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Synthesis and preliminary evaluation of pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidin-6(7*H*)-ones and related compounds, as benzodiazepine receptor ligands and anticonvulsant agents

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pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidin-6(7*H*)-ones / ethyl-7-methylpyrazolo[1,5-*a*]pyrimidin-6-carboxylates / 2 or 3-substituted 6-methylpyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidin-N^7-oxide / diethyl-7-methylpyrazolo[1,5-*a*]pyrimidin-3,6-dicarboxylates / synthesis / CNS activity / flunitrazepam binding study

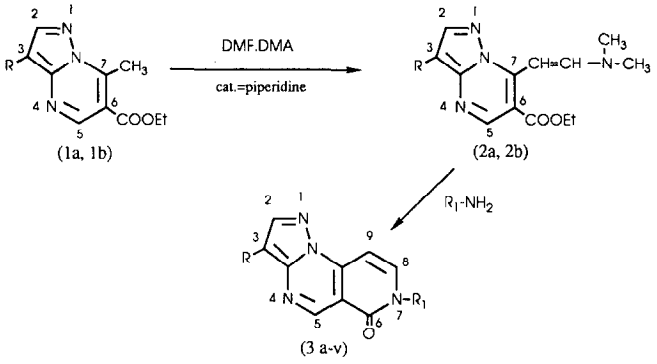
Introduction

As part of a program aimed at the synthesis and pharmacological study of condensed heterocyclic compounds containing the pyrazole[1,5-*a*] moiety [1], our interest has recently focused on the pyrazolo-[1,5-*a*]pyrido[3,4-*e*]pyrimidine system [2, 3].

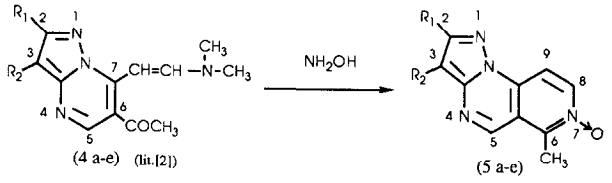
The interesting CNS properties of several compounds containing three angular fused heterocyclic systems are well known. The discovery of a CGS series at Ciba–Geigy as structures with high affinity for the benzodiazepine receptor [4] led us to investigate other tricyclic heterocycles of similar molecular shape. Recent papers report the antipsychotic of 1*H*-imidazo[1,2-*c*]pyrazolo[3,4-*e*]pyrimidines [5, 6], and the antidepressant effects of [1,2,4]-triazolo[4,3-*a*]quinoxalines [7, 8]. Other papers report the adenosine antagonism of [1,2,4]triazolo[1,5-*c*]-quinazolines [9] and BDZ binding activity of derivatives of the same system [10].

Chemistry

Following a described procedure [2], from **1a** or **1b** the respective 7-dimethylaminovinyl compounds **2a** or **2b** were obtained as a mixture of *Z–E* isomers. After recrystallization, **2a** and **2b** were isolated as



Radicals R and R_1 specified in table I.



Radicals R_1 and R_2 specified in table II.

Scheme 1.

Table I. Structure, chemical and ^{1}H-NMR data of pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyramidin-6(7*H*)-ones.

