Antiviral Assays. The were performed as reported previously.[35-37]

Acknowledgment. This research was supported by grants (to J.W.L.) from DHHS Grant 2-R01-CA21488-13 of the USPHS and from the Biotechnology Strategic Grants Programme of the Natural Sciences and Engineering Research Council of Canada; by Grant 3.0040.83 (to E.D.C.) from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek; by Grant 85/90-79 (to E. D.C.) from the Belgian Geconcerteerde Onderzoeksactics; and by Grant GM 2179-3 (to R.A.N.).

Registry No. 7, 97950-71-5; 8, 97950-77-1; 9, 121541-72-8; 9 (4-nitro derivative), 2522-28-3; 13, 121541-73-9; 13a·2HCl, 121541-86-4; 13a (free base), 121541-99-9; 14, 121541-74-0; 14a·2HCl, 121541-87-5; 14a (free base), 121542-00-5; 15, 121571-69-5; 15a·2HCl, 121541-88-6; 15a (free base), 121542-01-6; 16, 121541-75-1; 16a·2HCl, 121541-89-7; 16a (free base), 121542-02-7; 17, 121541-76-2; 17a·2HCl, 121541-90-0; 17a (free base), 121542-03-8; 18, 121541-77-3; 18a·2HCl, 121541-91-1; 18a (free base), 121542-04-9; 19, 121541-78-4; 19a·2HCl, 121541-92-2; 19a (free base), 121542-05-0; 20, 121541-79-5; 20a·2HCl, 121541-93-3; 20a (free base), 121542-06-1; 21, 121541-80-8; 21a·2HCl, 121541-94-4; 21a (free base), 121542-07-2; 22, 121541-81-9; 22a·2HCl, 121541-95-5; 22a (free base), 121571-70-8; 23, 121541-82-0; 23a·2HCl, 121541-96-6; 23a (free base), 121542-08-3; 25, 121541-84-2; 25a·2HCl, 121541-98-8; 25a (free base), 121542-10-7; $ClCOCH_2CH_2COCl$, 543-20-4; $ClCO(CH_2)_3COCl$, 2873-74-7; $ClCO(CH_2)_5COCl$, 142-79-0; $ClCO(CH_2)_6COCl$, 10027-07-3; $ClCO(CH_2)_7COCl$, 123-98-8; $ClCO(CH_2)_8COCl$, 111-19-3; $CH_2(COOH)_2$, 141-82-2; $HOOC(CH_2)_4COOH$, 124-04-9; R″-$COCH_2CH_2CO$-R″, 121541-83-1; R′-(Z)-COCH=CHCO-R′, 121541-85-3; R_2-$COCH_2CH_2CO$-R_2·2HCl, 121541-97-7; R_2-$COCH_2CH_2CO$-R_2 (free base), 121542-09-4; R_1-(Z)-COCH=CHCO-R_1·2HCl, 121542-11-8; maleic anhydride, 108-31-6.

Supplementary Material Available: Table giving analytical and spectroscopic data on linked oligopeptides and their precursors (5 pages). Ordering information is given on any current masthead page.

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Synthesis and Pharmacological Evaluation of CNS Activities of [1,2,3]Triazolo[4,5-*b*][1,5]-, Imidazolo[4,5-*b*][1,5]-, and Pyrido[2,3-*b*][1,5]benzodiazepines. 10-Piperazinyl-4*H*-1,2,3-Triazolo[4,5-*b*][1,5]benzodiazepines with Neuroleptic Activity[1]

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The synthesis of [1,2,3]triazolo[4,5-*b*][1,5]-, imidazolo[4,5-*b*][1,5]-, and pyrido[2,3-*b*][1,5]benzodiazepines is described. The antidopaminergic and anticholinergic activities of the compounds have been examined by the respective in vitro [^{3}H]spiperone and [^{3}H]QNB receptor binding assay. The neuroleptic potential has been further evaluated in terms of their ability to produce hypothermia and catalepsy in mice and a conditioned avoidance response in rats. Only compounds from the triazolobenzodiazepine series show antipsychotic potential. The lack of activity in the imidazolo- and pyridobenzodiazepine series indicates that the basicity of the heteroarene moiety may be determinant for activity.

It is known that a change in the electronic distribution in the two phenyl rings of the dibenzo–epine class of neuroleptics leads to a profound alteration in activity profile.[2-6] For example, clozapine (1) is a clinically effective antipsychotic which differs from typical neuroleptics by producing only minimal extrapyramidal symptoms (EPS), whereas its 2-chloro isomer HF-2046 (2) has a classical profile of activity.[7,8] In a previous publication[5] we reported that a profile of activity, in animal tests, similar to that of clozapine could be obtained if the relatively electron-rich phenyl ring, C, is replaced with an isosteric thiophene ring to give a corresponding thieno-[2,3-*b*][1,5]benzodiazepine. One of these compounds,

