POTENTIAL HYPNOTICS AND ANXIOLYTICS: 8-CHLORO-6-(2-CHLOROPHENYL)-1-[4-(2-METHOXYETHYL)-PIPERAZINO]-METHYL-4*H*-*s*-TRIAZOLO[4,3-*a*]-1,4-BENZODIAZEPINE AND RELATED COMPOUNDS*

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Ethyl esters of 4-substituted piperazinoacetic acids *IIIa–f* were prepared by alkylation of 1-(ethoxycarbonylmethyl)piperazine with 2-methoxyethyl bromide, 2-ethoxyethyl bromide, 2-methylthioethyl chloride, 2-phenoxyethyl bromide and 2-phenylthioethyl bromide or by reactions of 1-(3-methoxypropyl)piperazine and 1-(2-methylthioethyl)piperazine with ethyl chloroacetate. Reactions of the esters with hydrazine hydrate gave the hydrazides *IVa–f*. Their treatment with 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-thione resulted in the title compound *Ia* and analogues *Ib–f*. 1-(4-Methylpiperazinomethyl) derivatives *Ig* and *IIg* were similarly prepared from 4-methylpiperazinoacetic acid hydrazide (*IVg*). Compound *Ig* showed significant central depressant and anticonvulsant (electroshock) activity in mice. The enlargement of the substituent in position 4 of the piperazine residue results in an important decrease of these activities.

In the preceding communication of this series[1] we have described the synthesis of 8-chloro-6-(2-chlorophenyl)-1-[4-(2-methoxyethyl)piperazino]-4*H*-*s*-triazolo[4,3-*a*]-1,4-benzodiazepine and of some related compounds out of which especially the named basic substance showed a high degree of central depressant and anticonvulsant activity in mice and resembled by its pharmacological profile the very potent anxiolytic and hypnotic agents of the type of alprazolam[2] and triazolam[3]. The aim of the present work was to investigate the influence of the insertion of one methylene group between the position 1 of the 4*H*-*s*-triazolo[4,3-*a*]-1,4-benzodiazepine skeleton and the nitrogen atom of the piperazine residue on the activity. For this reason, the synthesis of the title compound *Ia* and its analogues *Ib–f* has been carried out and the products were subjected to pharmacological screening for the anticonvulsant and discoordinating effects.

Out of compounds of this type with a 1-piperazinomethyl residue the literature[4,5] described only one, 8-chloro-6-phenyl-1-(4-methylpiperazinomethyl)-4*H*-*s*-triazolo-[4,3-*a*]-1,4-benzodiazepine (*IIg*), which was obtained by substitution reactions of 1-chloromethyl- (ref.[4]) or 1-bromomethyl-8-chloro-6-phenyl-4*H*-*s*-triazolo[4,3-*a*]-

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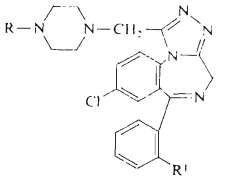
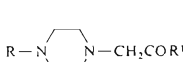
-1,4-benzodiazepine[5] with 1-methylpiperazine. In the effort to prepare compound *IIg* by an alternative method we carried out an attempt at acylating 7-chloro-2--hydrazino-5-phenyl-3*H*-1,4-benzodiazepine[6] with ethyl 2-(4-methylpiperazino)acetate (*IIIg*) (ref.[7,8]) in boiling chloroform. The 4-methylpiperazinoacethydrazide derivative (which would be a suitable precursor for the cyclization to the triazolobenzodiazepine *IIg*) was not obtained at all; the only identified product, which was isolated in a rather important amount, was the symmetrically disubstituted hydrazine *V*.



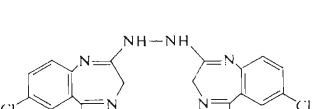
I, R¹ = Cl
II, R¹ = H

In Formulae *I*–*IV*:
a, R = $(CH_2)_2OCH_3$
b, R = $(CH_2)_3OCH_3$
c, R = $(CH_2)_2OC_2H_5$
d, R = $(CH_2)_2SCH_3$
e, R = $(CH_2)_2OC_6H_5$
f, R = $(CH_2)_2SC_6H_5$
g, R = CH_3

This compound is known[6] and it is easily formed by heating of the starting monosubstituted hydrazine. We have found an alternative and efficient synthesis of compound *IIg* in the reaction of 7-chloro-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2--thione[9–11] with 2-(4-methylpiperazino)acethydrazide (*IVg*) (ref.[12,13]) in boiling 1-butanol. Characterization of compound *IIg* was completed by recording the ^1H NMR spectrum and by preparing the fumarate. The new 1-(4-methylpiperazino)-methyl analogue of triazolam *Ig* was prepared by a similar way, *i.e.* by a reaction of 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-thione[9–11] with the hydrazide *IVg* in boiling 1-butanol.