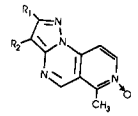
Compd	*R*	*R_1*	*Formula yield (%)*	*Mp (°C) recryst solv*	*^{1}H-NMR δ_{11} (*DMSO, § $CDCl_3$, # TFA, ¢ D_2O)*
3a	COOEt	H	$C_{12}H_{10}N_4O_3$ 76	305–306° (EtOH: H_2O)	1.30 (t, 3H, OCH_2CH_3); 4.60 (q, 2H, OCH_2); 7.15 (dd, J_{H9-H8} = 6.7 Hz, J_{H9-H5} = 0.8 Hz, 1H, H-9); 8.01 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.73 (d, 1H, H-2); 9.26 (d, J_{H5-H9} = 0.8 Hz, 1H, H-5); 12.50 (bs, 1H, NH, exch)*
3a'	COONa	H	$C_{10}H_5N_4O_3Na$		
3b	COOEt	CH_2COOCH_3	$C_{15}H_{14}N_4O_5$ 66	259–260° (EtOH: H_2O)	1.56 (t, 3H, OCH_2CH_3); 4.00 (s, 3H, OCH_3); 4.70 (q, 2H, OCH_2); 5.16 (s, 2H, NCH_2CO); 7.76 (d, 1H, H-9); 8.56 (d, 1H, H-8); 8.90 (s, 1H, H-2); 9.93 (s, 1H, H-5) #
3b'	COOEt	CH_2COOH	$C_{14}H_{12}N_4O_5$ 85	232–233° dec (EtOH: H_2O)	1.36 (t, 3H, OCH_2CH_3); 4.36 (q, OCH_2); 4.80 (s, 2H, NCH_2); 7.23 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 8.30 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.70 (s, 1H, H-2); 9.30 (s, 1H, H-5)*
3b''	COOEt	CH_2COONa	$C_{14}H_{11}N_4O_5Na$		
3c	COOEt	$(CH_2)_2COOEt$	$C_{17}H_{18}N_4O_5$ 68	202–203° dec (EtOH)	1.40 (m, 6H, OCH_2CH_3); 2.93 (t, 2H, CH_2CO); 4.35 (m, 6H, NCH_2, OCH_2); 7.20 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 8.00 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.72 (s, 1H, H-2); 9.50 (s, 1H, H-5) §
3c'	COOEt	$(CH_2)_2COOH$	$C_{15}H_{14}N_4O_5$ 83	> 300° EtOH	1.32 (t, 3H, OCH_2CH_3); 2.77 (t, 2H, CH_2COOH); 4.30 (m, 4H, NCH_2, CH_2O); 7.20 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 8.30 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.72 (s, 1H, H-2); 9.35 (1H, H-5); 12.5 (s, 1H, COOH)*
3d	COOEt	$CH(CH_2OH)COOCH_3$	$C_{16}H_{16}N_4O_6$ 45	225–226° (EtOH: H_2O)	
3e	COOEt	$(CH_2)_3COOH$	$C_{16}H_{16}N_4O_5$ 77	186–187° (EtOH: H_2O)	1.36 (t, 3H, OCH_2CH_3); 2.10 (m, 4H, CH_2); 4.23 (m, 4H, NCH_2, OCH_2); 7.20 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 8.30 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.76 (s, 1H, H-2); 9.30 (s, 1H, H-5)*
3e'	COOEt	$(CH_2)_3COONa$	$C_{16}H_{15}N_4O_5Na$		
3f	COOEt	$CH[CH(CH_3)_2]COOH$	$C_{16}H_{18}NO_5$	115–116° dec	0.82 (d, 3H, $CHCH_3$); 1.10 (d, 3H, $CHCH_3$); 1.38 (t, 3H, OCH_2CH_3); 2.45 (m, 1H, $CH(CH_3)_2$); 4.40 (q, 2H, OCH_2CH_3); 4.45 (d, 1H, NCH); 7.20 (d, J_{H9-H8} 6.7 Hz, 1H, H-9); 8.00 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.50 (s, 1H, H-2); 9.40 (s, 1H, H-5) §
