Pharmacological Properties of some Derivatives of 1,3-Benzoxazine

Among the compounds synthesized in our laboratories some of the 1,3-benzoxazine derivatives summarized in Table I become of particular interest.

The majority of the compounds reported in Table I were obtained according to a general procedure. An amino ester was reductively alkylated by means of hydrogenation in the presence of Pd/C in ethanol with 1 mole of an *o*-acyl-phenol to give a substituted *o*-hydroxybenzylamine which eventually underwent electrophylic substitution or was directly condensed with phosgene to yield a 3-carbalkoxyalkyl-1,3-benzoxazin-2-one. Alkaline hydrolysis afforded the carboxylic acids which were finally treated with ammonia, amines, hydroxylamine or hydrazine in the presence of dicyclohexylcarbodiimide and *N*-hydroxysuccinimide to yield the compounds I, II, VIII, IX, X, XI, XIII–XXI (direct aminolysis of the ester group was unsatisfactory owing to a concomitant opening of the benzoxazine ring). 1,3-Benzoxazin-2-one[1] was treated in xylene with NaH and phosgene and finally with anhydrous NH_3 to give compound III. Compound XII was obtained by refluxing II in acetic anhydride. The closure of the 1,3-benzoxazine-2-thione ring (compound XXI) was achieved by the use of $CSCl_2$ instead of $COCl_2$. Finally compound XXII was obtained by addition of H_2S to compound XII, in pyridine solution.

The compounds reported were studied by employing a general screening procedure according to IRWIN[2], slightly modified in our laboratories. Some of the derivatives of 1,3-benzoxazine demonstrated an effect upon the central nervous system. Besides various other tests, reserpine-induced depression was used to study the possibility of an antagonizing property in mice[3,4].

The compounds were administered orally by gavage suspended in gum acacia (5%). The dose levels were studied on a useful logarithmic scale: 30, 100, 300 and 1000 mg/kg. For each dose level 4 male albino mice of the Swiss strain (19–21 g) were employed. The animals were dosed with 2 ml/100 g and observed 30, 90 and 300 min after drug administration.

The anti-reserpine activity was determined in groups of 5 male albino mice (19–21 g)/dose level. The drugs were administered i.p. suspended in gum acacia (5%) at the volume of 1 ml/100 g of body weight. 2 h after the drugs were given, 3 mg/kg of reserpine was injected i.p. The room temperature during the experiment was maintained at 20°. 5 h after the drug administration the animals

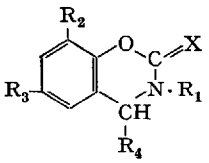
[1] F. W. HOOVER, H. B. STEVENSON and H. S. ROTHROCK, J. org. Chem. *28*, 1829 (1963).

[2] S. IRWIN, in *Screening Methods in Pharmacology* (Ed. R. A. TURNER; Academic Press, New York – London 1965), p. 26.

[3] E. COSTA, S. GARATTINI and L. VALZELLI, Experientia *16*, 461 (1960).

[4] F. SULSER, J. WATTS and B. B. BROODIE, Ann. N.Y. Acad. Sci. *96*, 279 (1962).

Table I



No.	R_1	R_2	R_3	R_4	X	m.p. (°C)	Elemental analysis					
							Found			Calculated		
							C	H	N	C	H	N
I	$-CH_2CH_2CONH_2$	H	H	H	O	165–166	60.0	5.6	12.6	60.0	5.5	12.7
II	$-CH_2CONH_2$	H	H	H	O	203–205	58.2	5.0	13.5	58.2	4.9	13.6
III	$-CONH_2$	H	H	H	O	181–183	56.3	4.3	14.6	56.2	4.2	14.6
IV	$-CH_2CH_2COOH$	H	H	H	O	130–131	60.0	5.1	6.3	59.8	5.0	6.3
V	$-CH_2COOH$	H	H	H	O	169–171	58.0	4.5	6.7	58.0	4.4	6.8
VI	$-CH_2CH_2COOC_2H_5$	H	H	H	O	42	62.9	6.1	5.6	62.6	6.0	5.6
VII	$-CH_2COOC_2H_5$	H	H	H	O	90– 91	61.5	5.5	5.8	61.3	5.6	5.9
VIII	$-CH_2CONHCH_3$	H	H	H	O	186–188	59.8	5.5	12.6	60.0	5.5	12.7
IX	$-CH_2CON(C_2H_5)_2$	H	H	H	O	90– 92	64.1	6.7	10.7	64.1	6.9	10.7
X	$-CH_2CONH\cdot CH_2CH_2OH$	H	H	H	O	183–184	57.6	5.5	11.1	57.6	5.6	11.2
XI	$-CH_2CONHNH_2$	H	H	H	O	180–183	54.5	5.1	19.0	54.3	5.0	19.0
XII	$-CH_2-C\equiv N$	H	H	H	O	136–137	63.7	4.5	14.5	63.8	4.3	14.9
XIII	$-CH_2CONHOH$	H	H	H	O	187–188	54.2	4.7	12.6	54.1	4.5	12.6
XIV	$-CH(CH_3)-CONH_2$	H	H	H	O	175–176	60.0	5.5	12.8	60.0	5.5	12.7
XV	$-CH(CH_2C_6H_5)-CONH_2$	H	H	H	O	165–168	68.7	5.4	9.0	68.9	5.4	9.5
XVI	$-CH_2CONH_2$	H	H	CH_3	O	144–145	59.8	5.3	12.4	60.0	5.5	12.7
XVII	$-CH_2CONH_2$	H	H	C_2H_5	O	185–186	61.9	6.3	12.1	61.5	6.0	12.0
XVIII	$-CH_2CONH_2$	$O\cdot CH_3$	H	H	O	219–220	55.7	5.0	11.8	55.9	5.1	11.9
XIX	$-CH_2CONH_2$	Br	Br	H	O	240–243	33.1	2.3	7.6	33.0	2.2	7.7
XX	$-CH_2CONH_2$	NO_2	NO_2	H	O	195–197	40.4	3.1	18.6	40.5	2.7	18.9
XXI	$-CH_2CONH_2$	H	H	H	S	235–237	54.1	4.6	12.5	54.0	4.5	12.6
XXII	$-CH_2CSNH_2$	H	H	H	O	180–182	53.9	4.4	12.6	54.0	4.5	12.6

