WO 99/67243 *159159* PCT/EP99/03863

ANTICONVULSIVE AND ANTIALLERGIC/ANTIASTHMATIC PYRAZOLO-(3,4-D)PYRIMIDINES

The invention relates to pyrazolo[3,4-d]pyrimidin-4(5H)-ones and pyrazolo[3,4-d]pyrimidine-4(5H)-thiones and their tautomers which contain a benzyl radical in the 2-position, processes for their preparation and their use as medicaments, in particular for the treatment both of epilepsies of various forms and of allergic diseases such as bronchial asthma, allergic rhinoconjunctivitis or atopic dermatitis.

The adenosine receptor is of importance as a target for influencing disregulations [sic] in various organ systems (e.g. the central nervous system, airways, etc.). On account of the structural similarities to adenine, pyrazolo[3,4-d]pyrimidines are pharmacologically interesting compounds.

2-Benzyl-substituted pyrazolo[3,4-d]pyrimidine-4(5H)-thiones and tautomers are not known.

Pyrazolo[3,4-d]pyrimidin-4(5H)-ones and tautomers which have a substituted benzyl radical in the 2-position are likewise not known.

Until now, only 2-benzylpyrazolo[3,4-d]pyrimidin-4(5H)-one has been described [R. Böhm, Pharmazie 1986, 41, 430; Th. Eisenächer, R. Pech, R. Böhm, Pharmazie 1991, 46, 747]. This compound is obtained by cyclization of ethyl 3-amino-1-benzylpyrazole-4-carboxylate with formamide. 2-Benzyl-substituted 3-amino-pyrazole-4-carboxylic acid esters can be obtained by benzylation of 3-amino-pyrazole-4-carboxylic acid esters [S. Senda, K. Hirota, G.-N. Yang, Chem. Pharm. Bull. 1972, 20(2) 391].

An actual pharmacological action has not been mentioned or suggested for 2-benzyl-pyrazolo[3,4-d]pyrimidin-4(5H)-one.

Known anticonvulsants on the one hand have the disadvantage that undesired side effects, such as

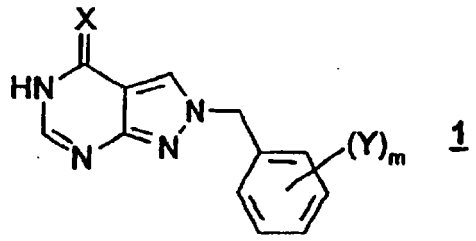
neurotoxicity and idiosyncrasies, occur and on the other hand these anticonvulsants are not active in certain forms of epilepsy.

Various forms of allergic/asthmatic disorders, such as bronchial asthma, can likewise be treated inadequately using the present medicaments.

Various forms of allergic/asthmatic disorders, such as bronchial asthma, can likewise be treated inadequately using the present medicaments. [sic]

The invention is therefore based on the object of making available compounds having favourable pharmacological properties, which can be employed as medicaments, in particular for the treatment of epilepsies and various allergic/asthmatic diseases.

According to the present invention, these new compounds are 2-ar(alkyl)pyrazolo[3,4-d]pyrimidin-4(5H)-ones and 2-ar(alkyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thiones of the general formula 1



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Formula 1

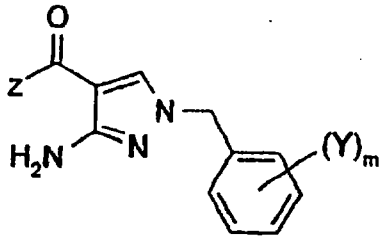
or their tautomers, where

X is oxygen and [sic] sulphur,
Y is halogen, C_1-C_4-alkyl, C_1-C_4-alkoxy, trifluoromethyl or trifluoromethoxy.

Examples of compounds of the general formula 1 which may be mentioned are:

2-(2-fluorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-chlorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-bromobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-iodobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-trifluoromethylbenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-methylbenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(3-trifluoromethylbenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2,6-difluorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-chloro-6-fluorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2,6-dichlorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2,4-dichlorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(4-methoxybenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-chloro-4,5-methylenedioxybenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-chlorobenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione
2-(2-bromobenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione
2-(2-iodobenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione
2-(2-trifluoromethylbenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione
2-(2,6-difluorobenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione

The process for the preparation of compounds of the formula 1 and their tautomers where X is oxygen is based on the cyclization of 3-aminopyrazole-4-carboxylic acid esters or 3-aminopyrazole-4-carboxamides of the general formula 2 in formamide at temperatures between 100 and 180°C.