3g	COOEt	$CH[CH_2CH(CH_3)_2]COOH$	$C_{18}H_{20}N_4O_5$ 36	316–318° (EtOAc)	0.90 (m, 6H, $CH(CH_3)_2$); 1.33 (t, 3H, OCH_2CH_3); 1.83 (m, 2H, CH_2CH); 2.35 (m, 1H, $CH(CH_3)_2$); 4.36 (q, 2H, OCH_2); 5.40 (t, 2H, NCH); 7.20 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 8.30 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.70 (s, 1H, H-2); 9.30 (s, 1H, H-5)*
3h	COOEt	$CH[CH(CH_3)CH_2CH_3]COOH$	$C_{18}H_{20}N_4O_5$ 40	115–116° (AmOH)	0.75 (m, 2H, $CH_2CH(CH_3)_2$); 0.98 (m, 6H, $CH(CH_3)_2$); 1.30 (t, 3H, OCH_2CH_3); 2.00 (m, 1H, $CH(CH_3)_2$); 4.03 (q, 2H, OCH_2); 5.10 (d, 1H, NCH); 7.15 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9), 8.50 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.70 (s, 1H, H-2); 9.30 (s, 1H, H-5)*
3i	COOEt	$CH(CH_2COOH)COOH$	$C_{16}H_{14}N_4O_7$ 50	> 300° dec (EtOH)	1.30 (t, 3H, OCH_2CH_3); 2.40 (d, 2H, CH_2COOH); 4.30 (q, 2H, OCH_2); 5.40 (t, 1H, NCH); 7.10 (dd, J_{H9-H8} = 6.7 Hz, 1H, H-9); 8.15 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.75 (s, 1H, H-2); 9.20 (s, 1H, H-5)*
3j	COOEt	$CH[(CH_2)_2COOH]COOH$	$C_{17}H_{16}N_4O_7$ 49	> 300° dec (EtOH)	1.30 (t, 3H, OCH_2CH_3); 1.70 (m, 2H, $CHCH_2$); 2.40 (m, 2H, CH_2COOH); 4.30 (q, 2H, OCH_2); 5.15 (m, 1H, NCHCOOH); 7.10 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 8.20 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.60 (s, 1H, H-2); 9.20 (s, 1H, H-5)*
3k	COOEt	$C(CHCH_3)COOH$	$C_{16}H_{14}N_4O_5$ 42	198–199° dec (EtOH: H_2O)	1.30 (t, 3H, OCH_2CH_3); 1.50 (d, 3H, $CH=CH_2$); 4.30 (q, 2H, OCH_2); 6.72 (m, 1H, CH=C); 7.10 (d, J_{H9-H8} = 5.8 Hz, 1H, H-9); 7.85 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.70 (s, 1H, H-2); 9.20 (s, 1H, H-5)*
3kk	COOEt	$C(=CH_2)COOCH_3$	$C_{16}H_{14}N_4O_4$ 53	220–221° (EtOH)	1.40 (t, 2H, CH_3); 3.85 (s, 3H, CH_3); 4.45 (q, 2H, CH_2); 6.00 (t, 1H, CH); 6.60 (d, 1H, CH); 7.40 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 7.60 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.60 (s, 1H, H-2); 9.40 (s, 1H, H-5) §
3l	COOEt	$(CH_2)_2N(C_2H_5)_2$	$C_{18}H_{23}N_5O_3$ 48	176–178° (EtOH: H_2O)	0.90 (m, 6H, NCH_2CH_3); 1.4 (t, 3H, OCH_2CH_3); 2.65 (m, 6H, $CH_2N(CH_2)_2$); 4.10 (t, 2H, NCH_2); 4.50 (q, 2H, OCH_2); 7.26 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 7.90 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.63 (s, 1H, H-2); 9.50 (s, 1H, H-5) §

