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Antibacterial Halogenoacetyl Derivatives of Amino Acids and Simple Peptides

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The vital role of D-alanine and L-lysine in the peptidoglycan crosslinking process in the bacterial cell wall prompted preparation of various small peptides incorporating these amino acids. *N*-Iodoacetyl or -bromoacetyl derivatives of the peptides were then prepared in the hope that they would serve as active-site-directed irreversible inhibitors of cell wall transpeptidases. Certain of the halogenoacetyl dipeptide esters, but not the corresponding free acids, showed slight antistaphylococcal activity. Subsequent structural variation showed that inclusion of D-alanine or L-lysine was not necessary, since antibacterial activity was at least as good when the dipeptide unit was replaced by glycylglycine or by an ω-aminoalkanoic acid. It was concluded that the observed antibacterial activity was probably not due to specific inhibition of a cell wall transpeptidase.

A vital process in the growth and division of bacterial cells is the construction of new peptidoglycan, a macromolecular framework which determines the integrity and shape of the cell wall.[1] The mucopeptide units which comprise the peptidoglycan are elaborated in the interior of the cells, but their assembly involves a peptide cross-linking reaction in the exterior situation. Penicillins and other β-lactam antibiotics are believed to exert their antibacterial action by inhibiting the membrane-bound transpeptidase enzyme or enzymes responsible for this reaction, probably by acylation at or near the active site.[2] In all bacteria so far studied cross-linking involves a C-terminal D-alanyl-D-alanine component of one peptide chain, the terminal D-alanine being displaced by the amino group of a second peptide chain, the following reaction in staphylococci being typical.

$$\text{R-L-Lys-D-Ala-D-Ala} + \text{R}^1\text{NH}_2 \xrightarrow{\text{enzyme}} \text{R-L-Lys-D-Ala-NHR}^1 + \text{D-Ala}$$

Certain small peptides structurally related to the terminal sequences involved in the cross-linking reaction act as competitive inhibitors of the DD-carboxypeptidase/transpeptidase of certain bacteria, but none of these peptides proved toxic to intact bacteria.[3] It occurred to us that such peptides might be converted into effective antibacterial agents by the attachment of a chemically reactive group, which could facilitate irreversible combination with the target enzyme according to Baker's concept[4] of active-site-directed irreversible enzyme inhibition. Other enzymes which have been irreversibly inhibited by chemically reactive peptide derivatives include elastase,[5] neurohypophyseal hormone-stimulated adenylate cyclase,[6] trypsin, and chymotrypsin.[4] The reactive groups which have proved valuable in designing effective inhibitors include *N*-bromoacetyl or -iodoacetyl groups. In this paper we describe the incorporation of such groups in certain amino acids or di- and tripeptides. In constructing the initial range of peptides, either D-alanine or L-lysine or both were included, since these two amino acids appear to play a central role in the cross-linking process in the staphylococcal cell wall. Later use was also made of D-glutamic acid, which had proved a useful C-terminus in the previously mentioned[3] reversible inhibitors, and of glycylglycine and various ω-aminoalkanoic acids as possible substitutes for a dipeptide unit.

Chemistry. Abbreviations used are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature Symbols for Amino Acid and Peptides: DCC, dicyclohexylcarbodiimide; Cbz, benzyloxycarbonyl; Bzl, benzyl; Boc, *tert*-butyloxycarbonyl; Bu, *tert*-butyl; Phth, phthalimidyl; Suc, succinyl; Ac, acetyl; TosOH, *p*-toluenesulfonic acid.

The protected peptide esters listed in Table I were synthesized by a standard coupling technique using DCC in methylene chloride. They were then converted into the corresponding compounds containing a single free amino group (Table II). For this purpose Cbz groups were removed by catalytic hydrogenation over 10% palladium on charcoal. Boc groups were removed by means of 1 equiv of *p*-toluenesulfonic acid, a procedure[7] which does not affect *tert*-butyl or benzyl ester functions, and in these cases (**18**, **22–26**, **30–32**, **34**, **35**) the product was frequently isolated as the TosOH salt.