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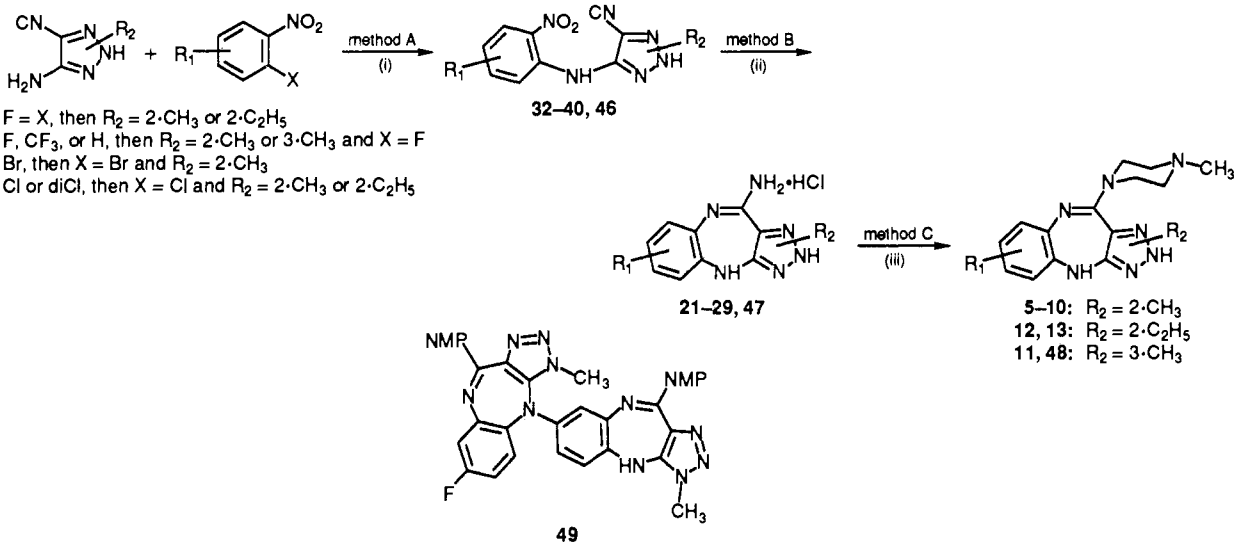
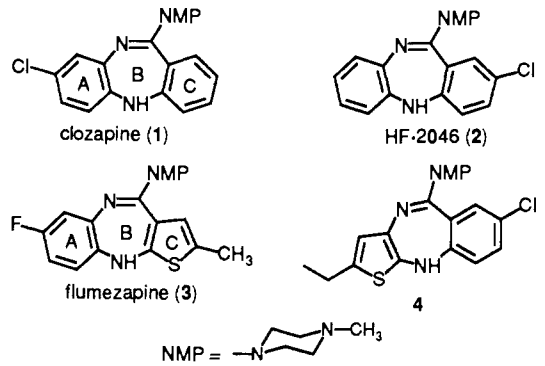
Scheme I[a]



[a] NMP = *N*-methylpiperazine. Conditions: (i) NaH, THF; (ii) $SnCl_2$, aqueous EtOH, HCl; (iii) NMP, DMSO, $PhCH_3$.

flumezapine[5] (3) in which the thiophene is substituted with an electron-donating methyl group is more potent than clozapine and was selected as a candidate for clinical trial.



Since transposition of the thiophene and phenyl rings, as with the isomeric thieno[1,4]benzodiazepines (4), produced greatly reduced neuroleptic activity,[5,6] it would appear that the electronic distribution, particularly of the C ring, is important for activity. It was of interest, therefore, to examine compounds where this ring was replaced with other heteroarene groups with different electronic characteristics. This report is concerned with the synthesis and pharmacological evaluation of some imidazolo[1,5]-, [1,2,3]triazolo[1,5]-, and pyrido[1,5]benzodiazepines. Experience in the SAR of the thienobenzodiazepines led us to the design and synthesis of only a limited number of compounds in each series.

Like other neuroleptics, the thienobenzodiazepines and clozapine are dopamine antagonists. It is now generally agreed that there are two dopamine receptors,[9] D_1 and D_2, and that antipsychotic activity is obtained by inhibiting the latter. In addition to being dopamine antagonists, these compounds also inhibit cholinergic (muscarinic) receptors, and in the case of clozapine, this activity is thought to contribute to the relative lack of EPS observed in the clinic.[8] For this reason, the compounds described in this paper were tested in vitro for their ability to interact with dopamine (D_2) and muscarinic receptors by assessing whether they competed with [^{3}H]spiperone and [^{3}H]QNB (quinuclidinyl benzylate), respectively, for binding sites in brain tissue.

In order to evaluate neuroleptic potential in vivo, these compounds were initially assessed for their ability to produce hypothermia and catalepsy in mice and then to block a conditioned avoidance response (CAR) in rats. This latter activity has been shown to be a common feature of clinically effective antipsychotics. As clozapine, like many other antipsychotics, produces muscular relaxation and incoordination, this activity was assessed by determining the length of time a rat could remain on a rotating rod.

Chemistry

4*H*-[1,2,3]Triazolo[4,5-*b*][1,5]benzodiazepines. There are three isomeric series of [1,2,3]triazolobenzodiazepines depending on which of the three triazole nitrogens carries substitution. We describe here the preparation of two of these isomers: the 2-alkyl- (**5–10**, **12**, **13**) and the 3-alkyl- (**11**, **48**) substituted [1,2,3]triazolobenzodiazepines (Scheme I). [The third isomer bearing an alkyl group on the triazole nitrogen at position 1 was not made. It has been shown[4] in the thienobenzodiazepine series that a methyl group substituted at the 3-position of the thiophene ring imposes a steric impedance to the piperazine ring, and reduces markedly the antidopaminergic activity.] For these isomers, we initially prepared the precursor 2-alkyl-4-amino[1,2,3]triazole-5-carbonitriles[10–12] and 3-alkyl-4-amino[1,2,3]triazole-5-carbonitriles[13,14] separately, but subsequent work showed that improved yields and a more convenient synthesis of these precursors were obtained in reactions[10,14] resulting in mixtures of the two isomers, which were then separated by column chromatography.

The intermediate (nitroanilino)triazolecarbonitriles **32–40**, **46** were prepared by reaction of the appropriate 4-amino[1,2,3]triazole-5-carbonitrile with 2-halonitrobenzenes with sodium hydride as a base in tetrahydrofuran at room temperature (method A), giving yields up to 91%.