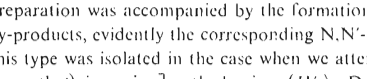
III, R¹ = OC_2H_5
IV, R¹ = $NH-NH_2$



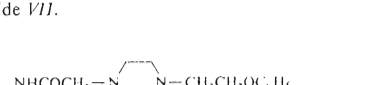
V

Similar methods like in the synthesis of compounds *Ig* and *IIg* were then used for preparing the title compound *Ia* and analogues *Ib*–*f*. To this end it was necessary in the first line to prepare the corresponding piperazine intermediates *IIIa*–*f* and *IVa*–*f* which have not been described yet. The starting substance was 1-(ethoxycarbonylmethyl)piperazine[14–16] which was alkylated with 2-methoxyethyl bromide[17],

2-ethoxyethyl bromide[18], 2-methylthioethyl chloride[19], 2-phenoxyethyl bromide[20] and 2-phenylthioethyl bromide[21] in boiling acetone in the presence of potassium carbonate (method *A*). There resulted the 4-substituted ethyl piperazinoacetates *IIIa* and *IIIc–f* which were characterized by spectra and in the case of compound *IIIa* also by a crystalline salt. In two cases the reversed approach was used: 1-(3-methoxypropyl)piperazine[1] and 1-(2-methylthioethyl)piperazine[1] were treated with ethyl chloroacetate in boiling ethanol in the presence of sodium hydrogen carbonate (method *B*); the piperazinoacetates *IIIb* and *IIId* were obtained. The esters *IIIa–f* afforded by reactions with 100% hydrazine hydrate in boiling ethanol (method *C*) the hydrazindes *IVa–f* which were characterized partly in the form of salts, partly by spectra. Their preparation was accompanied by the formation of small amounts of higher melting by-products, evidently the corresponding N,N'-diacylhydrazines[22]. Compound *VI* of this type was isolated in the case when we attempted to distill the crude 2-[4-(2-phenoxyethyl)piperazino]acethydrazine (*IVe*). Decomposition took place and the product *VI* was isolated as a crystalline tetramaleate. Crystallization of the dimaleate of the hydrazide *IVf* from acetone afforded the dimaleate of the isopropylidenehydrazide *VII*.



VI



VII

Reactions of 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-thione (ref.[9–11]) with hydrazides *IVa–f* in boiling 1-butanol (method *D*) afforded the final products *Ia–f*. The crude products were purified by chromatography on silica gel and their structures were corroborated by spectra (especially the ^{1}H NMR spectra). All of the bases *Ia–f* afforded by neutralization with fumaric acid the fumarates with the ratio of 2 molecules of the base per 3 molecules of fumaric acid (sesquifumarates) which were used for pharmacological tests. Compounds *Ia–f* and the intermediates *IIIa–f* and *IVa–f* are assembled in Table I with the usual experimental data. The Experimental contains then only examples of the preparations carried out by the general methods *A – D*.

TABLE I

8-Chloro-6-(2-chlorophenyl)-1-(piperazinomethyl)-4*H*-*s*-triazolo[4,3-*a*]-1,4-benzodiazepines (*Ia* to *f*) and Intermediates *IIIa*–*f* and *IVa*–*f*

Compound	Method (yield, %)	M.p., °C (Solvent) or b.p., °C/kPa	Formula (mol. wt.)	Calculated/Found		
				% C	% H	% N
IIIa	*A*[a] (44)	120–130/0·36	$C_{11}H_{22}N_2O_3$ (230·3)	57·36 57·62	9·63 9·67	12·16 12·36
IIIa-2 HM[b]		144–145 (ethanol)	$C_{19}H_{30}N_2O_{11}$ (462·4)	49·34 49·64	6·54 6·60	6·06 5·84
IIIb	*B*[c] (82)	124–126/0·4	$C_{12}H_{24}N_2O_3$ (244·3)	58·99 58·31	9·90 9·89	11·47 11·45
IIIc	*A* (45)	120–122/0·3	$C_{12}H_{24}N_2O_3$ (244·3)	58·99 58·76	9·90 9·84	11·47 12·19
IIId	*B*[d] (60)	130–132/0·27	$C_{11}H_{22}N_2O_2S$ (246·4)	53·62 54·19	9·00 9·00	11·37 11·34
IIIe	*A* (53)	160–165/0·4[e]	$C_{16}H_{24}N_2O_3$ (292·4)	65·73 65·15	8·27 8·37	9·58 9·58
IIIf	*A* (38)	170–175/0·4[f]	$C_{16}H_{24}N_2O_2S$[g] (308·4)	62·30 62·65	7·84 7·68	9·08 9·71
IVa-3 M[h]	*C*[i] (80)	126–127 (methanol)	$C_{21}H_{32}N_4O_{14}$ (564·5)	44·68 44·22	5·71 5·63	9·93 9·71
IVb-3 M[h]	*C*[j] (80)	108–110 (ethanol)	$C_{22}H_{34}N_4O_{14}$ (578·5)	45·67 45·73	5·92 6·14	9·69 9·24
IVc-3 M[h]	*C*[k] (45)	120–123 (ethanol)	$C_{22}H_{34}N_4O_{14}$ (578·5)	45·67 45·58	5·92 6·09	9·69 9·98
IVd	*C*[a] (91)	79–81 (ethanol)	$C_9H_{20}N_4OS$[l] (232·3)	46·52 46·39	8·68 8·73	24·12 24·72
IVd-3 M[h]		119–121 (ethanol)	$C_{21}H_{32}N_4O_{13}S$[m] (580·6)	43·44 43·75	5·56 5·62	9·65 8·89
IVe	*C* (56)	83·5–84·5[n] (benzene–hexane)	$C_{14}H_{22}N_4O_2$ (278·3)	60·41 60·26	7·97 8·02	20·13 20·49
IVf	*C* (70)	81–82[o] (benzene–light petroleum)	$C_{14}H_{22}N_4OS$[p] (294·4)	57·11 56·72	7·53 7·56	19·03 18·94
Ia-1·5 F[q]	*D*[r] (57)	213–216[s] (aqueous ethanol)	$C_{24}H_{26}Cl_2N_6O$[t] + 1·5 $C_4H_4O_4$ (659·6)	54·64 54·82	4·89 4·83	12·74 12·83
Ia-2 OxH[u]		207–210 (aqueous ethanol)	$C_{28}H_{30}Cl_2N_6O_9$[v] + H_2O (683·5)	49·21 49·73	4·72 4·52	12·30 12·20