The free amines were then treated with mixed anhydrides prepared from iodoacetic acid or bromoacetic acid to yield the *N*-halogenoacetyl derivatives listed in Table III. Finally the free acids (Table IV) were liberated by treating the *tert*-butyl esters with trifluoroacetic acid. In the case of certain lysine-containing peptides the TFA treatment also served to remove a Boc group and gave the peptides as TFA salts (**63–66** and **72**).

Table I. N-Protected Peptide Esters

No.	Structure	Yield, %	Mp, °C (solvent)[a]	$[\alpha]^{22}_D$, deg[b]	Formula[c]
1	Cbz-D-Ala-D-Ala-OBu	71	60 (EA-PE)	+54.0	$C_{18}H_{26}N_2O_5$
2	Boc-D-Ala-D-Ala-OBzl	68	69 (B-PE)	+53.1	$C_{18}H_{26}N_2O_5$
3	Cbz-D-Ala-D-Glu-$(OBu)_2$	68	Oil	+27.2	$C_{24}H_{36}N_2O_7$
4	Cbz-L-Lys(Boc)-D-Ala-OBu	66	64-65 (B-PE)	+7.3	$C_{26}H_{41}N_3O_7$
5	Cbz-L-Lys(Boc)-D-Ala-D-Ala-OBu	54	146-147 (B)	+28.1	$C_{29}H_{46}N_4O_8$
6	Boc-L-Lys(Cbz)-D-Ala-D-Ala-OBu	85	95 (EA-PE)	+23.7	$C_{29}H_{46}N_4O_8$
7	Boc-L-Lys(Ac)-D-Ala-D-Ala-OBu	82	115-116 (EA-PE)	+31.5	$C_{23}H_{42}N_4O_7$
8	Boc-L-Lys(Suc)-D-Ala-D-Ala-OBu	80	69-70 (EA-PE)	+22.3	$C_{25}H_{42}N_4O_8$
9	Boc-L-Lys(Phth)-D-Ala-D-Ala-OBu	52	132-133 (EA)	+26.6	$C_{29}H_{42}N_4O_8$
10	Boc-L-Lys(Cbz)-D-Ala-D-Glu-$(OBu)_2$	70	48	+14.5	$C_{35}H_{56}N_4O_{10}$
11	Boc-L-Lys(Phth)-D-Ala-D-Glu-$(OBu)_2$	41	130-131 (ET-PE)	+22.7	$C_{35}H_{52}N_4O_{10}$
12	Boc-L-Lys(Cbz)-NH$(CH_2)_4CO_2Bu$	97	Oil	+3.1	$C_{28}H_{45}N_3O_7$
13	Boc-L-Lys(Phth)-NH$(CH_2)_4CO_2Bu$	64	173-174 (ET-PE)	+1.2	$C_{28}H_{41}N_3O_7$
14	Boc-Gly-Gly-OBzl	88	77-79 (B-PE)		$C_{16}H_{22}N_2O_5$
15	Cbz-NH$(CH_2)_4CO_2Me$	60	Oil		$C_{14}H_{19}NO_4$
16	Cbz-NH$(CH_2)_4CO_2Bu$	87	Oil		$C_{17}H_{25}NO_4$

[a] B, benzene; EA, ethyl acetate; ET, ether; PE, petroleum ether (bp 60-80 °C). [b] Rotations were measured at concentrations of 0.9-3.0% in methanol. [c] Analyses (C, H, and N) were within ±0.4% of theory.

