_3O_2$	258-260	*a*	47
III-30	3-CF_3	$C_{13}H_9F_3N_2O_2$	205-207	B	85
III-31	3-Me	$C_{13}H_{12}N_2O_2$	199-200	B	80
III-32	2-NO_2[c]	$C_{12}H_9N_3O_4$	233-236	B	90
III-34	2-Cl	$C_{12}H_9ClN_2O_2$	192-194	B	85
III-35	2-Me	$C_{13}H_{12}N_2O_2$	170-172	B	80
III-36	2,6-F_2	$C_{12}H_8F_2N_2O_2$	247-248	B	55
III-37	3-F	$C_{12}H_9FN_2O_2$	214-215	*a*	50
III-38	4-NO_2	$C_{12}H_9N_3O_4$	307	*d*	57

[a] Like method B only used DMF and Et_3N instead of pyridine. [b] N: calcd, 9.56; found, 8.33. [c] C: calcd, 55.60; found, 55.13. [d] Like method B with added DMF to the pyridine.

Scheme I



disappointing in the follow-up studies. In both the [5,4-*b*] and [4,5-*b*] series only 2-substituents on the phenyl ring maintained or enhanced the activity of the unsubstituted compound, and only a few of these substituents gave really interesting activities. The activity-enhancing groups were the electronegative fluorine, chlorine, cyano, and nitro. Differences between the [5,4-*b*] and [4,5-*b*] isomers were not pronounced and dependent upon the assays.

Most of these compounds were also tested in the prostaglandin synthetase inhibition test in vitro.[7] Although all the compounds active in the edema assay were also active in this test, the contrary was not true. A number of compounds that were active in vitro were almost totally inactive in the edema assay. This difference may be attributed to their pharmacodynamics and a possible rapid metabolism of the compounds in vivo.

The compounds that showed the greatest activity in the edema assay (I-13, II-13, I-19, II-19, and I-22) were compared with aspirin, phenylbutazone, ibuprofen, and indomethacin in other standard antiinflammatory assays. As shown in Table IV, the oxazolopyridines were more active than phenylbutazone in the carrageenan-induced foot edema assay. In adjuvant arthritis[8,9] the compounds were several times as good as aspirin, somewhat better than ibuprofen, and of the same general potency as phenylbutazone. A similar order of activity was shown in the topical mouse ear assay.[10] As analgesics in the yeast-induced hyperesthesia assay,[11] one of the compounds (II-13) is as active as indomethacin; the others are only about one-third to one-half as active. As antipyretics,[12] the compounds were several times as active as phenylbutazone. Of particular interest was the observation that, as a group of potent antiinflammatories, they showed less tendency to cause irritation in the gastrointestinal tract[13] than the acidic antiinflammatories, indomethacin and ibuprofen. Whether this is related to their being nonacidic molecules with a lesser tendency to accumulate in the mucosa lining cells[14] remains to be ascertained. The acute oral toxicity tests in mice yielded LD_{50} values from 500 to 1500 mg/kg.

It appears that these oxazolopyridines have good activity as antiinflammatory and analgesic compounds with toxicity low enough to encourage their further trials, and more studies are in progress.

Experimental Section

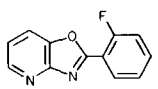
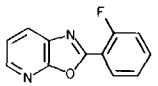
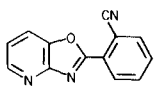
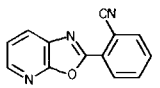
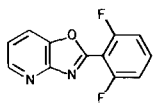
The compounds were prepared by three general methods. These methods are described by giving the preparation of specific compounds. The general method of synthesis is then designated in Tables I-III.

All melting points are corrected and were taken on a Thomas-Hoover capillary melting point apparatus. All compounds were analyzed for C, H, and N and were within 0.4% of calculated theoretical values unless designated otherwise. No attempt was made to maximize yields.