Table I. *Continued*

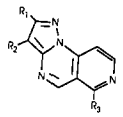
Compd	*R*	*R_1*	*Formula yield (%)*	*Mp (°C) recryst solv*	*^{1}H-NMR δ_{ll} (*DMSO, § $CDCl_3$, # TFA, ¢ D_2O)*
3m	COOEt	$(CH_2)_3N(C_2H_5)_2$	$C_{19}H_{25}N_5O_3$ 38	147–148° (iPrOH)	1.00 (m, 6H, NCH_2CH_3); 1.40 (t, 3H, OCH_2CH_3); 1.95 (q, 2H, CH_2); 2.50 (m, 6H, $CH_2N(CH_2)_2$); 4.18 (t, 2H, NCH_2); 4.5 (q, 2H, OCH_2); 7.23 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 7.90 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.60 (s, 1H, H-2); 9.50 (s, 1H, H-5) §
3n	COOEt	$(CH_2)_3N(CH_3)_2$	$C_{17}H_{21}N_5O_3$ 30	163–165° (iPrOH)	1.46 (t, 3H, OCH_2CH_3); 1.90 (m, 2H, CH_2 alif); 2.25 (m, 6H, $CH_2N(CH_2)_3$); 4.16 (t, 2H, NCH_2); 4.50 (q, 2H, OCH_2CH_3); 7.23 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 7.95 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.60 (s, 1H, H-2); 9.53 (s, 1H, H-5) §
3o	COOEt	$(CH_2)_2$-N(CH$_2$CH$_2$)$_2$N-$COCH_3$	$C_{20}H_{24}N_6O_4$ 40	210–211° (Cyclohex: EtOAc)	1.42 (t, 3H, OCH_2CH_3); 2.00 (s, 3H, CH_3CO); 2.50 (m, 4H, $N(CH_2)_2$); 2.78 (t, 2H, CH_2N); 3.40–3.60 (dt, 4H, $(CH_2)_2NCOCH_3$); 4.15 (t, 2H, CH_2N); 4.45 (q, 2H, OCH_2); 7.20 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 7.75 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.60 (s, 1H, H-2); 9.45 (s, 1H, H-5) §
3p	COOEt	$(CH_2)_2$-N(CH$_2$CH$_2$)$_2$O	$C_{18}H_{21}N_5O_4$ 30	195–196° (EtOH)	1.50 (t, 3H, OCH_2CH_3); 2.65 (m, 6H, $CH_2N(CH_2)_2$); 3.73 (t, 4H, CH_2OCH_2); 4.20 (t, 2H, NCH_2); 4.53 (q, 2H, OCH_2CH_3); 7.33 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 7.85 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.65 (s, 1H, H-2); 9.5 (s, 1H, H-5) §
3q	COOEt	CH_2CONH_2	$C_{14}H_{13}N_5O_4$ 53	249–250° (EtOH: EtOAc)	1.33 (t, 3H, OCH_2CH_3); 4.40 (q, 2H, OCH_2CH_3); 5.25 (s, 2H, NCH_2CO); 7.35 (d, J_{H9-H8} = 6.7 Hz, 1H, H-9); 8.40 (d, J_{H8-H9} = 6.7 Hz, 1H, H-8); 8.75 (s, 1H, H-2); 9.33 (s, 1H, H-5)*
3r	COOEt	$CH_2CONHCH_2COOH$	$C_{16}H_{15}N_5O_6$ 66	294–296° dec (EtOH: H_2O)	1.30 (t, 3H, OCH_2CH_3); 3.75 (s, 2H, CH_2COOH); 4.35 (q, 2H, OCH_2); 4.85 (s, 2H, NCH_2CO); 7.35 (d, 1H, J_{H9-H8} = 6.9 Hz, H-9); 8.10 (d, J_{H8-H9} = 6.9 Hz, 1H, H-8); 8.70 (s, 1H, H-2); 9.25 (s, 1H, H-5) ¢
3s	H	H	$C_9H_6N_4O$ 78	> 300° EtOH	6.86 (d, J_{H3-H2} = 2.5 Hz, 1H, H-3); 7.10 (dd, J_{H9-H8} = 7.3 Hz, J_{H9-H5} = 0.8 Hz, 1H, H-9); 7.93 (d, J_{H8-H9} = 7.3 Hz, 1H, H-8); 8.36 (d, J_{H2-H3} = 2.5 Hz, 1H, H-2); 9.00 (d, J_{H5-H9} = 0.8 Hz, 1H, H-5); 12.20 (bs, 1H, NH exch)*
3t	H	CH_2COOH	$C_{11}H_8N_4O_3$ 75	> 300° EtOH	4.35 (s, 2H, NCH_2COOH); 6.86 (d, J_{H3-H2} = 2.5 Hz, 1H, H-3); 7.15 (d, J_{H9-H8} = 7.3 Hz, 1H, H-9); 8.2 (d, J_{H8-H9} = 7.3 Hz, 1H, H-8); 8.38 (d, J_{H2-H3} = 2.5 Hz, 1H, H-2); 9.00 (s, 1H, H-5)*
3t'	H	CH_2COONa	$C_{11}H_7N_4O_3Na$		
3u	H	$(CH_2)_2COOH$	$C_{12}H_{10}N_3O_3$ 82	281–282° EtOH	2.75 (t, 2H, CH_2COOH); 4.20 (t, 2H, NCH_2); 6.85 (d, J_{H3-H2} = 2.5 Hz, 1H, H-3); 7.15 (d, J_{H9-H8} = 7.3 Hz, 1H, H-9); 8.20 (d, J_{H8-H9} = 7.3 Hz, 1H, H-8); 8.40 (d, J_{H2-H3} = 2.5 Hz, 1H, H-2); 9.00 (s, 1H, H-5)*
3u'	H	$(CH_2)_2COONa$	$C_{12}H_9N_4O_3Na$		
3v	H	$(CH_2)_3COOH$	$C_{13}H_{12}N_4O_3$ 78	199–200° EtOH	1.95 (m, 2H, CH_2); 2.30 (t, 2H, CH_2COOH); 4.00 (t, 2H, NCH_2); 6.85 (d, J_{H3-H2} = 2.5 Hz, 1H, H-3); 7.15 (d, J_{H9-H8} = 7.3 Hz, 1H, H-9); 8.20 (d, J_{H8-H9} = 7.3 Hz, 1H, H-8); 8.35 (d, J_{H2-H3} = 2.5 Hz, 1H, H-2); 9.00 (s, 1H, H-5)*
3v'	H	$(CH_2)_3COONa$	$C_{13}H_{11}N_4O_3Na$		