Table II. Deprotected Peptide Esters

No.	Structure	Yield, %	Mp, °C (solvent)[a]	$[\alpha]^{22}_D$, deg[b]	Formula[c]
17	D-Ala-D-Ala-OBu	97	Oil	+48.0	$C_{10}H_{20}N_2O_3$
18	D-Ala-D-Ala-OBzl·TosOH	82	165-166 (E-PE)	+21.8	$C_{20}H_{26}N_2O_6S$
19	D-Ala-D-Glu-$(OBu)_2$	94	Oil	+25.3	$C_{15}H_{28}N_2O_5$
20	L-Lys(Boc)-D-Ala-OBu	98	Oil	+25.2	$C_{18}H_{35}N_3O_5$
21	L-Lys(Boc)-D-Ala-D-Ala-OBu	91	Oil	+44.5	$C_{21}H_{40}N_4O_6$
22	L-Lys(Ac)-D-Ala-D-Ala-OBu·TosOH	90	93-95 (ET*)	+35.5	$C_{25}H_{42}N_4O_8S$
23	L-Lys(Suc)-D-Ala-D-Ala-OBu·TosOH·H_2O	65	80 (ET*)	+35.3	$C_{27}H_{40}N_4O_8S\cdot H_2O$
24	L-Lys(Phth)-D-Ala-D-Ala-OBu·TosOH·0.5H_2O	81	158-160 (E)	+39.1	$C_{31}H_{42}N_4O_9S\cdot 0.5H_2O$
25	L-Lys(Phth)-D-Ala-D-Glu-$(OBu)_2$·TosOH	44	97 (ET*)	+27.8	$C_{37}H_{52}N_4O_{11}S$
26	L-Lys(Phth)-NH$(CH_2)_4CO_2Bu$·TosOH	95	131 (EA-PE)	+7.4	$C_{30}H_{41}N_3O_8S$
27	Boc-L-Lys(NH_2)-D-Ala-D-Ala-OBu·0.5H_2O	95	79-80 (ET*)	+25.9	$C_{21}H_{40}N_4O_6\cdot 0.5H_2O$
28	Boc-L-Lys(NH_2)-D-Ala-D-Glu-$(OBu)_2$	99	58	+17.4	$C_{27}H_{50}N_4O_8$
29	Boc-L-Lys(NH_2)-NH$(CH_2)_4CO_2Bu$	100	Oil	+3.9	$C_{20}H_{39}N_3O_5$
30	Gly-Gly-OBzl·TosOH	57	145-148 (E-PE)		$C_{18}H_{22}N_2O_6S$
31	$H_2N(CH_2)_3CO_2Bzl$·TosOH	75	88-89 (EA)		$C_{18}H_{23}NO_5S$
32	$H_2N(CH_2)_4CO_2Me$·TosOH	81	86-87 (EA-PE)		$C_{13}H_{21}NO_5S$
33	$H_2N(CH_2)_4CO_2Bu$	99	Oil		$C_9H_{19}NO_2$
34	$H_2N(CH_2)_4CO_2Bzl$·TosOH	60	98-99 (EA-PE)		$C_{19}H_{25}NO_5S$
35	$H_2N(CH_2)_5CO_2Bzl$·TosOH	55	94-95 (EA-PE)		$C_{20}H_{27}NO_5S$

[a] E, ethanol; EA, ethyl acetate; PE, petroleum ether (bp 60-80 °C); ET*, trituration with ether. [b] Rotations were measured at concentrations of 0.5-3.0% in methanol. [c] Analyses were within ±0.4% of theory for C, H, and N and within ±0.5% for S.

Biology. All compounds listed in Tables III and IV were tested for their ability to inhibit growth of *Staphylococcus aureus* and *Escherichia coli* in Sensitivity Test Broth (Oxoid). None of the end products (Table IV) showed significant activity. Surprisingly, however, several of the intermediate esters (Table III) showed appreciable activity, especially against *S. aureus*. On the whole, the dipeptide esters tended to be more active than the tripeptide esters. The few bromoacetyl esters tested (**39**, **42**, and **44**) were slightly less active than the iodoacetyl analogues (**37**, **41**, and **43**). A further surprising feature of the antibacterial activities listed in Table III is that the constituent amino acids appear to have little importance. Indeed, central portions of the molecules of the most active compounds (**52**, **56**, **57**, and **59**) are simple nonchiral chains.