TABLE I
(*Continued*)

Compound	Method (yield, %)	M.p., °C (Solvent) or b.p., °C/kPa	Formula (mol. wt.)	Calculated/Found % C	% H	% N
Ih—1·5 F[q] [*]	*D* (96)	233—236[w] (aqueous ethanol)	$C_{25}H_{28}Cl_2N_6O^x$ · 1·5 $C_4H_4O_4$ (673·6)	55·28 55·24	5·09 5·19	12·48 12·46
Ic-1·5 F[q]	*D* (74)	194—195[y,z] (aqueous ethanol)	$C_{25}H_{28}Cl_2N_6O^{aa}$ · 1·5 $C_4H_4O_4$ (673·6)	55·28 55·27	5·09 5·14	12·48 12·46
Id	*D*[a] (95)	158—160 (ethanol)	$C_{24}H_{26}Cl_2N_6S^{bb}$ (501·5)	57·48 57·30	5·23 5·32	16·76 17·10
Id-1·5 F[q]		218—222[y] (aqueous ethanol)	$C_{24}H_{26}Cl_2N_6S^{cc}$ · 1·5 $C_4H_4O_4$ (675·6)	53·33 53·33	4·77 4·82	12·44 12·62
Ie-1·5 F[q]	*D* (95)	167—170[dd] (ethanol–ether)	$C_{29}H_{28}Cl_2N_6O^{ee}$ · 1·5 $C_4H_4O_4$ (721·6)	58·26 58·35	4·75 4·83	11·65 11·76
If-1·5 F[q]	*D* (27)	206—208[ff] (aqueous ethanol)	$C_{29}H_{28}Cl_2N_6S^{gg}$ · 1·5 $C_4H_4O_4$ (737·7)	56·99 57·31	4·65 4·66	11·39 11·25

[a] See Experimental. [b] Di(hydrogen maleate). [c] IR spectrum ($CHCl_3$): 1 030, 1 165, 1 298 (C—O in ester), 1 110 (R—OR'), 1 735 (C=O of ester), 2 820 cm^{-1} (CH_2—N); ^1H NMR spectrum: δ 4·17 (q, J = 7·0 Hz, 2 H, $COOCH_2$), 3·46 (t, J = 7·0 Hz, 2 H, CH_2O in ethoxyl), 3·49 (q, J = 7·0 Hz, 2 H, remaining CH_2N), 3·18 (s, 2 H, NCH_2O), 2·58 (bs, 8 H, 4 CH_2N of piperazine), 2·50 (t, 2 H, remaining CH_2N), 1·25 (t, 3 H, CH_3 in ester ethoxyl), 1·18 (t, 3 H, CH_3 in ether ethoxyl). [d] Was prepared also using method *A* in a yield of only 14%, b.p. 140—142°C/0·33 kPa. [e] IR spectrum ($CHCl_3$): 1 030, 1 170, 1 300 (C—O in ester), 1 240 (Ar—O—R), 1 735 (C=O in ester), 2 820 cm^{-1} (CH_2—N); ^1H NMR spectrum: δ 6·70—7·40 (m, 5 H, C_6H_5), 4·16 (q, J = 7·0 Hz, 2 H, $COOCH_2$), 4·08 (t, J = 7·0 Hz, 2 H, $ArOCH_2$), 3·19 (s, 2 H, NCH_2CO), 2·80 (t, 2 H, CH_2N outside of piperazine), 2·60 (bs, 8 H, 4 CH_2N of piperazine), 1·25 (t, 3 H, CH_3 in ethyl). [f] IR spectrum ($CHCl_3$): 1 165 (C—O in ester), 1 477, 1 580 (Ar), 1 740 (C=O in ester), 2 818 cm^{-1} (CH_2—N); ^1H NMR spectrum: δ 7·20 (m, 5 H, C_6H_5), 4·18 (q, 2 H, $COOCH_2$), 3·20 (s, 2 H, NCH_2CO), 3·02 (t, 2 H, CH_2S), c. 2·60 (m, 10 H, remaining 5 CH_2N), 1·25 (t, 3 H, CH_3). [g] Calculated: 10·40% S; found: 10·26% S. [h] Trimaleate. [i] The crude base was distilled, b.p. 160—163°C/0·26 kPa; the distillate crystallized to a low-melting solid. [j] The crude base melted at 68—70°C (benzene–light petroleum). [k] The crude base was distilled, b.p. 140—147°C/0·27 kPa; the distillate crystallized to a low-melting solid. [l] Calculated: 13·80% S; found: 13·50% S. [m] Calculated: 5·52% S; found: 5·47% S. [n] IR spectrum: 691, 755 (5 adjacent Ar—H), 1 005, 1 035, 1 165, 1 248 (Ar—O—R), 1 472, 1 490, 1 500, 1 582, 1 598, 3 020, 3 038, 3 050 (Ar), 1 535, 1 658, 1 677 (CONH), 2 785, 2 815 (ArOR, N—CH_2). 3 180, 3 290, 3 382 cm^{-1} (NH_2, NH); ^1H NMR spectrum: δ 8·15 (bs, 1 H, CONH), 6·80—7·50 (m, 5 H, C_6H_5), 4·12 (t, J = 6·0 Hz, 2 H, CH_2O), 3·90 (bs, 2 H, NH_2), 3·12 (s, 2 H, NCH_2CO), 2·85 (t, J = 6·0 Hz, 2 H, CH_2N