Method A. 2-(2-Fluorophenyl)oxazolo[4,5-*b*]pyridine (I-13). A mixture of 16.5 g (0.15 mol) of 2-amino-3-hydroxypyridine, 42 g (0.30 mol) of 2-fluorobenzoic acid, and 40 g of PPA was heated until the internal temperature was 185 °C (15 min) and maintained there for another 20 min. After cooling slightly, it was poured into ice water. After the PPA was dissolved a light tan solid was collected by filtration. The mother liquor was neutralized with $NaHCO_3$ to give more solid. The combined solid was extracted with $NaHCO_3$ solution and then dissolved in 100 mL of warm benzene. After concentrating to about 50 mL, Et_2O was added to the cloud point and the mixture filtered through Supercel. The filtrate was stirred with 5 g of Al_2O_3 which retained the yellow color. After concentrating, petroleum ether was added to cause crystallization: yield 16 g (50%); mp 126–127 °C.

Method B. 2-(2-Fluorophenyl)oxazolo[5,4-*b*]pyridine (II-13). To a cold solution of 3.3 g (0.03 mol) of 3-amino-2-pyridone in 75 mL of pyridine was added portionwise 6.3 g (0.04 mol) of 2-fluorobenzoyl chloride. After being stirred 15 h at room temperature the mixture was poured into ice water. The solid that separated was collected by filtration, washed with water, and

Table IV. Comparison of Oxazolopyridines in Standard Biological Assays

compd	carrageenan edema[a]	adjuvant arthritis[b]	antipyresia[c]	analgesia[d]	mouse ear topical[e]	PGE synth inhibn[f]	intestinal perforation, 3 days[g] (mg/kg)	LD_{50}, mg/kg (mice, oral)
 I-13	21[h] (4-78)	23[h] (1-6)	7[i] (3-18)	0.7[j] (6-54)	6[k] (1-6)	3[l]	0/5 (256)[m]	556
 II-13	22 (4-24)	10 (1-6)	7.6 (3-30)	1.1 (6-54)	2.1 (3-24)	3	0/5 (512)	1100
 I-19	17 (3-126)	20 (1-6)	7.7 (3-24)	0.35 (8-66)	2 (1-6)	14	1/9 (256)	1225
 II-19	11 (3-138)	44 (1-6)	13 (2-12)	0.44 (12-150)	3 (3-24)	16	0/5 (256)	1500
 I-22	22 (3-84)	7.4 (4-24)	4.6 (4-24)	0.45 (8-72)	2.4 (3-24)	15	0/5 (256)	709
aspirin	89 (3-756)	67 (3-794)	45 (3-180)	0.025 (4-390)	7.0 (3-168)	13.4	0/10 (1024)	1225
phenylbutazone	28 (3-306)	14 (3-96)	24 (4-90)	0.15 (9-72)	4.3 (3-138)	3	0/10 (400)	555
indomethacin	2.7 (3-834)	0.27 (4-468)	1.8 (3-630)	1.0 (4-546)	2.7 (3-72)	0.1	10/20 (5.2)	50
ibuprofen	15 (3-126)	27 (4-24)	5.9 (4-42)	0.85 (3-18)	5.0 (5-72)	0.4	3/5 (256)	1370

[a] Assay described in ref 4. [b] Assay described in ref 8 and 9. [c] Assay described in ref 12. [d] Assay described in ref 11. [e] Assay described in ref 10, except a mouse ear was substituted for the rat ear. [f] Assay described in ref 7. [g] Assay described in ref 13. [h] Estimated median effective dose (mg/kg) required to give 50% inhibition of swelling. The numbers in parentheses are the number of doses tried and the total number of animals used. [i] Estimated median effective dose (mg/kg) required to give a decrease of 1 °C in fever. [j] Ratio of activity compared to indomethacin. [k] Estimated median effective dose (mg) required to give 50% inhibition of weight increment per ear. [l] Estimated median effective dose (μg/mL) to give 50% inhibition of synthetase. [m] Number of perforations of ulcers per number of rats tested at indicated dosage level.

dried to give 6.4 g (90%) of 3-(2-fluorobenzoylamino)-2-pyridone, mp 218 °C. The melting point was raised to 223 °C by crystallization from absolute ethanol. A solution of 5.4 g of the above crude amide in 55 mL of $POCl_3$ was heated under reflux for 5 h. After removing most of the solvent the residue was carefully treated with ice. The solid that separated was collected and dissolved in 50 mL of warm benzene. After adding 50 mL of Et_2O the mixture was filtered through Supercel and the filtrate concentrated. The addition of petroleum ether induced crystallization: yield 3.1 g (62%); mp 119–120 °C.