conformationally pure *E* isomers, the *trans* geometry being ascertained by the coupling constants between the olefinic protons. Reaction of **2a** or **2b** with ammonium acetate in glacial acetic acid, afforded **3a** or **3b**. A lactam carbonyl band at 1630–1660 cm^{-1} confirmed the structure proposed. We thought it interesting to prepare a series of pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidin-6-ones with a functionalized side chain at the N-7 position, and to evaluate their CNS activity. Thus, **2a** or **2b** were reacted with a series of dialkylamino alkylamines, natural aminoacids, or their esters. To further explore the structure–activity relationship, we decided to develop a series of 6-methylpyrazolo-[1,5-*a*]pyrido[3,4-*e*]pyrimidin-7-oxides (**5a–e**) according to a procedure described in our previous paper [3]. The structures of the synthesized compounds were confirmed by IR and ^{1}H-NMR data (see *Experimental protocols* and table II).

Table II. Inhibition[a] of [^{3}H]-flunitrazepam binding.



5 a-e



6 a-h

No	R_1	R_2	R_3	*Inhibition % (10 μM)*
5a	H	H	–	64.0 ± 3.0
5b	CH_3	H	–	0
5c	H	C_6H_5	–	0
5d	H	CN	–	9.0 ± 0.5
5e	H	$COOC_2H_5$	–	26.1 ± 1.1
6a	H	H	CH_3	0
6b	CH_3	H	CH_3	6.0 ± 0.4
6c	H	C_6H_5	CH_3	38.9 ± 2.0
6d	H	CN	CH_3	0
6e	H	COOEt	CH_3	0
6f	H	H	H	0
6g	H	H	$COOCH_3$	0
6h	H	H	CH_2OAc	0

[a]Percent of inhibition of specific [^{3}H]-flunitrazepam binding at 10 μM concentration are means ± SEM of five determinations.

Pharmacological results

The sodium salts **3a'**, **3b''**, **3e'**, **3l'**, **3t'**, **3u'**, **3v'** and the amide **3q** together with **5a**, **5e** were evaluated for CNS activity in a preliminary screening based on observation of the gross behavior of mice injected intraperitoneally (ip) with 100 mg/kg or treated per os (300 mg/kg). Animals were observed 60-90 min after treatment. None of the treated mice revealed any behavioural alterations and none died. The above compounds were tested for their anticonvulsant effect using PTZ (Pentylenetetrazole) as convulsant agent. The compounds **3a'**, **3l'**, **3t'** and **5a** showed an anticonvulsant activity (31–34%) only at very high doses (100 mg/kg ip or 300 mg/kg po). None of the mice pretreated with sodium phenobarbital 25 mg/kg ip developed convulsions.

Binding studies

The ability of pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidine derivatives to interact with benzodiazepine receptor sites was investigated by evaluating their displacing effect on [^{3}H]-flunitrazepam binding to bovine brain membranes.