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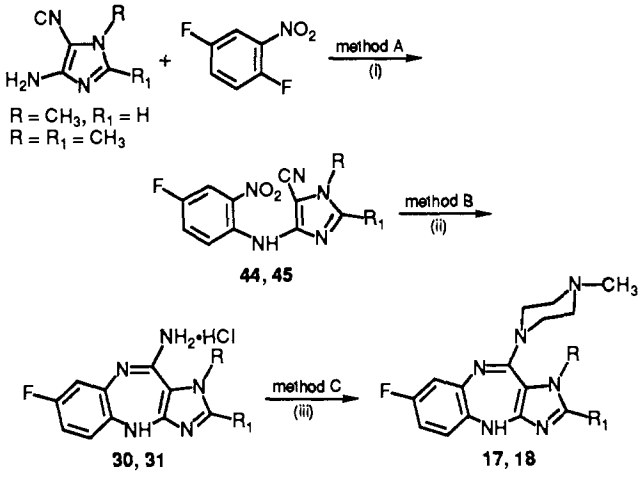
Scheme II[a]



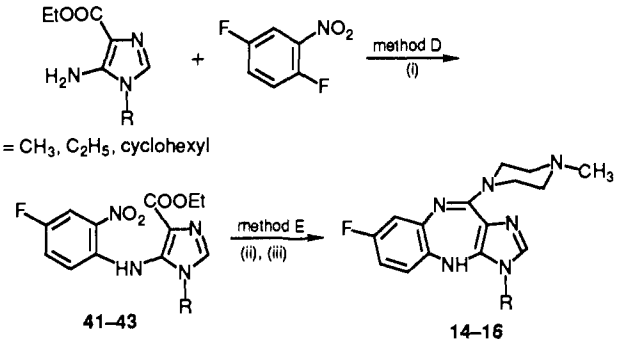
[a] Conditions: (i) NaH, THF; (ii) $SnCl_2$, aqueous EtOH, HCl; (iii) NMP, DMSO, $PhCH_3$.

It is interesting to note that proton NMR spectra ($CDCl_3$) of the (nitroanilino)-2-methyltriazolecarbonitriles showed an absorption of the phenyl H-6 proton at an exceptionally low field. This downfield shift may be explained by the deshielding influence of the lone pair of electrons from the unsubstituted N at position 3, which is in proximity to the H-6 proton of the aniline ring. For example, the signal due to this proton in the 3-methyl compound (**38**) appears at δ 6.8, which is consistent with its position ortho to the NH, but shifts to δ 8.2 in the 2-methyl isomer (**32**). This is similar to the proton deshielding effect observed in the corresponding anilinoalkylpyrazole series of compounds published previously.[15]

Reduction and cyclization to the cyclic aminoamidines **21–29**, **47** were carried out with anhydrous stannous chloride in aqueous ethanolic hydrochloric acid (method B) to give directly the hydrochloride salts. Transamination of these aminoamidines with *N*-methylpiperazine was facilitated by DMSO, and by the use of the salt in preference to the free base. The transamination was best achieved with toluene as a cosolvent at 125 °C (method C), while removing traces of water via a Dean–Stark apparatus. It was found necessary to purge the solvent mixture with N_2 before addition of the aminoamidine (Scheme I).

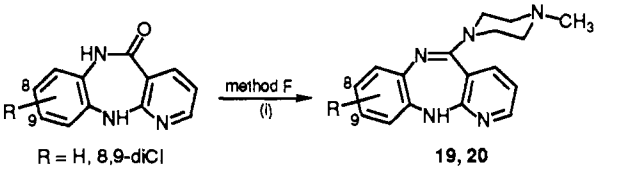
An interesting reaction involving the fluorine atom at the 7-position and the 4-bridge NH function occurred when 10-amino-7-fluoro-3-methyl-3,4-dihydro-4*H*-1,2,3-triazolo[4,5-*b*][1,5]benzodiazepine hydrochloride (**47**) was used in the above transamination reaction. GC-MS analysis of the reaction mixture showed two main components in almost equal ratios. The fore fraction gave a mass ion of 315, corresponding to the expected product, 7-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-3,4-dihydro-4*H*-1,2,3-triazolobenzodiazepine (**48**), the structure of which was also confirmed by spectral evidence. The second fraction gave a mass ion of 610, suggesting a dimeric product less the elements of HF. ^1H NMR ($CDCl_3$ and DMSO-d_6) showed two different piperazine *N*-methyl groups at δ 2.2 and 2.3, and also two different *N*-methyl groups on the triazole ring at δ 3.8 and 4.0. The spectrum in $CDCl_3$ showed only one NH. This compound must therefore be a nonsymmetrical dimer. ^{13}C NMR in DMSO-d_6 showed only one carbon to fluorine signal in two aromatic ring systems, which leads us to believe the dimer is 7-{7-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-3,4-dihydro-1,2,3-triazolo[4,5-*b*][1,5]benzodiazepin-4-yl}-3-methyl-10-(4-methyl-1-piperazinyl)-3,4-dihydro-4*H*-1,2,3-triazolo[4,5-*b*][1,5]benzodiazepine (**49**).

Scheme III[a]



[a] Conditions: (i) K_2CO_3, DMSO; (ii) 10% Pd–C, H_2, EtOH, 60 psi; (iii) NMP, $TiCl_4$, anisole.

Scheme IV[a]



[a] Conditions: (i) NMP, $TiCl_4$, PhMe.

Due to the strength of the aromatic carbon to fluorine bond, substitution with a fluorine atom is often used to block metabolic hydroxylation. Thus a defluorination under these conditions is surprising.

4*H*-Imidazolo[4,5-*b*][1,5]benzodiazepines. Examples of the two isomeric imidazolobenzodiazepine series have been synthesized. Compounds **17** and **18**, with 1-methyl and 1,2-dimethyl substituents, were prepared from the appropriate 4-aminoimidazole-5-carbonitrile[16,17] according to the methods described above for the triazolobenzodiazepines (Scheme II). The examples of the series with 3-alkyl substituents (**14–16**) were prepared from the appropriate 4-aminoimidazole-5-carboxylates[18] (Scheme III). The intermediate (nitroanilino)imidazolecarboxylates **41–43** were prepared by reaction of the appropriate imidazole carboxylate with 2,5-difluoronitrobenzene using potassium carbonate as a base, in dry DMSO at 90 °C (method D). It was found necessary to predry the potassium carbonate at 110 °C for several hours and to use a finely divided form of carbonate; otherwise yields were much lower. The nitro group of **41–43** was hydrogenated by using 10% Pd/C as catalyst. In most cases, the unstable diamino esters were used in the next stage without purification. As with the analogous thienobenzodiazepines,[5] reacting the diamino esters with excess *N*-methylpiperazine and titanium tetrachloride in refluxing anisole (method E) gave reasonable yields of the imidazolo[4,5-*b*][1,5]benzodiazepines **14–16** (Scheme III).