2

Formula 2

in which

Z is hydroxyl, alkoxy or amino,

Y is halogen, C_1-C_4-alkyl, C_1-C_4-alkoxy, trifluoromethyl, trifluoromethoxy. [sic]

The process for the preparation of compounds of the formula 1 and their tautomers where X is sulphur is based on the substitution of compounds of the formula 1 and their tautomers where X is oxygen by sulphur by means of phosphorus pentasulphide or 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulphide.

The process for the preparation of compounds of the formula 2 starts from 3-aminopyrazole-4-carboxylic acid derivatives. The compounds of the formula 2 are obtained by alkylation under phase-transfer conditions using a suitably substituted benzyl halide.

The compounds according to the invention or their pharmaceutically utilizable salts are suitable for the production of pharmaceutical compositions. The pharmaceutical compositions or medicaments can contain one or more of the compounds according to the invention. The customary pharmaceutical excipients and auxiliaries can be used for the production of the pharmaceutical preparations. The pharmaceuticals can be administered, for example, parenterally (e.g. intravenously, intramuscularly, subcutaneously), topically (intranasally, by inhalation) or orally.

The administration forms can be prepared by processes which are generally known and customary in pharmaceutical practice.

The compounds according to the invention have strong anticonvulsive or antiallergic/antiasthmatic actions.

1. Anticonvulsive activity

The compounds according to the invention were tested in vivo for their anticonvulsive action (Table 1) after i.p. administration to mice or rats (p.o. administration) according to the internationally customary standard (Pharmac. Weekblad, Sc. Ed. 14, 132 (1992) and Antiepileptic Drugs, Third Ed., Raven Press, New York 1989).

Analogous results were obtained for the oral action. For example, for the compound 2 (2-(2-chlorobenzyl)-pyrazolo[3,4-d]pyrimidin-4(5H)-one) in the rat, the ED_{50} (p.o.) was determined in maximal electroshock to be 32 mg/kg and the NT_{50} was determined to be > 250 mg/kg for the neurotoxicity. Compound 15 (2-(2-chlorobenzyl)-pyrazolo[3,4-d]pyrimidine-4(5H)-thione) is likewise strongly anticonvulsive together with great therapeutic breadth (ED_{50} (rat p.o.) = 12 mg/kg, NT_{50} > 460 mg/kg).

Table 1: Anticonvulsive action of selected pyrazolo[3,4-d]pyrimidines

Compound[1]	Log P[2]	Test[3]	Dose[4]	Action[5]
1	0.2	MES	30	100
		PTZ	100	100
2	1.01	MES	100	100
		PTZ	100	60
3	0.56	MES	100	100
		PTZ	300	80
4	0.74	MES	300	100
		PTZ	300	--

5	1.08	MES PTZ	30 30	100 20
6	0.75	MES PTZ	30 100	100 20
7	1.00	MES PTZ	30 100	100 60
8	0.18	MES PTZ	30 30	100 40
9	0.93	MES PTZ	30 300	100 --
10	1.16	MES PTZ	100 300	60 --
11	1.68	MES PTZ	100 100	100 20
12	0.62	MES PTZ	100 300	15 40
13	1.21	MES PTZ	100 300	30 --
15	1.54	MES PTZ	100 100	100 60
16	1.20	MES PTZ	100 300	30 --
17	1.40	MES PTZ	100 300	30 --
18	1.60	MES PTZ	100 100	100 80
19	1.57	MES PTZ	30 100	100 60
2-benzyl-pyrazolo-[3,4-d]-pyrimidine	0.40	MES PTZ	100 100	100 --
Comparison substances				
Carbamazepine		MES PTZ	100 100	100 0
Valproate		MES PTZ	100 100	0 30

Comments for Table 1:

1) Numbering of the compounds corresponding to the examples in Table 2 and Table 3
2) Octanol/water partition coefficient
3) Mouse i.p.: MES = maximal electroshock, PTZ = s.c. pentetrazole
4) In mg/kg
5) In % of the protected animals; n.t. = not tested

2. Antiallergic/antiasthmatic activity:

The compounds according to the invention were tested in vivo for antiasthmatic action after oral administration to male guinea-pigs, the inhibition of the infiltration of the eosinophilic granulocytes into the lungs being determined.