TABLE I
(*Continued*)

outside of piperazine), 2·64 (s, 8 H, 4 CH_2N of piperazine). [o] IR spectrum: 690, 740 (5 adjacent Ar—H), 1 532, 1 575, 1 665 (CONH), 2 767, 2 813 (CH_2—N), 3 163, 3 250, 3 295 cm^{-1} (NH_2 and NH); [p] ^1H NMR spectrum: δ 8·12 (bs, 1 H, NH), 7·10—7·50 (m, 5 H, C_6H_5), 3·88 (bs, 2 H, NH_2), 3·08 (s, 2 H, NCH_2CO), 3·05 (m, 2 H, CH_2S), 2·65 (m, 2 H, CH_2N outside of piperazine), 2·54 (s, 8 H, 4 CH_2N of piperazine). [p] Calculated: 10·89% S; found: 10·68% S. [q] Sesquifumarate. [r] The base was released from the salt and used for recording the ^1H NMR spectrum: δ 8·50 (d, J = 8·5 Hz, 1 H, 10-H), 7·20—7·70 (m, 5 H, 9-H and 4 ArH of chlorophenyl), 7·18 (d, J = = 2·0 Hz, 1 H, 7-H), 5·55 and 4·20 (ABq, J = 13·0 Hz, 1 + 1 H, 4,4-H_2), 3·80 and 3·50 (ABq, J = 13·0 Hz, 1 + 1 H, 1-CH_2—N), 3·55 (t, 2 H, CH_2O), 3·40 (s, 3 H, CH_3O), c. 2·60 (bm, 10 H, 5 CH_2N). [s] Mass spectrum, m/z (%): 484 (M^+ corresponding to $C_{24}H_{26}Cl_2N_6O$), 127 (25), 114 (58), 111 (100), 97, 95 (93), 69 (31), 56 (38). [t] Calculated: 10·75% Cl; found: 11·00% Cl. [u] Dioxalate monohydrate. [v] Calculated: 10·38% Cl; found: 10·33% Cl. [w] The base was released from the salt and used for recording the spectra; IR spectrum: 748, 830, 887 (4 and 2 adjacent and solitary Ar—H), 1 480, 1 530, 1 564 (Ar), 1 610 cm^{-1} (Ar—C=N); [t] ^1H NMR spectrum: δ 8·49 (d, J = 8·5 Hz, 1 H, 10-H), 7·20—7·70 (m, 5 H, 9-H and 4 ArH of chlorophenyl), 7·16 (d, J = 2·0 Hz, 1 H, 7-H), 5·55 and 4·20 (ABq, J = 13·0 Hz, 1 + 1 H, 4,4-H_2), 3·80 and 3·60 (ABq, J = 13·0 Hz, 1 + 1 H, 1-CH_2N), 3·41 (t, J = 7·0 Hz, 2 H, CH_2O), 3·32 (s, 3 H, CH_3O), 2·50 (bm, 10 H, remaining 5 CH_2N), 1·85 (bm, 2 H, CH_2 in the middle of the propane chain). [x] Calculated: 10·53% Cl; found: 10·68% Cl. [y] With decomposition. [z] The base was released from the salt and used for recording the ^1H NMR spectrum: δ 8·49 (d, J = 8·5 Hz, 1 H, 10-H), 7·30 to 7·80, m, 5 H, 9-H and 4 Ar-H of chlorophenyl), 7·18 (d, J = 2·0 Hz, 1 H, 7-H), 5·55 and 4·20 (ABq, J = 13·0 Hz, 1 + 1 H, 4,4-H_2), 3·80 and 3·60 (ABq, J = 13·0 Hz, 1 + 1 H, 1-CH_2—N), c. 3·50 (m, 4 H, CH_2OCH_2), c. 2·60 (m, 10 H, remaining 5 CH_2N), 1·20 (t, J = 7·0 Hz, 3 H, CH_3). [aa] Calculated: 10·53% Cl; found: 10·90% Cl. [bb] Calculated: 14·14% Cl, 6·39% S; found: 14·32% Cl, 6·60% S. [cc] Calculated: 10·50% Cl, 4·75% S; found: 10·62% Cl, 5·00% S. [dd] The base was released from the salt and used for recording the spectra; UV spectrum: λ_{max} 221 nm (log ε 4·67), inflexes at 251 nm (4·00) and 270 nm (3·52); IR spectrum: 690, 752, 815, 830, 885 (5, 4 and 2 adjacent and solitary Ar—H), 1 230 (Ar—O—R), 1 486, 1 531, 1 569, 1 585, 1 599 (Ar), 1 614 (Ar—C=N), 2 770, 2 810 cm^{-1} (ArOR, CH_2—N); [t] ^1H NMR spectrum: δ 6·80—7·70 (m, 12 H, ArH), 5·60 and 4·20 (ABq, J = 13·0 Hz, 1 + 1 H, 4,4-H_2), 4·15 (t, J = 6·0 Hz, 2 H, OCH_2), 3·85 and 3·58 (ABq, J = 13·0 Hz, 1 + 1 H, 1-CH_2—N), 2·85 (t, J = 6·0 Hz, 2 H, CH_2N outside of piperazine), 2·68 (bm, 8 H, 4 CH_2N of piperazine). [ee] Calculated: 9·83% Cl; found: 9·84% Cl. [ff] The base was released from the salt and used for recording the spectra; IR spectrum: 695, 752, 838, 895 (5, 4 and 2 adjacent and solitary Ar—H), 1 490, 1 535, 1 568, 1 582, 3 040 (Ar), 1 614 (Ar—C=N), 2 785, 2 820 cm^{-1} (CH_2-N); ^1H NMR spectrum: δ 8·45 (d, J = 8·5 Hz, 1 H, 10-H), 7·10—7·70 (m, 11 H, remaining ArH), 5·58 and 4·20 (ABq, J = 13·0 Hz, 1 + 1 H, 4,4-H_2), 3·80 and 3·50 (ABq, J = 13·0 Hz, 1 + 1 H, 1-CH_2—N), 2·40—3·20(m, 12 H, 4 CH_2N of piperazine and SCH_2CH_2N). [gg] Calculated: 9·61% Cl, 4·35% S; found: 9·67% Cl, 4·63% S.