11*H*-Pyrido[2,3-*b*][1,5]benzodiazepines. Two compounds were prepared in this series, from the benzodiazepinones[19] (Scheme IV), by using excess *N*-methyl-

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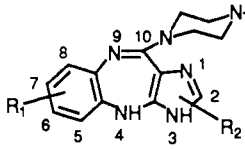
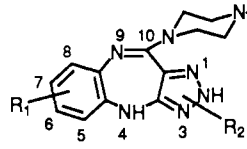
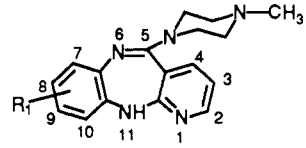
Table I. *N*-Methylpiperazino-1,2,3-triazolo-, *N*-Methylpiperazinoimidazolo-, and *N*-Methylpiperazinopyrido[1,5]benzodiazepines



14–18



5–13



19, 20

no.	R_1	R_2	% yield (method)	mp, °C	recrystn solvent[a]	formula	anal.
5	H	2-CH_3	50 (C)	182–184	EA/nH	$C_{15}H_{19}N_7$	C, H, N
6	7-F	2-CH_3	65 (C)	195–197	EA/nH	$C_{15}H_{18}FN_7$	C, H, N, F
7	7-Cl	2-CH_3	72 (C)	200–203	A	$C_{15}H_{18}ClN_7$	C, H, N, Cl
8	7-Br	2-CH_3	89 (C)	183–184	EA/nH	$C_{15}H_{18}BrN_7$	C, H, N, Br
9	7-CF_3	2-CH_3	71 (C)	109–111	EA/nH	$C_{16}H_{18}F_3N_7$	C, H, N, F
10	6,7-diCl	2-CH_3	83 (C)	217–218	EA/nH	$C_{15}H_{17}Cl_2N_7$	C, H, N, Cl
11	H	3-CH_3	46 (C)	243–245	EA/nH	$C_{15}H_{19}N_7$	C, H, N
12	7-F	2-C_2H_5	49 (C)	178–180	EA/nH	$C_{16}H_{20}FN_7$	C, H, N, F
13	7-Cl	2-C_2H_5	54 (C)	180–182	EA/nH	$C_{16}H_{20}ClN_7$	C, H, N, Cl
14	7-F	3-cyclohexyl	52 (E)	186–187	C/nH	$C_{21}H_{27}FN_6$	C, H, N, F
15	7-F	3-C_2H_5	30 (E)	210 dec	EA/nH	$C_{17}H_{21}FN_6$	C, H, N, F
16	7-F	3-CH_3	83 (E)	>280	EA	$C_{16}H_{19}FN_6$	C, H, N, F
17	7-F	1-CH_3	24 (C)	246–247	A	$C_{16}H_{19}FN_6$	C, H, N, F
18	7-F	1,2-diCH_3	12 (C)	194–196	EA/nH	$C_{17}H_{21}FN_6$	C, H, N, F
19	H	H	90 (F)	147	B	$C_{17}H_{19}N_5$	C, H, N
20	8,9-diCl	H	31 (F)	170[b]	P	$C_{17}H_{17}Cl_2N_5$·, $C_4H_4O_4$	C, H, N, Cl

[a] Solvent of crystallization: A = CH_3CN; B = benzene; EA = ethyl acetate; nH = *n*-hexane; P = 2-propanol. [b] Crystallized as the maleate salt.

Table II. Pharmacology of Triazolo-, Imidazolo-, and Pyridobenzodiazepines

no.	[^{3}H]spiperone binding IC_{50}, μM[a]	[^{3}H]QNB binding IC_{50}, μM[a]	ED_{min}, mg/kg po: rat rotarod[b]	ED_{min}, mg/kg po: CAR[b]	mouse behavior[c] ED_{min} mg/kg po: hypo	mouse behavior[c] ED_{min} mg/kg po: cat
5	1.42	>1.0	>50	5	25	25
6	0.84	>1.0	20	5	12.5	25
7	0.21	2.6	>10	4	6	12.5
8	0.30	4.0	5	5	12.5	12.5
9	1.10	>1.0	25	>25	50	50
10	2.30	>1.0	>50	>20	50	100
11	>10			>50		
12	0.18	>1.0	10	5	12.5	12.5
13	0.067	2.4	>5	5	12.5	12.5
14	>10	>100	>50	>50	200	>400
15	>10	61	>50	>50	>200	>200
16	>100	122	>50	>50	>400	>400
17	>10	>1.0	40	>20	25	100
18	>10	>1.0	>50	>50	50	>200
19	>10				100	
20	9.20				100[d]	50
1[e]	0.25	0.18	40	30	50	100
3[e]	0.02	0.08	10	5	1.6	6.25

[a] Drug concentrations were assayed in triplicate and the IC_{50}'s calculated from the regression line of the mean of the results. [b] Groups of five animals per dose level. [c] Groups of three animals per dose level. [d] Dose i.p. [e] 1 = clozapine; 3 = flumezapine.

piperazine and titanium tetrachloride in refluxing toluene (method F), to give the pyrido[2,3-*b*][1,5]benzodiazepines **19**, **20**.