The above findings led us to doubt whether the compounds owed their activity to the mechanism originally postulated, i.e., inhibition of cell-wall transpeptidases. We therefore submitted a selection of the compounds comprising one of the most active iodoacetyl dipeptide esters (**52**), two iodoacetyltripeptide esters (**47** and **48**), and seven free iodoacetyl peptides (**62**, **67**–**71**, and **73**) to Dr. P. E. Reynolds (Department of Biochemistry, University of Cambridge, England) who kindly tested their ability to inhibit the action of three DD-carboxypeptidase/transpeptidases in releasing C-terminal D-alanine (estimated using D-amino acid oxidase) from suitable peptide substrates. Ac_2-L-Lys-D-Ala-D-Ala served as substrate for the *Streptomyces* R61 enzyme and UDP-*N*-acetylmuramyl-L-alanyl-*meso*-diaminopimeloyl-D-alanyl-D-alanine for the enzymes of *Bacillus stearothermophilus* and *Salmonella typhimurium*. The compounds were tested at concentrations of 10 and 100 μg/mL but in no case was any significant inhibition observed. We are consequently unable to explain the biochemical basis for the observed antibacterial activity of certain of the compounds in Table III.

Experimental Section

Materials and Methods. All amino acids were purchased from Sigma Chemical Co. or Fluka A.G. Basle. Melting points were determined on a Büchi Tottoli melting point apparatus and are uncorrected. All new compounds were characterized by infrared and NMR spectra. Elemental microanalyses were determined to within ±0.4% of theoretical values for C, H, and N; for S and halogen the limits were ±0.5%. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. Thin-layer chromatography was performed on silica gel plates in MeOH–DMF–NH_4OH (12:3:5) or MeOH and developed with ninhydrin, bromocresol green (0.05% in methanol), or iodine (1% solution in methanol).

The following preparative procedures are representative. Details of analogues are given in Tables I–IV.