Compounds *Ia—g* and *IIg* in the form of fumarates were subjected to a preliminary pharmacological evaluation in mice using oral administration (all doses in mg/kg). The acute toxicity (LD_{50}) has been determined only with three compounds: *Ia*, 721; *Ig*, 635 (starting with a dose of 200 mg/kg a strong sedation of the animals in the experiment takes place; the toxic symptoms are convulsive twitches and ataxia); *IIg*,

c. 1 000. Assuming a possible considerable discoordinating effect in the rotarod test the compounds *Ia–f* were administered in a dose of 1 mg/kg. They appeared, however, to be almost inactive under these conditions (ataxia in 10–20% of the animals at the maximum). For the most active compound *Ig* the medium effective dose was determined; ED_{50} = 25·9 mg/kg. Compound *IIg* was administered in this test in a dose of 30 mg/kg which only exceptionally brought about ataxia. The central depressant effect manifested by the inhibition of the locomotor activity, evaluated by the photo-cell method of Dews, was estimated only with two compounds: compound *Ig* in a dose of 10 mg/kg inhibited the locomotor activity very intensively (to 30% of the control value); compound *IIg* brings about a statistically significant inhibition in this test only in a relatively high dose of 50 mg/kg (a dose of 10 mg/kg is practically without effect). The anticonvulsant activity towards pentetrazole was evaluated likewise only with the just two mentioned compounds: *Ig*, a dose of 1 mg/kg lacks any protective effect towards the convulsant action but it decreases significantly the toxicity of pentetrazole; *IIg*, a dose of 10 mg/kg is inactive towards pentetrazole. All compounds were tested for the protective action towards convulsions elicited by electroshock. In this test compounds *Ia–f* were administered in a dose of 1 mg/kg. Compounds *Ia* and *Ib* showed signs of effect but the dose given protected less than 50% animals in the experiment. Compounds *Ic–f* were practically inactive. For the most active compound *Ig* the medium protective dose was determined; PD_{50} = = 1·23 mg/kg. Compound *IIg* was inactive in a dose of 50 mg/kg. In comparison with triazolam[3,11], even the most active compound of our series (*Ig*) is thus in all tests used less active approximately by two degrees of magnitude. When enlarging the N-substituent (*Ia–f*) and with the absence of the atom of chlorine in the *o*-position of the 6-phenyl (*IIg*) the activity continues to decrease.

Evaluation of the antimicrobial activity (Dr J. Turinová and Dr V. Holá, bacteriological department of this institute) showed signs of inhibitory effects of some compounds towards some microorganisms (numbers of compounds and the minimum inhibitory concentrations in μg/ml — unless they exceed 100 μg/ml — are given): *Streptococcus faecalis*, *Ie* 100; *Staphylococcus pyogenes* aureus, *Ie* 100, *If* 25; *Trichophyton mentagrophytes*, *Ia* 50, *If* 50, *IIg* 50. All compounds were inactive in concentrations of 50–100 μg/ml towards *Streptococcus* β-*haemolyticus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Saccharomyces pasterianus*, *Candida albicans* and *Aspergillus niger*.

EXPERIMENTAL

The melting points of analytical samples were determined in Kofler's block and are not corrected; the samples were dried *in vacuo* of about 60 Pa over P_2O_5 at room temperature or at 77 °C. The UV spectra (in methanol) were recorded with a Unicam SP 8000 spectropohtometer, the IR spectra (in Nujol unless stated otherwise) with a Unicam SP 200G spectrophotometer, the ^1H NMR spectra (mostly in C^2HCl_3) with a Tesla BS 487C (80 MHz) spectrometer and the mass spectra with MCH-1320 and Varian MAT 44S spectrometers. The homogeneity of the compounds and composition of the reaction mixtures and crude products were checked by thin-layer chromato-

graphy on silica gel (Silufol). The preparative separations were carried out on columns of silica gel (Merck 60).