Male guinea-pigs (Dunkin Hartley Shoe) weighing 200-250 g were actively sensitized by an s.c. injection of ovalbumin (10 µg + 1 mg of aluminium hydroxide) and boosted 2 weeks later. One week after boosting with ovalbumin, the animals were challenged with an aerosol of 0.5% strength ovalbumin solution for 20-30 sec. 24 h later, a bronchoalveolar lavage (BAL) was carried out in the animals under urethane anaesthesia using 2 x 5 ml of saline solution. The lavage fluid was collected and centrifuged at 400 x g for 10 min and the cell pellets were suspended in 1 ml of saline solution. The eosinophilic granulocytes were counted microscopically in a Neubauer chamber. The Becton Dickinson test kit (No. 5877) for eosinophils was used for staining. The test kit used phloxin B as a selective stain for eosinophils. The eosinophils in the BAL were counted for each animal and the eosinophils were calculated in millions/animal. The test substances were administered orally 2 hours before allergen challenge.

The percentage inhibition of eosinophilia of the group treated with substance is calculated according to the following formula:

(A - C) - (B - C) / (A - C) x 100 = % inhibition

A = eosinophils in the untreated challenge control group

B = eosinophils in the challenge group treated with substance

C = eosinophils in the unchallenged control group

For example, compound 3 at a dose of 100 mg/kg inhibits the infiltration of the eosinophilic granulocytes into the lung, which is characteristic of bronchial asthma, by 74%.

The compounds according to the invention are suitable, for example, for the treatment of bronchial asthma, rhinoconjunctivitis and atopic dermatitis.

The compounds according to the invention bind subtype-specifically (A_3) to the adenosine receptor.

Adenosine A_3 receptor antagonists can prevent mediator secretion by the blockage of their receptors. Recently, it has been shown that adenosine A_3 receptor antagonists prevent the lowering of the intracellular cAMP concentration even in the eosinophilic granulocytes and thereby also the release of the cytokines and other mediators from these cells (Jacobson et al., 1995). As a result, on the one hand the unpleasant, acute symptoms severely impairing quality of life can be alleviated and on the other hand the inflammatory processes underlying the disease can also be suppressed.

Table 2: K_i values [μM] at the adenosine receptor (A_3) and selectivities

Compound[1)]	K_i values [μM]	A_3/A_1	A_3/A_2
1	3.2	0.7	0.01
2	2	0.02	0.06
3	0.43	0.008	0.005
4	0.49	0.008	<0.0005
5	0.79	0.002	0.007

6	4	0.01	0.08
9	1	0.002	0.004
10	1.6	0.02	0.02
11	0.71	0.04	0.01
12	9.8	0.5	0.01
13	8.3	0.4	0.02
15	0.5	0.007	<0.001
16	0.34	0.005	<0.001
17	0.5	0.008	<0.001

Comments for Table 2:

1) Numbering of the compounds corresponding to the examples in Table 3 and Table 4

The K_i values shown in Table 2 demonstrate that the compounds according to the invention bind to the receptors which show selectivity values (columns 2 and 3) that selectively bind these compounds. A biological activity can possibly be mediated by means of this novel binding mechanism.

The following examples serve to illustrate the invention further without restricting it.

General procedure for the preparation of compounds of the formula 1 and their tautomers where X is oxygen according to Table 3, Examples 1-13.

30 mmol of compound of the formula 2 are added to 40 ml of formamide and the mixture is heated at between 100 and 200°C for 8-16 hours. After cooling, the precipitated crude product is filtered off with suction and a recrystallization (RC) is carried out using a suitable solvent, e.g. ethanol or DMF.

Table 3: Pyrazolo[3,4-d]pyrimidines, X = O, Examples 1-13

Compound	Y	Yield (%)	M.p. (°C)	Recrystallization from:
1	2-F	40	230-234	EtOH
2	2-Cl	51	228-231	EtOH
3	2-Br	26	227-232	EtOH
4	2-I	28	265-268	EtOH
5	2-CF_3	48	224-226	EtOH
6	2-CH_3	65	262-264	EtOH
7	3-CF_3	56	243-244	EtOH
8	2,6-F_2	42	251-253	EtOH
9	2-Cl-6-F	61	254-256	EtOH
10	2,6-Cl_2	76	268-271	EtOH
11	2,4-Cl_2	41	255-257	EtOH
12	4-OCH_3	64	260-261	EtOH
13	2-Cl-4,5-OCH_2O	42	258-260	DMF

General procedure for the preparation of the compounds of the formula 1 and their tautomers where X is sulphur according to Table 4, Examples 14-20.

