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Il Farmaco 53 (1998) 579–585

IL FARMACO

Structure–activity relationship investigations of the modulating effect of core substituents on the affinity of pyrazoloquinolinone congeners for the benzodiazepine receptor

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Received 26 June 1998; accepted 20 October 1998

Abstract

A series of 6- and 7-substituted-2-arylpyrazolo[4,3-*c*]quinolin-3-ones was synthesized and tested in vitro for binding with the benzodiazepine receptor in competition with [^{3}H]flunitrazepam. Electronic parameters (molecular electrostatic potential (MEP), charge distribution on the nitrogen atoms, dipole moment μ, and ionization potential (IP)) were calculated for the compounds by semi-empirical quantum chemistry methods. Lipophilicity of the compounds, expressed as logarithm of the octanol–water partition coefficient (log *P*), was calculated by the program Pallas. A quantitative correlation of the biological data with molecular parameters revealed a significant dependence ($r = 0.95$) of the activity on hydrophobic constants of the substituents, log *P*, and magnitude of the MEP minimum associated with the carbonyl oxygen atom. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Pyrazoloquinolinones; Benzodiazepine receptor; QSAR; Substituent effects

1. Introduction

Benzodiazepines and other chemical families capable of binding with high affinity to the benzodiazepine receptor (BzR) have held the attention of researchers in the fields of medicine, pharmacology, and chemistry for several years. Many are used as indispensable therapeutic agents to treat specific diseases in the central nervous system (CNS). Many more are still the subject of pharmacological and/or clinical testing and their fate is in the balance. Since BzR is located on the $GABA_A$ receptor channel, the major inhibitory neurotransmitter system in the CNS, it is a matter of utmost importance to gain a better knowledge of its structure at the molecular level and thus to reach a more precisely outlined target for the design of new active compounds. Numerous structure–activity relationship studies that recently addressed this problem have revealed that many BzR ligands, in spite of marked differences in the structure of their heterocyclic cores, have some geometric, electronic, and/or hydrophilic/lipophilic structural features in common. This knowledge, although still limited, has made it possible to attempt to delineate different models of the pharmacophore for the BzR binding site [1–10]. In almost all of them, the pyrazolo[4,3-*c*]quinolin-3-one family ligands were considered as a subject for study and discussion.

Many members of this family have been synthesized and pharmacologically tested to date [11–15]. However, only three top activity compounds derived from the original Yokoyama's synthesis [11,12] and commonly known under the CGS code notation arouse particular interest, although none of them have gained new drug approval (Fig. 1).

CGS-9895 and CGS-9896 exhibit strong anxiolytic and anticonvulsant activity with an almost total lack of neurological deficits and are therefore classified as partial agonists [16]. On the contrary, CGS-8216 is an inverse agonist which reveals anxiogenic, somnolytic, and proconvulsant actions [17]. Attempts were made to rationalize this rather unusual effect of the substituent R in terms of either tautomeric equilibria [1] or reversed relative lipophilicity of both the pyridine-fused benzene ring and the 2-phenyl ring [13]. Either concept calls for assuming the possibility of an inverted orientation of the molecules within the receptor pocket [13]. The problem does not seem to be definitively

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PII S0014-827X(98)00072-X

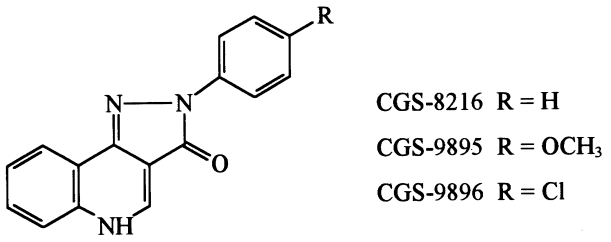
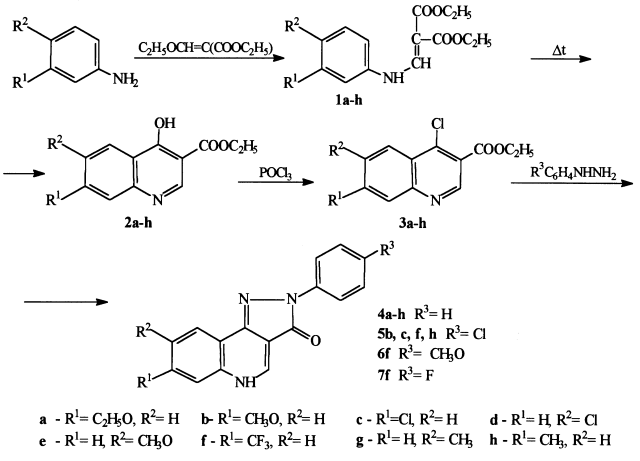


Fig. 1. The known pyrazolo[4,3-*c*]quinolin-3-ones with top affinity for the benzodiazepine receptor.

settled, however. Even so, the cyclic substituent at N(2) is generally recognized as the crucial determinant of both the affinity for the receptor and the activity profile and all research programs are consequently on that basis. Much less is known about the effects of substituents in the benzene fragment of the heterocyclic core.