8-Chloro-6-(2-chlorophenyl)-1-(4-methylpiperazino)methyl-4*H*-*s*-triazolo[4,3-*a*]-1,4-benzodiazepine (*Ig*)

A mixture of 6·4 g 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-thione[9-11], 3·45 g 2-(4-methylpiperazino)acethydrazide (*IVg*) (ref.[12,13]) and 80 ml 1-butanol was stirred and refluxed for 8 h. After standing overnight the mixture was heated with charcoal, filtered and butanol was evaporated *in vacuo*. The residue was dissolved in 50 ml benzene, the solution was filtered through a column of 40 g neutral Al_2O_3 (activity II) which was washed with 160 ml benzene. Evaporation of the filtrate gave 8·2 g crude oily product which crystallized slowly from a solution in 20 ml benzene to which 10 ml hexane were added. After 2 days standing in a refrigerator the product was filtered, washed with hexane and dried; 5·0 g (57%), m.p. 173–175°C. Analytical sample, m.p. 175–176°C (benzene–hexane). IR spectrum (KBr): 755, 770, 826, 833, 895 (4 and 2 adjacent and solitary Ar–H), 1 490, 1 540, 1 570 (Ar), 1 610 (Ar–C=N), 2 725, 2 748, 2 780, 2 790 cm^{-1} (CH_3–N, CH_2–N). ^1H NMR spectrum ($C^2H_3SOC^2H_3$): δ 8·36 (d, J = 9·0 Hz, 1 H, 10-H), 7·30–8·00 (m, 5 H, 9-H and 4 ArH of chlorophenyl), 7·11 (d, J = 2·5 Hz, 1 H, 7-H), 5·31 and 4·31 (ABq, J = 13·0 Hz, 1 + 1 H, 4,4-H_2), 3·90 and 3·60 (ABq, J = 14·0 Hz, 1 + 1 H, 1-CH_2–N), 2·55 (bm, 4 H, $CH_2N^1CH_2$ of piperazine), 2·46 (bm, 4 H, $CH_2N^4CH_2$ of piperazine), 2·19 (s, 3 H, NCH_3). For $C_{22}H_{22}Cl_2N_6$ (441·4) calculated: 59·87% C, 5·02% H, 16·07% Cl, 19·04% N; found: 59·24% C, 5·13% H, 16·30% Cl, 18·76% N.

Sesquifumarate, m.p. 220–225°C with decomposition (aqueous ethanol). For $C_{22}H_{22}Cl_2N_6$ + + 1·5 $C_4H_4O_4$ (615·5) calculated: 54·64% C, 4·59% H, 11·52% Cl, 13·66% N; found: 54·73% C, 4·79% H, 11·38% Cl, 13·80% N.

Oxalate sesquihydrate, m.p. 207–209°C with decomposition (aqueous ethanol). For $C_{24}H_{24}$·$Cl_2N_6O_4$ + 1·5 H_2O (558·4) calculated: 51·62% C, 4·87% H, 12·70% Cl, 15·05% N; found: 51·66% C, 4·57% H, 12·37% Cl, 14·61% N.

8-Chloro-1-(4-methylpiperazino)methyl-6-phenyl-4*H*-*s*-triazolo[4,3-*a*]-1,4-benzodiazepine (*IIg*)

A mixture of 2·9 g 7-chloro-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-thione[9,11], 1·7 g 2-(4-methylpiperazino)acethydrazide (*IVg*) (ref.[12,13]) and 50 ml butanol was refluxed for 4 h, allowed to stand overnight and evaporated under reduced pressure. The residue was dissolved in 30 ml ethanol, the solution was filtered with charcoal and evaporated. Crystallization of the residue from a mixture of 15 ml ethanol and 15 ml cyclohexane and processing of the mother liquor gave 3·1 g (72%) *IIg*, m.p. 201–205°C. ^1H NMR spectrum: δ 8·38 (d, J = 8·0 Hz, 1 H, 10-H), 7·20 to 7·70 (m, 7 H, 7,9-H_2 and C_6H_5), 5·48 and 4·08 (ABq, J = 12·0 Hz, 1 + 1 H, 4,4-H_2), 3·81 and 3·53 (ABq, J = 13·0 Hz, 1 + 1 H, 1-CH_2–N), 2·20–2·90 (m, 8 H, 4 CH_2N of piperazine), 2·25 (s, 3 H, NCH_3). Lit.[4,5], m.p. 294–205·5°C, and 203–208°C, respectively.

Fumarate, m.p. 145–149°C with decomposition (ethanol–ether). For $C_{26}H_{27}ClN_6O_4$ (523·0) calculated: 59·71% C, 5·20% H, 6·78% Cl, 16·07% N; found: 58·91% C, 5·18% H, 7·09% Cl, 15·51% N.