Table III. Halogenoacetyl Peptide Esters

No.	Structure	Yield, %	Mp, °C (solvent)[a]	$[\alpha]^{22}_D$, deg[b]	Formula[c]	Antibacterial act.[d] (MIC in μg/mL) *S. aureus*	*E. coli*
36	ICH_2CO-D-Ala-OBu	90	Oil	+39.6	$C_9H_{16}INO_3$	31	>500
37	ICH_2CO-D-Ala-D-Ala-OBu	45	158–160 (B-PE)	+64.9	$C_{12}H_{21}IN_2O_4$	31	>500
38	ICH_2CO-D-Ala-D-Ala-OBzl	76	176 (EA-PE)	+67.3	$C_{15}H_{19}IN_2O_4$	7.5	>500
39	$BrCH_2CO$-D-Ala-D-Ala-OBu	44	148–150 (B-PE)	+67.8	$C_{12}H_{21}BrN_2O_4$	62	>500
40	ICH_2CO-D-Ala-D-Glu-$(OBu)_2$	60	41–42	+41.2	$C_{18}H_{31}IN_2O_6$	31	>500
41	ICH_2CO-L-Lys(Boc)-D-Ala-OBu	59	150–151 (B-PE)	+0.5	$C_{20}H_{36}IN_3O_6$	15	>500
42	$BrCH_2CO$-L-Lys(Boc)-D-Ala-OBu	61	140–141 (B-PE)	+2.2	$C_{20}H_{36}BrN_3O_6$	62	>500
43	ICH_2CO-L-Lys(Boc)-D-Ala-D-Ala-OBu	40	136 (B-PE)	+16.3	$C_{23}H_{41}IN_4O_7$	250	500
44	$BrCH_2CO$-L-Lys(Boc)-D-Ala-D-Ala-OBu	61	125–127 (B)	+25.8	$C_{23}H_{41}BrN_4O_7$	500	500
45	ICH_2CO-L-Lys(Ac)-D-Ala-D-Ala-OBu	55	162–164 (EA-PE)	+31.3	$C_{20}H_{35}IN_4O_6$	500	>500
46	ICH_2CO-L-Lys(NH_2)-D-Ala-D-Ala-OBu·TosOH	86	103–104 (E-EA)	+12.0	$C_{25}H_{41}IN_4O_8S$	250	>500
47	ICH_2CO-L-Lys(Suc)-D-Ala-D-Ala-OBu	80	167–169 (EA-PE)	+17.2	$C_{22}H_{35}IN_4O_7$	>500	>500
48	ICH_2CO-L-Lys(Phth)-D-Ala-D-Ala-OBu	85	180–182 (EA)	+9.7 (HOAc)	$C_{26}H_{35}IN_4O_7$	62	>500
49	ICH_2CO-L-Lys(Phth)-D-Ala-D-Glu-$(OBu)_2$	50	152–153 (EA)	+11.4	$C_{32}H_{45}IN_4O_9$	62	>500
50	ICH_2CO-L-Lys(Phth)-$NH(CH_2)_4CO_2Bu$·0.5H_2O	60	138 (EA)	−13.5	$C_{25}H_{34}IN_3O_6$	>500	>500
51	Boc-L-Lys($COCH_2I$)-D-Ala-D-Ala-OBu	65	85 (EA-PE)	+21.4	$C_{23}H_{41}IN_4O_7$	125	>500
52	ICH_2CO-Gly-Gly-OBzl	75	171–172 (E-EA)		$C_{13}H_{15}IN_2O_4$	0.9	62
53	ICH_2CO-Gly-OBzl	75	111 (B)		$C_{11}H_{12}INO_3$	7.5	125
54	ICH_2CO-$NH(CH_2)_2CO_2Bzl$	60	36–38 (PE*)		$C_{12}H_{14}INO_3$	7.5	500
55	ICH_2CO-$NH(CH_2)_3CO_2Bzl$	40	50 (EA-PE)		$C_{13}H_{16}INO_3$	7.5	250
56	ICH_2CO-$NH(CH_2)_4CO_2Bzl$	40	45–46 (EA-PE)		$C_{14}H_{18}INO_3$	<0.4	62
57	ICH_2CO-$NH(CH_2)_4CO_2Me$	75	42 (EA-PE)		$C_8H_{14}INO_3$	3.7	7.5
58	ICH_2CO-$NH(CH_2)_4CO_2Bu$	70	58 (EA-PE)		$C_{11}H_{20}INO_3$	7.5	500
59	ICH_2CO-$NH(CH_2)_5CO_2Bzl$	60	53–54 (EA-PE)		$C_{15}H_{20}INO_3$	3.7	62

[a] B, benzene; E, ethanol; EA, ethyl acetate; PE, petroleum ether (bp 60–80 °C); PE*, trituration with petroleum ether. [b] Rotations were measured at concentrations of 0.5–3.0% in methanol unless indicated otherwise. [c] Analyses were within ±0.4% of theory for C, H, and N and within ±0.5% for halogens. [d] 0.5-mL vol of Sensitivity Test Broth (Oxoid Ltd., Basingstoke, England) inoculated with 0.2 mL of 10^{-4} dilution of 18-h broth culture. Minimum inhibitory concentration (MIC) recorded after 18-h incubation.