Results and Discussion

The ability of neuroleptics to combine with dopamine (D_2) receptors is thought to correlate with their antipsychotic activity[20] and can be evaluated by assessing whether they compete in vitro with [^{3}H]spiperone for binding sites in calf caudate tissue. Clinically active antipsychotics are also known to block a conditioned avoidance response in rats. The results obtained in these two tests are shown in Table II, where it can be seen that only the triazolobenzodiazepine series contains compounds with a significant level of activity. In general, the in vitro activity of the triazolobenzodiazepines on [^{3}H]spiperone binding is about 10 times less than that obtained with similarly substituted thieno[2,3-*b*][1,5]benzodiazepines,[4,21] cf. **3**.

As in the case of the thienobenzodiazepines,[5] the neuroleptic activity is enhanced by halogen substitution in the 7-position as in compounds **6**–**8**, **12**, and **13**, although further halogen substitution as in the dichloro derivative **10** reduces the ability of the compound to compete with [^{3}H]spiperone. 7-CF_3 substitution in compound **9** has little effect on in vitro activity. All the active compounds have a short alkyl group at the 2-position, with ethyl being more active than methyl. Compound **11**, with a methyl group at the 3-position is inactive. This may be explained by a steric requirement or an increased basicity of the 3-methyltriazole moiety as compared with the 2-methyl analogue (**5**). A methyl group substituted on the nitrogen at the 1- or 3-position in 1,2,3-triazole is more basic than

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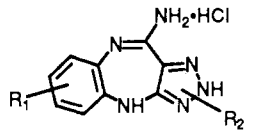
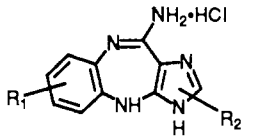
Table III. 10-Amino-1,2,3-triazolo- and 10-Aminoimidazolo[1,5]benzodiazepines



21–29, 47

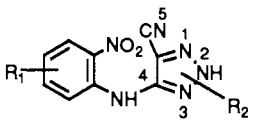


30, 31

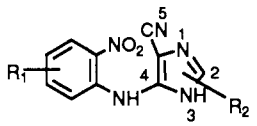
no.	R_1	R_2	% yield (method)	mp, °C[a]	formula	anal.
21	H	2-CH_3	100 (B)	>270	$C_{10}H_{11}ClN_6$	C, H, N, Cl
22	7-Cl	2-CH_3	91 (B)	>300	$C_{10}H_{10}Cl_2N_6$	C, H, N, Cl
23	7-F	2-CH_3	100 (B)	>275	$C_{10}H_{10}ClFN_6$	C, H, N, F
24	7-Br	2-CH_3	78 (B)	287–291	$C_{10}H_{10}BrClN_6$	C, H, N, Br
25	7-CF_3	2-CH_3	92 (B)	>250	$C_{11}H_{10}ClF_3N_6$	C, N, H, F
26	6,7-diCl	2-CH_3	100 (B)	>255	$C_{10}H_9Cl_3N_6$	C, H, N, Cl
27	H	3-CH_3	100 (B)	295–296 dec	$C_{10}H_{11}ClN_6$	C, H, N, Cl
28	7-F	2-C_2H_5	100 (B)	>270	$C_{11}H_{12}ClFN_6$	C, H, N, F
29	7-Cl	2-C_2H_5	88 (B)	>270	$C_{11}H_{12}Cl_2N_6$	C, H, N, Cl
30	7-F	1-CH_3	100 (B)	>275	$C_{11}H_{11}ClFN_5$	C, H, N, F
31	7-F	1,2-diCH_3	100 (B)	>275	$C_{12}H_{13}ClFN_5$	C, H, N, F
47	7-F	3-CH_3	100 (B)	>275	$C_{10}H_{10}ClFN_6$	*b*

[a] All compounds were recrystallized from ethanol. [b] MS and NMR evidence only.

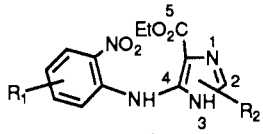
Table IV. Triazolo and Imidazolo Nitro Nitriles and Imidazolo Nitro Esters



32–40, 46



44, 45



41–43

no.	R_1	R_2	% yield (method)	mp, °C	recrystn solvent[a]	formula	anal.
32	H	2-CH_3	43 (A)	144–145	E	$C_{10}H_8N_6O_2$	C, H, N, O
33	4′-Cl	2-CH_3	33 (A)	166–168	E	$C_{10}H_7ClN_6O_2$	C, H, N, Cl
34	4′-F	2-CH_3	79 (A)	159–160	E/EA	$C_{10}H_7FN_6O_2$	C, H, N, O, F
35	4′-Br	2-CH_3	90 (A)	162–164	E	$C_{10}H_7BrN_6O_2$	C, H, N, O, Br
36	4′-CF_3	2-CH_3	80 (A)	116–117	E	$C_{11}H_7F_3N_6O_2$	C, H, N, F
37	4′,5′-diCl	2-CH_3	91 (A)	148–150	E	$C_{10}H_6Cl_2N_6O_2$	C, H, N, O, Cl
38	H	3-CH_3	86 (A)	121–123	E	$C_{10}H_8N_6O_2$	C, H, N, O
39	4′-F	2-C_2H_5	61 (A)	115–116	E	$C_{11}H_9FN_6O_2$	C, H, N, F
40	4′-Cl	2-C_2H_5	22 (A)	130–132	E	$C_{11}H_9ClN_6O_2$	C, H, N, O, Cl
41	4′-F	3-cyclohexyl	47 (D)	157–158	E	$C_{18}H_{21}FN_4O_4$	C, H, N, F
42	4′-F	3-C_2H_5	43 (D)	144–145	E	$C_{14}H_{15}FN_4O_4$	C, H, N, F
43	4′-F	3-CH_3	41 (D)	155–156	E	$C_{13}H_{13}FN_4O_4$	C, H, N, F
44	4′-F	1-CH_3	44 (A)	158–159	EA/nH	$C_{11}H_8FN_5O_2$	C, H, N, F
45	4′-F	1,2-diCH_3	47 (A)	185–186	E	$C_{12}H_{10}FN_5O_2$	C, H, N, F
46	4′-F	3-CH_3	77 (A)	171–172	E	$C_{10}H_7FN_6O_2$	C, H, N, F

[a] Solvent of crystallization: E = ethanol; EA = ethyl acetate; nH = *n*-hexane.

the 2-methyl analogue due to the former's ability to form an imidazolium type cation, while the latter can only form a pyrazolium type cation. Imidazole is a much stronger base than pyrazole, probably due to the resonance stabilization of its protonated form. It will also be evident from Table II that compounds containing basic components, i.e., imidazole (14–18) and pyridine (19, 20), show lack of activity.