Ethyl 2-[4-(2-Methoxyethyl)piperazino]acetate (*IIIa*) (Method *A*)

A mixture of 25·8 g 1-(ethoxycarbonylmethyl)piprazine[14-16], 23·5 g 2-methoxyethyl bromide[17] 20·6 g K_2CO_3 and 150 ml acetone was stirred and refluxed for 6 h. After standing overnight the solid was filtered off and washed with acetone. The filtrate was evaporated, the oily residue diluted

with 100 ml benzene and the solution was washed with water, dried with K_2CO_3, filtered with charcoal and distilled; 15·0 g (44%), b.p. 120–130°C/0·36 kPa. For analysis a sample was redistilled, b.p. 95–98°C/70 Pa. IR spectrum ($CHCl_3$): 1 030, 1 168, 1 300 (C–O of ester), 1 112 (R–O–R'), 1 735 (C=O of ester), 2 820 cm^{-1} (CH_2–N). ^1H NMR spectrum: δ 4·18 (q, J = 7·0 Hz, 2 H, $COOCH_2$), 3·49 (t, J = 7·0 Hz, 2 H, the remaining OCH_2), 3·32 (s, 3 H, OCH_3), 3·18 (s, 2 H, NCH_2CO), 2·58 (bs, 8 H, 4 NCH_2 of piperazine), 2·49 (t, J = 7·0 Hz, 2 H, CH_2N of methoxyethyl), 1·25 (t, J = 7·0 Hz, 3 H, CH_3 of ethyl). Di(hydrogen maleate), m.p. 144–145°C (ethanol). The analyses in Table I.

Ethyl 2-[4-(3-Methoxypropyl)piperazino]acetate (*IIIb*) (Method *B*)

A stirred mixture of 20 g 1-(3-methoxypropyl)piperazine[1], 40 ml ethanol and 19 g $NaHCO_3$ was treated dropwise over 20 min at 50°C with a solution of 12·2 g ethyl chloroacetate in 40 ml ethanol. The mixture was then stirred and refluxed for 16 h. Ethanol was evaporated, the residue was diluted with 50 ml water and extracted with chloroform. The extract was washed with water, dried with $MgSO_4$ and distilled; 20 g (82%), b.p. 124–126°C/0·4 kPa. IR spectrum ($CHCl_3$): 1 010, 1 030, 1 164, 1 295 (C–O of ester), 1 112 (R–O–R'), 1 735 (C=O of ester), 2 820 cm^{-1} (CH_2–N). ^1H NMR spectrum: δ 4·18 (q, J = 7·0 Hz, 2 H, $COOCH_2$), 3·39 (t, J = 7·0 Hz, 2 H, remaining OCH_2), 3·30 (s, 3 H, OCH_3), 3·18 (s, 2 H, NCH_2CO), 2·55 (bs, 8 H, 4 NCH_2 of piperazine), 2·40 (t, 2 H, CH_2N of methoxypropyl), 1·85 (bm, 2 H, CH_2 in the middle of the propane residue), 1·25 (t, J = 7·0 Hz, 3 H, CH_3 of ethyl). The analysis in Table I.

2-[4-(2-Methylthioethyl)piperazino]acethydrazide (*IVd*) (Method *C*)

A stirred solution of 1·7 g 100% $N_2H_4.H_2O$ in 7 ml ethanol was treated dropwise over 15 min with a solution of 7·4 g *IIId* in 8 ml ethanol and the mixture was refluxed for 8 h. After standing overnight at room temperature a solid by-product (0·9 g, m.p. 145–153°C) was filtered off and the filtrate was evaporated; 6·4 g (91%), m.p. 68–72°C. Analytical sample, m. p.79–81°C (ethanol). IR spectrum (KBr): 1 532, 1 579, 1 663 (CONH), 2 760, 2 800 (CH_2–N), 3 160, 3 210, 3 285 cm^{-1} (NH_2 and NH). ^1H NMR spectrum ($C^2H_3SOC^2H_3$): δ 8·85 (bs, 1 H, NH), 4·20 (bs, 2 H, NH_2), 2·95 (s, 2 H, NCH_2CO), 2·54 (s, 4 H, SCH_2CH_2N), 2·45 (s, 8 H, 4 CH_2N of piperazine), 2·10 (s, 3 H, SCH_3). Tri(hydrogen maleate), m.p. 119–121°C (ethanol), Analyses in Table I.

N,N'-Bis[4-(2-phenoxyethyl)piperazinoacetyl]hydrazine (*VI*)

A solution of 5·35 g 100% $N_2H_4.H_2O$ in 25 ml ethanol was stirred and treated with a solution of 27·5 g *IIIe* in 25 ml ethanol and the mixture was refluxed for 8 h. Ethanol was evaporated and it was attempted to distill the residue. There were signs of decomposition, the heating was discontinued and the crude product (18 g) was neutralized with 30 g maleic acid in 80 ml ethanol; 41 g tetra(hydrogen maleate) of *VI*, m.p. 144–146°C (ethanol). For $C_{44}H_{56}N_6O_{20}$ (989·0) calculated: 53·43% C, 5·71% H, 8·50% N; found: 52·92% C, 5·78% H, 8·42% N.

N-Isopropylidene-2-[4-(2-phenylthioethyl)piperazino]acethydrazide (*VII*)

IVd (0·6 g) was neutralized with 0·71 g maleic acid in 8 ml ethanol and the precipitated maleate was crystallized from acetone; dimaleate of *VII*, m.p. 135–138°C. Mass spectrum, *m/z*: 335 (M^+ corresponding to $C_{17}H_{26}N_4OS$), 334, 333, 332, 235, 225 ($C_{11}H_{21}N_4O$), 211 ($C_{10}H_{19}N_4O$, 100%), 137, 111, 99, 98, 97, 70, 58, 56, 55. For $C_{25}H_{34}N_4O_9S$ (566·6) calculated: 52·99% C, 6·05% H, 9·89% N, 5·66% S; found: 52·60% C, 6·02% H, 9·79% N, 6·14% S.