The compounds **3a**, **3b'**, **3c**, **3e**, **3l**, **3q**, **3r**, **3s**, **3t**, **3u**, **3v**, **5a**, **5b**, **5c**, **5d**, **5e** together with those listed in table II, whose synthesis has been previously reported [2, 3] were tested at a concentration of 10 μM in the presence of 2% ethanol to solubilize the pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidines (table II). None of the tested compounds belonging to the **3** series showed any ability to displace [^{3}H]-flunitrazepam binding. The only compound that showed an affinity was **5a**. The present findings, even if disappointing, allowed us to establish some more structure–activity relationships. From our preliminary results it appears that there is no correlation between the **3** or **6** and **5** series; moreover, within this latter subseries, a prerequisite for retaining some activity to displace specific [^{3}H]-flunitrazepam from the BZR is the presence of the H at 2 and 3 positions. The replacement with other substituents, regardless of their nature and position, infact dramatically prevents binding to the receptor site.

Experimental protocols

Chemistry

Melting points were determined with a Gallenkamp apparatus and are uncorrected. IR spectra were recorded using a Perkin–Elmer 681 spectrophotometer, in nujol mulls. ^{1}H-NMR spectra were measured with a Varian Gemini at 200 MHz and chemical shifts are expressed in δ (ppm), using DMSO–d_6 or $CDCl_3$, D_2O, TFA as solvents. ^5J values are expressed only for **3a** and **3s**. The purity of samples was determined by means of TLC, which was performed using Merck (Darmstadt) silica gel 60 F254 plates. Microanalyses were performed with a Perkin–Elmer Model 240 C Elemental Analyzer and values are within ± 0.4% of the theoretical values.

Diethyl 7-methylpyrazolo[1,5-a]pyrimidin-3,6-dicarboxylate ***1a***
From ethyl-3-aminopyrazole-4-carboxylate and ethyl-2-acetyl-3-ethoxyacrylate [12] according to a reported procedure [2]. Mw = 205.22, mp = 142–143°C (EtOH), yield = 56%. Anal (C, H, N): $C_{13}H_{15}N_3O_4$. ^{1}H-NMR (200 MHz; DMSO) δ ppm: 1.20 (t, 3H, OCH_2CH_3); 1.75 (t, 3H, OCH_2CH_3); 3.01 (s, 3H, 7-CH_3); 4.40 (dq, 4H, OCH_2CH_3); 8.60 (s, 1H, H-2); 9.00 (s, 1H, H-5). IR (Nujol): 1750–1720 cm^{-1} (C=O).

Ethyl 7-methylpyrazolo[1,5-a]pyrimidin-6-carboxylate ***1b***
From 3-aminopyrazole and ethyl-2-acetyl-3-ethoxyacrylate as indicated above. Mw = 205.22, mp = 90–91°C (EtOH) (lit [11] 94–95°C EtOH/H_2O 1/1), yield = 70%. Anal (C, H, N): $C_{10}H_{11}N_3O_2$. ^{1}H-NMR (200 MHz; DMSO) δ ppm: 1.70 (t, 3H, OCH_2CH_3); 3.01 (s, 3H, 7-CH_3); 4.35 (q, 2H, OCH_2CH_3); 6.70 (d, 1H, H-3); 8.30 (d, 1H, H-2); 8.90 (s, 1H, H-5). IR (Nujol): 1730 cm^{-1} (C=O).

General procedure for the preparation of ***2a*** *or* ***2b***
Compounds **2a** or **2b** were obtained according a described method [2]. A catalytic amount of piperidine (0.5 ml) was added.

Diethyl 7-(2-dimethylaminovinyl)pyrazolo[1,5-a]pyrimidin- 3,6-dicarboxylate ***2a***
Mw = 332.37, mp = 117-118°C (cyclohexane), yield = 72%. Anal (C, H, N): $C_{16}H_{20}N_4O_4$. ^{1}H-NMR (200 MHz; $CDCl_3$) δ ppm: 1.25 (t, 3H, OCH_2CH_3); 1.75 (t, 3H, OCH_2CH_3); 3.15 (s, 3H, NCH_3); 3.53 (s, 3H, NCH_3); 4.43 (dq, 4H, OCH_2); 7.20 (d, J_{trans} = 12.55 Hz, 1H, $CHCHN(CH_3)_2$); 8.50 (d, 1H, H-2); 9.10 (d, 1H, H-5); 9.65 (d, J_{trans} = 12.55 Hz, 1H, $CHCH\text{-}N(CH_3)_2$). IR (Nujol): 1750–1730 cm^{-1} (C=O).