Method A:

20 mmol of the compound of the formula 1 where X is oxygen and 80 mmol of phosphorus pentasulphide are added to 100 ml of pyridine and the mixture is heated at between 80 and 115°C for 4 to 8 hours. After cooling, the precipitated crude product is purified from a suitable solvent, preferably ethanol, by means of recrystallization (RC).

Method B:

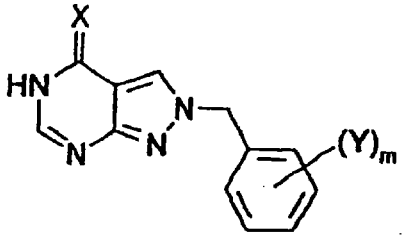
10 mmol of the compound of the formula 1 where X is oxygen and 20 mmol of 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulphide are added to 100 ml of xylene and the mixture is heated for 8 to 24 hours. The crude product is filtered off and purified as in Method A.

Table 4: Pyrazolo[3,4-d]pyrimidines, X = S, Examples 14-20

Compound	Y	Yield (%)	M.p. (°C)	Method	Recrystallization from
14	2-F	62	272-275	A	EtOH
15	2-Cl	98	247-249	B	DMF
16	2-Br	66	263-264	A	EtOH
17	2-I	64	278-281	A	DMF
18	2-CF_3	93	303-305	B	EtOH
19	2,6-F_2	72	293-297	B	EtOH
20	2-Cl-6-F	47	252-253	A	AcOH

Patent Claims

1. New compounds of the general formula 1



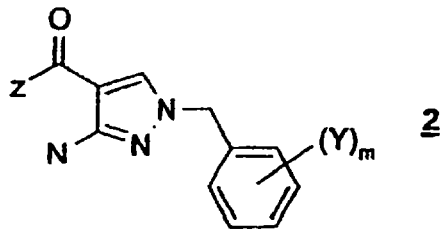
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or their tautomers, in which
X is oxygen or sulphur and
Y is halogen, C_1-C_4-alkyl, C_1-C_4-alkoxy, trifluoromethyl or trifluoromethoxy.

2. Compounds of the general formula 1:
2-(2-fluorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-chlorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-bromobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-iodobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-trifluoromethylbenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-methylbenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(3-trifluoromethylbenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2,6-difluorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-chloro-6-fluorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2,6-dichlorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2,4-dichlorobenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one

2-(4-methoxybenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-chloro-4,5-methylenedioxybenzyl)pyrazolo[3,4-d]pyrimidin-4(5H)-one
2-(2-chlorobenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione
2-(2-bromobenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione
2-(2-iodobenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione
2-(2-trifluoromethylbenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione
2-(2,6-difluorobenzyl)pyrazolo[3,4-d]pyrimidine-4(5H)-thione

3. Process for the preparation of pyrazolo[3,4-d]-pyrimidines of the general formula 1 where X = oxygen, characterized in that a compound of the formula 2 is cyclized with formamide



2

Formula 2

in which Z is hydroxyl, alkoxy or amino
and Y is halogen, C_1-C_4-alkyl, C_1-C_4-alkoxy, trifluoromethyl or trifluoromethoxy. [sic]

4. Process according to Claim 3 characterized in that alkyl orthoformates or formic acid/acetic anhydride mixtures are used for the cyclization of 3-aminopyrazole-4-carboxamides of the formula 2.

5. Process for the preparation of pyrazolo[3,4-d]-pyrimidines of the general formula 1 where X = sulphur, characterized in that a compound of the

formula 1 where X = oxygen is reacted with phosphorus pentasulphide or 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulphide.

6. Pharmaceutical compositions, characterized in that they contain one or more pyrazolo[3,4-d]-pyrimidines of the formula 1 according to Claim 1 or their pharmaceutically utilizable salts as active compounds in addition to one or more physiologically tolerable auxiliaries and/or excipients and, if appropriate, a diluent.

7. Pharmaceutical compositions, characterized in that they contain one or more pyrazolo[3,4-d]-pyrimidines according to Claim 2 or their pharmaceutically utilizable salts as active compounds in addition to one or more physiologically tolerable auxiliaries and/or excipients and, if appropriate, a diluent.

8. Use of pyrazolo[3,4-d]pyrimidines according to formula 1 for the production of medicaments for the treatment, in particular, of epilepsies of various forms.

9. Use of pyrazolo[3,4-d]pyrimidines according to formula 1 for the production of medicaments for the treatment, in particular, of allergic/asthmatic diseases.