With the possible exception of 5, a reasonable correlation exists between the ability of the triazolobenzodiazepines to compete in vitro with [^{3}H]spiperone and their in vivo activity at blocking CAR in rats and producing hypothermia and catalepsy in mice. It should be noted, however, that compounds 5, 6, 8, 12, and 13 which are equipotent in vivo with flumezapine (3) at blocking a CAR are all less active at displacing [^{3}H]spiperone in vitro.

The antimuscarinic activity of clozapine (1) is thought to contribute to the lack of EPS observed with this compound in the clinic.[9] Relative to their ability to interact with dopamine receptors in vitro and in vivo, the triazolobenzodiazepines are much less active than clozapine (1) or flumezapine (3) at competing with [^{3}H]QNB for binding sites in rat brain tissue in vitro. This profile indicates their potential as antipsychotics, but the relative lack of antimuscarinic activity would suggest that they may produce EPS in a greater propensity than clozapine in the clinic. The production of muscular incoordination, as shown only by this series of compounds, is consistent with their antipsychotic profile.

This study shows the importance of the electronic characteristics and basicity of the heteroarene group in modulating the activity profile. It has been shown previously[5] that the level of both antidopaminergic and anticholinergic activities similar to the dibenzodiazepine clozapine (1) is maintained in the thieno[1,5]benzodiazepines, where a suitably oriented thiophene isosteric with benzene is incorporated. By replacing thiophene with an amphoteric [1,2,3]triazole moiety, the level of antidopaminergic activity is retained, but the anticholinergic property is greatly diminished. It seems that basicity of a particular heteroarene ring also plays a vital role. As mentioned above, a 2-methyl-1,2,3-triazole is a weaker base than its 3-isomer. This makes a dramatic difference in the activity of the two isomeric triazolobenzodiazepines, and

incorporation of a more basic heteroarene, e.g., imidazole and pyridine, in the tricyclic system almost abolishes the activity.

Experimental Section

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. All compounds were characterized by physical methods using IR, UV, NMR, and MS. Column chromatography was carried out using Florisil or Sorbsil U30 grade silica gel. Microanalyses were within ±0.4% of calculated values except where noted.

Method A. 4-[(4-Fluoro-2-nitrophenyl)amino]-2-methyl-1,2,3-triazole-5-carbonitrile (34). To a solution of 2-alkyl-4-amino-2-methyl-1,2,3-triazole-5-carbonitrile[10-12,14] (1.75 g, 14.2 mmol) in dry tetrahydrofuran (30 mL) under nitrogen was added sodium hydride (1.02 g, 50% oil dispersion, 1.5 mol equiv) at room temperature. After 15 min, 2,5-difluoronitrobenzene (2.22 g, 14.2 mmol) was added to the mixture, which was stirred overnight under nitrogen. The deep red solution was then quenched in ice-cold concentrated HCl and filtered to give an orange solid. The solid was chromatographed on a Florisil column with dichloromethane; the purified product was recrystallized from ethyl acetate–ethanol to give an orange crystalline solid: yield 2.94 g (79%); mp 159–160 °C. Anal. ($C_{10}H_7FN_6O_2$) C, H, N, O, F.

Method B. 10-Amino-7-fluoro-2-methyl-2,4-dihydro-1,2,3-triazolo[4,5-*b*][1,5]benzodiazepine Hydrochloride (23). To a slurry of 4-[(4-fluoro-2-nitrophenyl)amino]-2-methyl-1,2,3-triazole-5-carbonitrile (34) (2.62 g, 10 mmol) in ethanol (25 mL) was added anhydrous stannous chloride (5.7 g, 3 mol equiv) in concentrated HCl (25 mL), the solution was heated to reflux for 1 h and cooled, and the resulting mixture was filtered to give a pale yellow crystalline solid: yield 2.68 g (100%); mp >275 °C. Anal. ($C_{10}H_{10}ClFN_6$) C, H, N, F.

Method C. 7-Fluoro-2-methyl-10-(4-methyl-1-piperazinyl)-2,4-dihydro-4*H*-1,2,3-triazolo[4,5-*b*][1,5]-benzodiazepine (6). 10-Amino-7-fluoro-2-methyl-2,4-dihydro-1,2,3-triazolo[4,5-*b*][1,5]benzodiazepine hydrochloride (23) (2.68 g, 10 mmol) was added to a mixture of dry dimethyl sulfoxide (10 mL), toluene (10 mL), and dry *N*-methylpiperazine (3.3 mL), which had been purged with nitrogen for 20 min. The stirred solution was then heated at 125 °C (oil bath) under nitrogen for 5 h and cooled to room temperature, and distilled water (33 mL) was added, keeping the temperature below 25 °C. After stirring at 5 °C for 30 min, the suspension was filtered and dried at 70 °C under reduced pressure to leave a yellow crystalline solid, which was recrystallized from ethyl acetate–hexane: yield 2.05 g (65%); mp 195–197 °C. Anal. ($C_{15}H_{18}FN_7$) C, H, N, F.