Table IV. Halogenoacetyl Peptides

No.	Structure	Yield, %	Mp, °C (solvent)[a]	$[\alpha]^{22}_D$, deg[b]	Formula[c]	Antibacterial act. (MIC in μg/mL)[d] *S. aureus*	*E. coli*
60	ICH_2CO-D-Ala-D-Ala	90	175 (ET*)	+60.0	$C_8H_{13}IN_2O_4$	500	>500
61	$BrCH_2CO$-D-Ala-D-Ala	85	164 (ET*)	+68.0	$C_8H_{13}BrN_2O_4$	500	>500
62	ICH_2CO-D-Ala-D-Glu	80	Oil	+46.9	$C_{10}H_{15}IN_2O_6$	500	>500
63	ICH_2CO-L-Lys(NH_2)-D-Ala·CF_3CO_2H	62	55 (ET*)	−12.0	$C_{12}H_{21}F_3IN_3O_6$	>500	>500
64	$BrCH_2CO$-L-Lys(NH_2)-D-Ala·CF_3CO_2H	78	51–53 (ET*)	−11.3	$C_{13}H_{21}BrF_3N_3O_6$	>500	>500
65	ICH_2CO-L-Lys(NH_2)-D-Ala-D-Ala·CF_3CO_2H	90	190 (ET*)	+2.0 (HOAc)	$C_{16}H_{26}F_3IN_4O_7$	>500	>500
66	$BrCH_2CO$-L-Lys(NH_2)-D-Ala-D-Ala·CF_3CO_2H	100	100 (ET*)	−26.6 (HOAc)	$C_{16}H_{26}BrF_3N_4O_7$	>500	>500
67	ICH_2CO-L-Lys(Ac)-D-Ala-DAla	98	212 (E-ET)	+4.1	$C_{16}H_{27}IN_4O_6$	>500	>500
68	ICH_2CO-L-Lys(Suc)-D-Ala-D-Ala	90	125–127 (ET*)	+3.6 (HOAc)	$C_{18}H_{27}IN_4O_7$	500	>500
69	ICH_2CO-L-Lys(Phth)-D-Ala-D-Ala	98	189–190 (EA)	+5.9 (HOAc)	$C_{22}H_{27}IN_4O_7$	500	>500
70	ICH_2CO-L-Lys(Phth)-D-Ala-D-Glu	90	135 (ET*)	+6.8	$C_{24}H_{29}IN_4O_9$	250	>500
71	ICH_2CO-L-Lys(Phth)-$NH(CH_2)_4CO_2H$	85	124 (ET*)	−13.6	$C_{21}H_{26}IN_3O_6$	>500	>500
72	H_2N-L-Lys($COCH_2I$)-D-Ala-D-Ala·CF_3CO_2H	80	110 (E-ET)	+31.9 (HOAc)	$C_{16}H_{26}F_3IN_4O_7$	500	>500
73	$ICH_2CONH(CH_2)_4CO_2H$	60	94–95 (ET)		$C_7H_{12}INO_3$	125	500

[a] E, ethanol; EA, ethyl acetate; ET, ether; ET*, trituration with ether. [b] Rotations were measured at concentrations of 0.5–3.0% in methanol unless indicated otherwise. [c] Analyses were within ±0.4% of theory for C, H, and N and within ±0.5% for halogens. [d] 0.5-mL vol of Sensitivity Test Broth (Oxoid Ltd., Basingstoke, England) inoculated with 0.2 mL of 10^{-4} dilution of 18-h broth culture. Minimum inhibitory concentration (MIC) recorded after 18-h incubation.

N-Protected Peptide Esters (Table I). The N-protected amino acid (0.01 mol) was dissolved in dry CH_2Cl_2 (50 mL) and a solution of amino acid ester *p*-tosylate (0.01 mol) in dry CH_2Cl_2 (25 mL) containing N-methylmorpholine (0.01 mol) was added. The solution was cooled to 0 °C and dicyclohexylcarbodiimide (DCC) (0.01 mol) was added. The solution was allowed to warm to room temperature, stirred overnight, and filtered, and the filtrate was washed with 20% citric acid solution (2 × 50 mL) and 3% sodium bicarbonate (3 × 50 mL). The organic phase was dried over $MgSO_4$, the solvent evaporated, and the residual oil crystallized from the appropriate solvent (Table I).

Removal of N-Protecting Groups (Table II). (a) The *N*-Cbz peptide *tert*-butyl ester (0.01 mol) was dissolved in MeOH (10 mL) and hydrogenated at ambient temperature and pressure over 10% Pd/C (500 mg). When the theoretical amount of hydrogen had been adsorbed, the solution was filtered through Kieselguhr and the solvent evaporated to leave the free amine.

(b) The *N*-Boc peptide ester (0.01 mol) in EtOH (50 mL) was cooled to 0 °C and treated dropwise with *p*-toluenesulfonic acid (0.01 mol) in EtOH (10 mL). The solution was allowed to warm to room temperature; then the solvent was evaporated and the residual oil triturated with ether to give the TosOH salt of the amine.