Table II

Compound No.	Spontaneous behaviour	Influence on nociceptive reflexes mg/kg os	Death occurs at mg/kg os	Antireserpine activity			
				Antagonism of ptosis mg/kg i.p.	Antagonism of diarrhea mg/kg i.p.	Restored spontaneous motility mg/kg i.p.	Restored body temperature mg/kg i.p.
I	↓1	–	1000 (1/4)	≅ 300	≅ 300	300	300
II	↓2	–	> 1000	34	50	50	50
III	↑2	–	> 1000	300 ia	300 ia	300 ia	300 ia
IV	–	–	> 1000	300 ia	300 ia	300 ia	300 ia
V	–	–	> 1000	300 ia	300 ia	300 ia	300 ia
VI	↓2	–	> 1000	300 ia	300 ia	300 ia	300 ia
VII	–	–	> 1000	300 ia	300 ia	300 ia	300 ia
VIII	↓2	–	> 1000	300 ia	300 ia	300 ia	300 ia
IX	↓3	1000	> 1000	150 ia	150 ia	100 ia	150 ia
X	↓1	–	> 1000	100 ia	100 ia	100 ia	100 ia
XI	↓1	–	> 1000	300 ia	300 ia	300 ia	300 ia
XII	↓2	–	> 1000	100 ia	100 ia	100 ia	100 ia
XIII	↓1	–	> 1000	43	100	100	50
XIV	↓3	1000	> 1000	100	≅ 100	≅ 100	100
XV	–	–		150 ia	150 ia	150 ia	150 ia
XVI	↓2	1000	1000 (4/4)	42	100	100	100
XVII	↓1	–	> 1000	100 ia	100 ia	100 ia	100
XVIII	↑1	–	> 1000	150	≅ 300	≅ 300	150
XIX	↓2	–	> 1000	150 ia	150 ia	150 ia	150 ia
XX	–	–	> 1000	150 ia	150 ia	150 ia	150 ia
XXI	↓1	–	> 1000	50	100	100	100
XXII	↓3	–	300 (4/4)	100	> 100	> 100	> 100

↓ Decreased; ↑ increased; ia, inactive; spontaneous behaviour: 1 = slight, 3 = strong response.

were evaluated. The following parameters were recorded: degree of ptosis on an arbitrary scale (eye closure: 0, open; 1, one fourth; 2, one half; 3, three quarters; 4, closed; appearance of diarrhea, spontaneous motility and rectal temperature (°C). The compounds were considered active if the reserpine syndrome was antagonized. As median active dose we chose the amount of the drug in mg/kg necessary to reduce the ptosis to 50%.

Table II shows that compounds I, II, VI, VIII–XIV, XVI, XVII, XXI and XXII reduced the spontaneous activity; only III and XVIII induced excitation, whereas the rest did not influence the normal behaviour. High doses (1000 mg/kg) of IX, XIV and XVI inhibited partially nociceptive reflexes (Pinna reflex).

The most toxic derivative was XXII followed by XVI; all the others were well tolerated.

The highest anti-reserpine activity was displayed by compound II; compounds XIII, XVI and XXI were slightly less active. Some activity was present in the compounds I, XIV, XVIII and XXII. All the active compounds likewise antagonized the reserpine-induced symptoms, as for instance diarrhea, decreased motility and body temperature. Blepharoptosis decreased more readily and at smaller dose-levels.

With regard to the relationship between chemical structure and pharmacological activity, the following conclusions can be drawn. The number n of carbon atoms separating the amide group from the benzoxazine ring seems to be of essential importance (compounds I–III). Its optimum is represented by $n = 1$; since when $n = 2$ the analogue is much less active; when $n = 0$ the compound is inactive (III). A straight chain (XIV, XV), as well as a free amide group (IV–XIII), seems to be essential for activity. An exception is presented by compound XIII; however it seems possible that the –NHOH group is reduced to $-NH_2$ in the organism. A substitution in R_4 diminishes the activity or may lead to its disappearance (XVI, XVII). Similar behaviour is observed in compounds substituted in the benzene ring (XVIII–XX).

The substitution of the CO group in the benzoxazine ring by a CS group does not interfere essentially with the pharmacological activity (XXI); the substitution of the amide by a thioamide group reduces considerably the anti-reserpine activity and increases the toxicity of the compound (XXII).

Riassunto. Sono stati studiati 22 derivati della 1,3-benzossazina, 8 dei quali sono risultati attivi sulla depressione da reserpina. Tra essi il più efficace è stato il 4H-3-carbossamidometil-1,3-benzossazin-2-one. Correlando la struttura chimica all'attività farmacologica si è visto che la distanza del gruppo amidico dall'anello benzossazinico, la linearità della catena, come pure la presenza di un gruppo amidico libero sono essenziali per l'esplicarsi dell'effetto. Anche sostituzioni in R_4 o nell'anello benzenico portano a perdita dell'attività. Il carbonile in 2 può essere sostituito da un gruppo CS senza sostanziali variazioni di attività.

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