Method D. Ethyl 4-[(4-Fluoro-2-nitrophenyl)amino]-3-methylimidazole-5-carboxylate (43). To a solution of ethyl 4-amino-3-methylimidazole-5-carboxylate[18] (3.1 g, 20 mmol) and 2,5-difluoronitrobenzene (3.38 g, 20 mmol) in dry dimethyl sulfoxide (50 mL) heated to 70 °C (oil bath) was added finely divided anhydrous potassium carbonate (5.47 g, 40 mmol, dried at 110 °C), and the reaction mixture was stirred at 90 °C for 4 h. The cooled suspension was poured onto ice–water containing concentrated HCl. The nitro ester was extracted into dichloromethane, washed with water, dried over magnesium sulfate, filtered, and evaporated under reduced pressure to give a red solid. The solid was chromatographed on a Florisil column, eluting with ethyl acetate. The purified product was recrystallized from ethyl acetate–hexane to give 43, an orange-red crystalline solid: yield 2.5 g (41%); mp 155–156 °C. Anal. ($C_{13}H_{13}FN_4O_4$) C, H, N, F.

Method E. 7-Fluoro-10-(4-methyl-1-piperazinyl)-3-methyl-3,4-dihydro-4*H*-imidazolo[4,5-*b*][1,5]benzodiazepine (16). To a solution of ethyl 4-[(4-fluoro-2-nitrophenyl)amino]-3-methylimidazole-5-carboxylate (43) (2.2 g, 7.13 mmol) in ethanol (100 mL) was added a slurry of 10% palladium on charcoal catalyst (200 mg) in ethanol (30 mL). The suspension, in a Parr bottle, was hydrogenated on a Parr apparatus at 60 psi and room temperature, until the required amount of hydrogen was taken up. The suspension was filtered through a Celite pad and quickly evaporated under reduced pressure to give a brown solid: yield 1.98 g (100%).

The above diamino ester (1.98 g, 7.13 mmol), *N*-methylpiperazine (21 mL), and anisole (37 mL) were stirred at 0 °C under nitrogen. A solution of titanium tetrachloride (3.2 mL, 21 mmol) in anisole (37 mL) was added dropwise at 0 °C. The mixture was then heated at 100 °C for 2 h and further heated to reflux at 150 °C overnight, under nitrogen. The cooled mixture was poured onto water–ammonia–ethyl acetate and filtered, and the layers were separated. The aqueous phase was washed with ethyl acetate, and the combined organic phase was washed with water, dried over magnesium sulfate, and evaporated under reduced pressure to leave a brown oil. The oil was chromatographed on a Florisil column with ethyl acetate to give an orange solid; the purified product was recrystallized from ethyl acetate to give a yellow crystalline solid: yield 1.86 g (83%); mp >280 °C. Anal. ($C_{16}H_{19}FN_6$) C, H, N, F.

Method F. 5-(4-Methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,5]benzodiazepine (19). To 6*H*-pyrido[2,3-*b*][1,5]benzodiazepin-5(11*H*)-one[19] (2.2 g, 0.011 mol) in *N*-methylpiperazine (25 mL) was added a solution of titanium tetrachloride (1.25 mL) in toluene (10 mL). The amide dissolved to a clear brown solution which was heated under reflux for 3 h, cooled, and poured onto a mixture of ice and 30% aqueous ammonia solution. The mixture was extracted with dichloromethane, washed with water, and dried over magnesium sulfate. After removal of the solvent under reduced pressure the residue was crystallized from benzene: yield 2.73 g (90%); mp 147 °C. Anal. ($C_{17}H_{19}N_5$) C, H, N.

Pharmacological Methods. All compounds were dissolved in distilled water or suspended in 0.5% (carboxymethyl)cellulose. Solutions or suspensions were administered orally except where mentioned otherwise.

Mouse Behavior. Groups of three CFW mice (21–23 g) were assessed for changes in body temperature and for the presence of catalepsy at 0.25, 2.5, and 5.0 h after oral administration of the compound.

(a) Catalepsy. Animals were tested for their ability to remain with one hind limb on a rubber bung (2 cm high) and also to remain on a vertical grid. The animal was considered cataleptic, if, in the opinion of a trained observer, it remained in the set position for a period significantly longer than a control animal. The ED_{min} value is the dose below which no catalepsy was observed at any time period.

(b) Hypothermia. The rectal temperature of the three mice in each group was measured at the three time periods after compound administration. The mean temperature change from the initial mean temperature of each group for all three time periods were summed. Hypothermia was considered to be present if the sum of the mean temperature reduction was >4 °C.

Muscular Incoordination in Rats (Rat Rotarod). Groups of five male Olac Wistar rats (140–150 g), fed and watered ad libitum, were assessed for the presence of muscular incoordination at 1 and 2 h after the oral administration of the compound. The animals were placed individually on a horizontal rotating rod (2 rpm) formed from a kymograph spindle covered with corrugated paper to a mean diameter of 32 mm. The time the animals remained on the rod, up to a maximum of 30 s, was recorded and the mean time for each group compared with that of a vehicle-treated control group. The compound was considered to have produced muscular incoordination if the mean time on the rod was significantly ($p < 0.05$, Mann–Whitney "U" test) lower than that of the controls.

Conditioned Avoidance Response (CAR) in Rats. The method used was essentially that described by Jacobsen and Sonne.[22] Olac Wistar rats (120–130 g) were trained to pass from one side of a shuttle box to the other on hearing a 5-s buzzer. Failure to respond within 1 s from the end of the buzzer resulted in the animals receiving a mild electric shock. The compound under test was administered to only those animals that showed a high level of conditioned response. Groups of five animals were dosed orally 110 min prior to placing them individually in the shuttle boxes. After a 10-min habituation period, they were tested for 20 min. During this period the number of times the buzzer sounded, as well as the number of shocks received by the animal, was recorded. The degree of conditioned avoidance blockade was calculated by expressing the number of shocks received as a percentage of the number of stimuli presented.

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Dopaminergic Receptor Binding ([^{3}H]Spiperone). The assay was carried out in the striatum of the calf brain according to the method described previously.[23]

Muscarinic Cholinergic Receptor Binding ([^{3}H]QNB). This assay was also carried out on male Olac rat brain by the method previously described.[4]

Acknowledgment. We thank Dave Rackham, Sarah Morgan, Juliet Brown, and Julie Smith for the spectroscopic data and Nick Moore, Francesca Risius, Susan Wedley, Linda Horsman, and Jeremy Findlay for pharmacological assays. Elemental analyses were carried out at the Lilly Microanalytical Lab, Indianapolis, IN.