8-Chloro-6-(2-chlorophenyl)-1-[4-(2-methylthioethyl)piperazino]methyl-4*H*-*s*-triazolo[4,3-*a*]--1,4-benzodiazepine (*Id*) (Method *D*)

A mixture of 6·4 g 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-thione[9-11], 5·8 g *IVd* and 70 ml 1-butanol was stirred and refluxed for 10 h. After standing overnight butanol was evaporated *in vacuo* and the residue was chromatographed on a column of 60 g silica gel. Elution with benzene removed 0·3 g starting thione. Continued elution with chloroform and chloroform containing 2% methanol resulted then in 9·6 g (95%) crude oily base *Id* which crystallized from ethanol, m.p. 158—160°C. UV spectrum: inflex at 248 nm (log ε 4·11). IR spectrum: 750, 834, 848, 880 (4 and 2 adjacent and solitary Ar—H), 1 485, 1 540, 1 565, 1 588 (Ar), 1 610 (Ar—C=N), 2 810 cm^{-1} (CH_2—N). ^1H NMR spectrum: δ 8·50 (d, J = 8·5 Hz, 1 H, 10-H), 7·30—7·70 (m, 5 H, 9-H and 4 ArH of chlorophenyl), 7·20 (d, J = 2·5 Hz, 1 H, 7-H), 5·60 and 4·25 (ABq, J = 13·0 Hz, 1 + 1 H, 4,4-H_2), 3·82 and 3·55 (ABq, J = 13·0 Hz, 1 + 1 H, 1-CH_2—N), 2·60 (m, 12 H, 4 CH_2N of piperazine and SCH_2CH_2N), 2·18 (s, 3 H, SCH_3). Sesquifumarate, m.p. 218—222°C with decomposition (aqueous ethanol). Analyses in Table I.

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REFERENCES

1. Polívka Z., Ryska M., Holubek J., Svátek E., Metyš J., Protiva M.: This Journal *48*, 2395 (1983).
2. Castañer J., Chatterjee S. S.: Drugs Future *1*, 551 (1976); *2*, 826 (1977); *4*, 904 (1979); *6*, 788 (1981).
3. Pakes G. E., Brogden R. N., Heel R. C., Speight T. M., Avery G. S.: Drugs *22*, 81 (1981).
4. Hester J. B. jr, Rudzik A. D., VonVoigtlander P. F.: J. Med. Chem. *23*, 392 (1980).
5. Meguro K., Kuwada Y. (Takeda Chemical Industries, Ltd.): Ger. Offen. 2 159 242 (Japan Appl. 11. 12. 70); Chem. Abstr. *78*, 72 239 (1973).
6. Meguro K., Kuwada Y.: Chem. Pharm. Bull. *21*, 2 375 (1973).
7. Morren H., Trolin S., Denayer R., Grivsky E.: Bull. Soc. Chim. Belg. *59*, 228 (1950); Chem. Abstr. *45*, 6 210 (1951).
8. Zaugg H. E., Weston A. W., Freifelder M., Michaels R. J. jr (Abbott Laboratories): U.S. 2 980 683 (18.04.61); Chem. Abstr. *55*, 17 663 (1961).
9. Archer G. A., Sternbach L. H.: J. Org. Chem. *29*, 231 (1964).
10. Hester J. B. jr, Rudzik A. D., Kamdar B. V.: J. Med. Chem. *14*, 1 078 (1971).
11. Vejdělek Z., Metyš J., Protiva M.: This Journal *48*, 123 (1983).
12. Massarani E., Nardi D., Degen L. (Société d'Exploitation Chimiques et Pharmaceutiques Seceph S. A.): Swiss 473 826 (Appl. 11.09.67); S. African 69/01 435; Chem. Abstr. *72*, 21 712 (1970).
13. Massarani E., Nardi D., Tajana A., Degen L.: J. Med. Chem. *14*, 633 (1971).
14. Braker W., Christiansen W. G.: J. Amer. Pharm. Assoc. *22*, 950 (1933); Chem. Zentralbl. *1934*, I, 1 819.
15. Barrett P. A., Caldwell A. G., Walls L. P.: J. Chem. Soc. *1961*, 2 404.
16. Barrett P. A., Caldwell A. G., Walls L. P. (Wellcome Foundation Ltd.): Brit. 921 315 (Appl. 31.03.58); Chem. Abstr. *60*, 2 967 (1964).
17. Palomaa M. H., Kenetti A.: Ber. Deut. Chem. Ges. *64*, 797 (1931).
18. Harrison G. C., Diehl H.: Org. Syn., Coll. Vol. *3*, 370 (1955).

19. Kirner W. R., Windus W.: Org. Syn., Coll. Vol. *2*, 136 (1943).
20. Marvel C. S., Tanenbaum A. L.: Org. Syn., Coll. Vol. *1*, 435 (1932).
21. Scherlin S. M., Jakubowitsch A. I.: J. Prakt. Chem. (N. F.) *138*, 23 (1933).
22. Vejdělek Z., Svátek E., Holubek J., Metyš J., Bartošová M., Protiva M.: This Journal *46*, 148 (1981).

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Note added in proof: The base *Ib* is also crystalline; m. p. 94-96°C with decomposition (ethanol). For $C_{25}H_{28}Cl_2N_6O$ (499·5) calculated: 60·12% C, 5·65% H, 14·20% Cl, 16·83% N; found: 59·64% C, 5·96% H, 14·19% Cl, 16·43% N.