Ethyl 7-(2-dimethylaminovinyl)pyrazolo[1,5-a]pyrimidin-6-carboxylate ***2b***
Mw = 260.3, mp = 109–110°C (cyclohexane), yield = 78%. Anal (C, H, N): $C_{13}H_{16}N_4O_2$. ^{1}H-NMR (200 MHz; $CDCl_3$) δ ppm: 1.70 (t, 3H, OCH_2CH_3); 3.15 (s, 3H, NCH_3); 3.55 (s, 3H, NCH_3); 4.40 (q, 2H, OCH_2); 6.50 (d, 1H, H-3); 7.25 (d, J_{trans} = 12.65 Hz, 1H, $CHCHN(CH_3)_2$); 8.10 (d, 1H, H-2); 8.70 (s, 1H, H-5); 9.70 (d, J_{trans} = 12.65 Hz, 1H, $CHCH\text{-}N(CH_3)_2$). IR (Nujol): 1730 cm^{-1} (C=O).

General procedure for the preparation of ethyl 6-oxopyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-3-carboxylate ***3a*** *or pyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-6(7H)-one* ***3s***
To a solution of **2a** or **2b** (1 mmol) in acetic acid (10 ml) ammonium acetate (26 mmol) was added and the mixture was refluxed for 1 h.

General procedure for the preparation of 7-substituted ethyl 6-oxo-pyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-3-carboxylates ***3b-3r*** *and 7-substituted pyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-6(7H)-ones* ***3t-3v'***
Method A. A suspension of **2a** or **2b** (2 mmol) in acetic acid (25 ml) containing anhydrous sodium acetate (4 mmol) was treated with suitable aminoacid (2 mmol) or glycylglycine and refluxed for 2 h under magnetic stirring. After concentration of the solvent the residue was filtered and recrystallized (see table I).
Method B. This procedure was used when the amino-acid are glycine methyl ester. HCl and β-alanine ethyl ester HCl. In these cases a suspension of **2a** or **2b** (2 mmol) and equimolar amounts of aminoacid and pyridine (1:1:1) were refluxed in MeOH (20 ml) for 6 hours.

After cooling, the solid formed was separated by filtration and recrystallized from EtOH (**3b**, **3c**).
Method C. A suspension of **2a** or **2b** (2 mmol) and the appropriate amines in large excess were refluxed for 2 hours. After cooling, a filtrable and crystallizable product was obtained (table I). This general procedure was used for all the amines except for 1-(2-aminoethyl)piperazine, which was reacted according to procedure A and characterized as an acetyl-derivate.

Synthesis of the acetylderivative ***3o***
To a solution of **2a** (2 mmol) in acetic acid (20 ml) containing 1-(2-aminoethyl)piperazine (2 mmol), acetic anhydride was added in excess and the mixture was stirred for 30 min. After stirring and cooling the residue was filtered, a deliquescent product was obtained which was treated with $CHCl_3$ and water to eliminate the excess of acid. After evaporation of the chloroform the light brown residue was collected by filtration and purified by recrystallization from cyclohexane: ethyl acetate, affording white/cream crystals.

General procedure for the preparation of ***5b–e***
Compounds **5b–e** were prepared according to a procedure previous described for **5a** [3].

6-Methylpyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-N^7-oxide ***5a***
Mw = 200.20, mp = 269–270°C (H_2O) (lit [3] 269–270°C).