Method C. 2-(2-Cyanophenyl)oxazolo[5,4-*b*]pyridine (II-19). A mixture of 2 g (0.007 mol) of II-16, 1.6 g (0.18 mol) of CuCN, and 15 mL of 1-methyl-2-pyrrolidinone was purged with N_2 and heated in an oil bath until the bath temperature was 170 °C. After 3 h the mixture was cooled and diluted with 75 mL of 10% NH_4OH. The solid that separated was collected and weighed 2.3 g. This was extracted with 100 mL of boiling CH_2Cl_2 leaving 600 mg of dark-brown material. The CH_2Cl_2 solution was concentrated and diluted with petroleum ether to the cloud point and filtered through Supercel. Crystals separated in the filtrate: yield 1.1 g (68%); mp 146–147 °C.

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Potential Anticancer Agents. 16. Methotrexate Analogues with a Modified Peptide Side Chain

Dan Carol Suster,[1] Eutanţa Tărnăuceanu, Dumitru Ionescu, Vasile Dobre, and Ion Niculescu-Duvăz*

Department of Cytostatics, Oncological Institute, Bucharest, Romania.

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Nine analogues of methotrexate, in which the side chain is modified by replacement of the terminal glutamyl moiety with other amino acids, were synthesized from 2,4-diamino-6-(chloromethyl)pteridine. None of these compounds exhibited significant activity against L1210.

The synthesis of new methotrexate (MTX) analogues, in order to find more effective anticancer agents and increase the number of compounds available for structure–activity analysis, has been one of the main goals of our laboratory since 1972.[2-4]

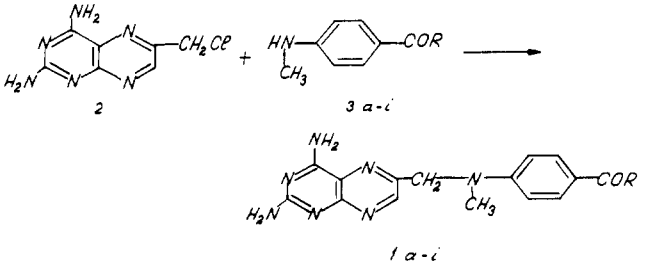
Both the position of attachment of the peptide side chain to the pteridine ring[3] and the influence of the optically active center[4,5] on the biological properties of these analogues were investigated previously. In this paper our attention is therefore focused on other changes in the peptide side chain.

Replacement of the terminal glutamic acid by other amino acids has been accomplished both in folic acid[6-8] and in aminopterin.[6,8,9] Surprisingly, only lysine, β-aminoglutaric acid, aspartic acid, α-aminoadipic acid, and α-aminopimelic acid congeners of MTX are known,[5,10-12] although a considerable number of alkyl ester and amide analogues have been prepared.[13-19] None of these is any more effective than MTX, although some of them are powerful inhibitors of dihydrofolate reductase (DHFR).[15,17] It has been demonstrated that rapid cleavage of the ester function occurs after in vivo administration.[15,16]

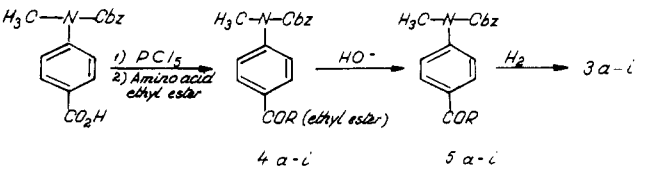
There is evidence that the glutamyl residue is involved in active transport of MTX across cell membranes.[20-23] This could explain the fact that attempts to replace it (except with α-aminoadipate and α-aminopimelate)[24] have not been successful, giving only inactive compounds. On the other hand, the role of the α- and γ-carboxyl groups is not clear.

Accordingly, we directed our efforts toward the synthesis and biological evaluation of some MTX analogues, in which the glutamyl residue was replaced with monobasic

Scheme I



Scheme II



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