Registry No. **5**, 83448-96-8; **6**, 83448-87-7; **7**, 83448-93-5; **8**, 83448-94-6; **9**, 83448-95-7; **10**, 83448-90-2; **11**, 121845-20-3; **12**, 83448-97-9; **13**, 83448-98-0; **14**, 121845-21-4; **15**, 121845-22-5; **16**, 121845-23-6; **17**, 121865-29-0; **18**, 121845-24-7; **19**, 121845-25-8; **20**, 121845-26-9; **20**·maleate, 121845-27-0; **21**, 83449-02-9; **22**, 83448-99-1; **23**, 83448-88-8; **24**, 83449-00-7; **25**, 83449-01-8; **26**, 83448-91-3; **27**, 121845-28-1; **28**, 83449-03-0; **29**, 83449-04-1; **30**, 121845-29-2; **31**, 121845-30-5; **32**, 83449-08-5; **33**, 83449-05-2; **34**, 83448-89-9; **35**, 83449-06-3; **36**, 83449-07-4; **37**, 83448-92-4; **38**, 121845-31-6; **39**, 83449-09-6; **40**, 83449-10-9; **41**, 121845-32-7; **42**, 121845-33-8; **43**, 121845-34-9; **44**, 121845-35-0; **45**, 121845-36-1; **46**, 121845-37-2; **47**, 121845-38-3; 5-amino-4-cyano-2-methyl-2*H*-1,2,3-triazole, 28539-27-7; 5-amino-4-cyano-2-ethyl-2*H*-1,2,3-triazole, 121845-39-4; *o*-fluoronitrobenzene, 1493-27-2; 2,5-dichloronitrobenzene, 89-61-2; 2,5-difluoronitrobenzene, 364-74-9; 2,5-dibromonitrobenzene, 3460-18-2; 2-fluoro-5-(trifluoromethyl)nitrobenzene, 367-86-2; 2,4,5-trifluoronitrobenzene, 89-69-0; 5-amino-4-carbethoxy-1-cyclohexyl-1*H*-imidazole, 37842-61-8; 5-amino-4-cabethoxy-1-ethyl-1*H*-imidazole, 121845-40-7; 5-amino-4-carbethoxy-1-methyl-1*H*-imidazole, 54147-04-5; 4-amino-5-cyano-1-methyl-1*H*-imidazole, 40637-80-7; 4-amino-5-cyano-1,2-dimethyl-1*H*-imidazole, 58192-81-7; 5-amino-4-cyano-1-methyl-1*H*-1,2,3-triazole, 23085-12-3; 1-methylpiperazine, 109-01-3; ethyl 5-[(2-amino-4-fluorophenyl)amino]-1-methyl-1*H*-imidazole-4-carboxylate, 121845-41-8; 6*H*-pyrido[2,3-*b*][1,5]-benzodiazepin-5(11*H*)-one, 10189-78-3; 8,9-dichloro-6*H*-pyrido-[2,3-*b*][1,5]benzodiazepin-5(11*H*)-one, 10321-11-6.

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Long-Acting Dihydropyridine Calcium Antagonists. 3. Synthesis and Structure–Activity Relationships for a Series of 2-[(Heterocyclylmethoxy)methyl] Derivatives

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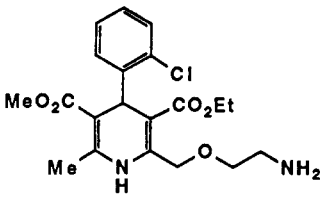
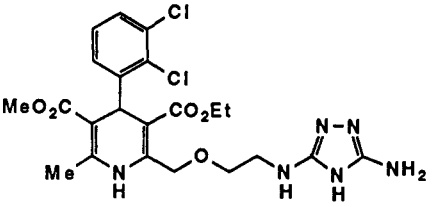
The preparation of 1,4-dihydropyridines containing (heterocyclylmethoxy)methyl groups in the 2-position is described and the structural identification of certain of the compounds using ^{1}H NMR spectroscopic methods is reported. The calcium antagonist activity of the compounds on rat aorta is listed and is compared with the negative inotropic potency as determined by using a Langendorff-perfused guinea pig heart model. Several compounds are more potent than nifedipine and show greater selectivity for the vasculature over the heart. One compound, 2-[[(2-amino-4-hydroxypyrimidin-6-yl)methoxy]methyl]-4-(2,3-dichlorophenyl)-3-(ethoxycarbonyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydropyridine (**27**, UK-56,593), was identified as a potent ($IC_{50} = 1.6 \times 10^{-9}$ M), tissue-selective calcium antagonist which proved to have a markedly longer duration of action (>4.5 h) than nifedipine in the anesthetized dog on intravenous administration.

We have recently reported[1] the synthesis and structure activity relationships (SARs) of a series of novel 1,4-dihydropyridine (DHP) calcium antagonists bearing basic side chains at the 2-position of the DHP ring. Our aim in this study was to modify the physicochemical properties of the DHP system so as to improve bioavailability and duration of action over the agents available at that time. Amlodipine (**1**) was identified as fulfilling our objectives



1, amlodipine

and is currently in late-stage clinical evaluation for the once-daily treatment of angina and hypertension.[2-4] In a subsequent publication,[5] we reported that a basic center in the amlodipine series was not an absolute requirement for good calcium antagonist activity and that the amino group could be substituted by a number of five- or six-membered heterocycles. The excellent calcium antagonist potency and selectivity for the vasculature over cardiac tissue seen for these compounds was thought to arise from enhanced hydrogen-bonding interactions between the polar heterocycles and the DHP receptor. As a result of these studies, UK-52,831 (**2**) was selected for clinical development. In order to extend these SARs and to identify additional structural features compatible with potent



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