2,6-Dimethylpyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-N^7-oxide ***5b***
Mw = 214.23, mp = 253–254°C (iPrOH), yield = 78%. Anal (C, H, N): $C_{11}H_{10}N_4O$. ^{1}H-NMR (200 MHz; DMSO) δ ppm: 2.96 (bs, 6H, 6-CH_3, 2-CH_3); 6.79 (s, 1H, H-3); 8.15 (d, J_{H9-H8} = 7.1 Hz, 1H, H-9); 8.63 (d, J_{H8-H9} = 7.1 Hz, 1H, H-8); 9.25 (s, 1H, H-5).

6-Methyl-3-phenylpyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-N^7-oxide ***5c***
Mw = 276.31, mp = 276–277°C (EtOH), yield = 82%. Anal (C, H, N): $C_{16}H_{12}N_4O$. ^{1}H-NMR (200 MHz; DMSO) δ ppm: 2.96 (s, 3H, 6-CH_3); 7.45 (m, 3H, ArH_3); 7.99–820 (m, 2H, ArH_2); 8.25 (d, J_{H9-H8} = 7.4 Hz, 1H, H-9); 8.40–8.45 (s, 1H, H-2); 8.62 (d, J_{H8-H9} = 7.4 Hz, 1H, H-8); 9.08 (s, 1H, H-5).

3-Cyano-6-methylpyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-N^7-oxide ***5d***
Mw = 225.16, mp = 284–285°C ($CH_3OCH_2CH_2OH$), yield = 86%. Anal (C, H, N): $C_{11}H_7N_5O$. ^{1}H-NMR (200 MHz; DMSO) δ ppm: 2.87 (s, 3H, 6-CH_3); 8.32 (d, J_{H9-H8} = 6.5 Hz, 1H, H-9); 8.78 (d, J_{H8-H9} = 6.5 Hz, 1H, H-8); 8.91 (s, 1H, H-2); 9.64 (s, 1H, H-5).

3-Ethoxycarbonyl-6-methylpyrazolo[1,5-a]pyrido[3,4-e]pyrimidin-N^7-oxide ***5e***
Mw = 272.27, mp = 269–270°C (EtOH), yield = 89%. Anal (C, H, N): $C_{13}H_{12}N_4O_3$. ^{1}H-NMR (200 MHz; DMSO) δ ppm: 1.35 (t, 3H, CH_2CH_3); 2.86 (s, 3H, 6-CH_3); 4.36 (q, 2H, CH_2CH_3); 8.30 (d, J_{H9-H8} = 7.3 Hz, 1H, H-9); 8.71 (s, 1H, H-2); 8.75 (d, J_{H8-H9} = 7.3 Hz, 1H, H-8); 9.61 (s, 1H, H-5).

Pharmacology

General procedure for evaluating anticonvulsant activity

The experiments were carried out on 206 adult male Swiss mice from the Morini S Polo breeding farm, body weight 25–35 g, commercially fed and with water *ad libitum* prior to

the test. Animals were randomly divided into groups. Each mouse was used only once. All drugs were given in volumes of 10 ml/kg: ip in 0.9% NaCl 15 min prior to PTZ (Pentylenetetrazole, Sigma) or po in carboxymethylcellulose 1% 30 min prior to PTZ. The dose of PTZ is 70 mg/kg sc (convulsant dose 74%) this being the dose at which a sodium phenobarbital ('Gardenale' Farmitalia) 25 mg/kg ip, was able to prevent convulsions. The animals were observed closely for 45 min after receiving PTZ. The criterion used to indicate a convulsive response was a generalized clonic or tonic-clonic seizure. The tested compounds were used as sodium salts to increase their solubility in physiological saline and administered ip.

Binding studies

[^{3}H]-Flunitrazepam (79.6 Ci/mmol; radiochemical purity > 99%) was obtained from New England Nuclear (Dreieichenain, West Germany). Membranes from bovine brains were prepared after homogenization and differential centrifugation as described in [13]. The estimation of proteins was based on Lowry's method [14], after membrane solubilization with 0.75 M NaOH. Benzodiazepine receptor binding studies were performed by using a filtration technique and [^{3}H]-flunitrazepam as ligand according to a method previously described [15]. Water insoluble pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidines were dissolved in ethanol and added to the assay mixture to a final volume of 500 µl. Blank experiments were carried out to determine the effect of ethanol (2%) on the binding.

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