Introduction of *N*-Halogenoacetyl Groups (Table III). Iodo- or bromoacetic acid (0.01 mol) in dry CH_2Cl_2 (50 mL) containing *N*-methylmorpholine (0.01 mol) was cooled to −5 °C and isobutyl chloroformate (0.01 mol) was added over 15 min. A solution of the amine (0.01 mol) (or of the amine TosOH salt plus *N*-methylmorpholine) in CH_2Cl_2 was added portionwise at −5 °C and the mixture was stirred thus for 30 min, then allowed to warm to room temperature, and washed with 20% citric acid followed by 3% sodium bicarbonate solution. The organic phase was dried ($MgSO_4$) and evaporated to leave the desired amide.

Halogenoacetyl Peptides (Table IV). The *N*-halogenoacetyl peptide *tert*-butyl ester (0.01 mol) was stirred with trifluoroacetic acid (2 mL) at room temperature for 1 h. Dry ether (50 mL) was added, the suspension was stirred for a further hour, and the solid product was collected by filtration.

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Synthesis of β-Spiro[pyrrolidinoindolines], Their Binding to the Glycine Receptor, and in Vivo Biological Activity

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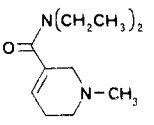
A series of β-spiro[pyrrolidinoindolines], **3a–d**, was prepared and evaluated for their ability to bind to the glycine receptor. These compounds were also tested in vivo to determine if they would produce convulsant or anxiolytic effects. The target indolines were chosen because they represent rings A, B, E, and a portion of ring C of strychnine. Results of this study indicate that, in this series, an acetylindoline in the endo configuration and a tertiary amine, such as that of the pyrrolidine ring nitrogen, are required for biological activity. In all of the cases studied, the activity was of a convulsant rather than a relaxant nature. Excellent correlation was found to exist between the binding affinities to the strychnine site of the glycine receptor and clonic convulsions (ED_{50}) and death (LD_{50}) in the mouse.

The excitatory actions of strychnine on the central nervous system (CNS) have been attributed to its ability to interact with postsynaptic receptors which are sensitive to glycine, thereby blocking the inhibitory effects of this amino acid neurotransmitter.[1] Snyder et al. have demonstrated that [^{3}H]strychnine binds to synaptosomal membrane fragments obtained from the rat brain stem and spinal cord[2] and that the regional location of this binding within the CNS correlates with endogenous glycine concentrations.[3]

The inhibition of [^{3}H]strychnine binding to synaptosomal preparations has thus far been reported for glycine and β-alanine,[2] a group of strychnine alkaloids,[4] and a series of 1,4-benzodiazepine derivatives.[5] The convulsant activities of the series of strychnos alkaloids studied were found to be highly correlated with their respective binding affinities. Likewise, Snyder was able to show that the rank order of potency of a series of 21 1,4-benzodiazepines in a variety of animal and human pharmacological and behavioral tests correlated with the ability of these compounds to displace bound [^{3}H]strychnine. This observation led to the hypothesis that the 1,4-benzodiazepines exert their anxiolytic, muscle-relaxant, and anticonvulsant effects by mimicing glycine at its receptor site.[5]

The purpose of this study was twofold. First, we were interested in determining what structural features within the strychnine molecule were responsible for the high binding affinity to the glycine receptor and, secondly, to establish if a portion of the strychnine skeleton would bind to this receptor and elicit anxiolytic, rather than convulsant, effects. Although the likelihood of finding a strychnine antagonist (glycine agonist) within the framework of the strychnine molecule might seem remote, such an approach has been successful elsewhere. The tetrahydropyridine, 1, which constitutes a portion of the LSD molecule, 2, was recently reported to bind with high affinity to the LSD receptor and, more importantly, was found to antagonize the behavioral effects of LSD.[6]



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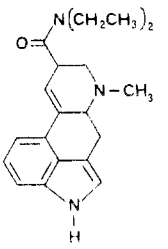

2

Furthermore, Rees and Smith[7] reported that certain seco derivatives of strychnine, strychnidine, and brucidine,