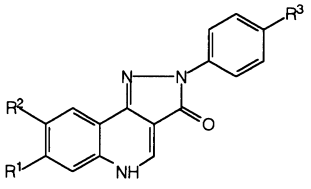
We have previously described [18] a quantitative relationship between the structure and in vitro affinity for the BzR in another group of ligands, namely 6-aryltriazolo[4,3-*b*]pyridazines substituted in the phenyl and triazole moieties. The excellent correlation obtained involved the Hansch constants for both substituents and the ionization potential of the molecule as the most significant components of the correlation equation. This means that in compounds with roughly the same overall geometry and almost exactly the same localization of the anchoring sites, the binding capacity greatly depends on modulation of the electronic properties of the molecules by the substituents.



Scheme 1.

In the present research we have synthesized a series of pyrazolo[4,3-*c*]quinolin-3-ones carrying various 7- or 8-substituents (R^1 and R^2, respectively); some also have *p*-substituents (R^3) in the 2-phenyl moiety (Table 1). All the compounds have been subsequently tested for their affinity for the benzodiazepine receptor by determining in vitro the inhibition of [^{3}H]flunitrazepam binding in rat brain homogenates. The results have been quantitatively correlated with molecular hydrophobic and electronic parameters.

Table 1
Pyrazoloquinolinones[a]



No.	R^1	R^2	R^3	M.p. (°C)[b]	Yield (%)
4a	OC_2H_5	H	H	289–292	57
4b	OCH_3	H	H	300–301	63
4c	Cl	H	H	389–392	72
4d	H	Cl	H	396–400[c]	65
4e	H	OCH_3	H	317–320[d]	78
4f	CF_3	H	H	368–369	84
4g	H	CH_3	H	364–366	68
4h	CH_3	H	H	360–362	61
5b	OCH_3	H	Cl	334–336	74
5c	Cl	H	Cl	389–393	87
5f	CF_3	H	Cl	356–358	70
5h	CH_3	H	Cl	352–356	66
6f	CF_3	H	OCH_3	305–308	54
7f	CF_3	H	F	344–347	73

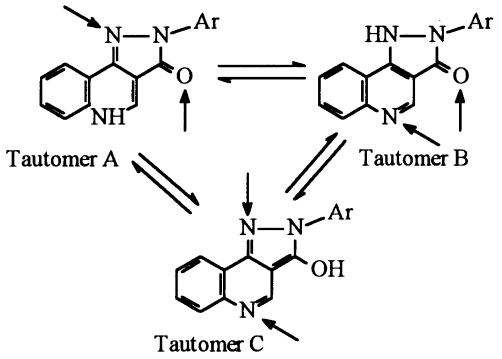
[a] Analyses C, H, N for all compounds.
[b] All melting points with decomposition.
[c] Literature [13] m.p. > 400° (dec.).
[d] Literature [13] m.p. 322–325°C.

2. Chemistry

The synthetic route, which is shown in Scheme 1, followed in general that used by Yokoyama [11,12]. Thus, condensation of diethyl ethoxymethylenemalonate with an appropriately substituted aniline yielded the corresponding diethyl [(arylamino)methylene]malonates (**1**), most of which have been synthesized previously. Compound **1** cyclized at ~ 250°C to the corresponding ethyl 7- (or 8-) substituted-4-hydroxyquinoline-3-carboxylates (**2**). Subsequent replacement of the hydroxy function with chlorine by treatment with phosphorus oxychloride yielded the corresponding ethyl 7- (or 8-) substituted-4-chloroquinoline-3-carboxylates (**3**) which, in the reaction with an appropriately substituted phenylhydrazine, gave the final products (**4–7**) (Scheme 1 and Table 1). In order to secure a maximum comparability of the biological results, three known pyrazoloquinolines (CGS-8216, **4d**, and **4e**) were also resynthesized as reference compounds and included in the SAR and QSAR analysis and discussion.

3. Results and discussion

The recent knowledge of the ligand BzR interaction assumes for certain that at least two sites on the ligand molecule are required to act as acceptors of protons from



Compd.	Tautomer Heat of formation [kcal/mol]		
	A	B	C
CGS 8216	-53.39	-45.92	-39.45
CGS 9895	-60.87	-53.67	-48.24
CGS 9896	-95.23	-87.63	-82.34

Fig. 2. Possible tautomeric forms of the three well known 2-arylpyrazolo[4,3-*c*]quinolin-3 ones and their calculated heat of formation. Arrows point to atoms presumed to act as the proton acceptor sites.

the receptor protein [9]. However, the compounds investigated may theoretically exist in three tautomeric forms, the prospective binding sites in each being associated with different atom pairs (Fig. 2). In order to estimate which tautomer is thermodynamically preferred and therefore most likely to exist in an aqueous phase, the structures of the particular tautomeric forms of the three CGS ligands were optimized by molecule modeling and the values of the heats of formation (H_f) were calculated for each of them. The calculations, carried out by using the semi-empirical method AM1 from the MOPAC-93 program [19], referred to geometry optimization in an aqueous medium so the dielectric constant of water (78.4) was included. As seen in Fig. 2, the tautomer A of the investigated molecules is thermodynamically preferred; it was assumed that the same preference was true for other congeneric compounds. Moreover, the choice of tautomer A is consistent with opinions of other authors working in the domain of BzR ligands [9,13–15].

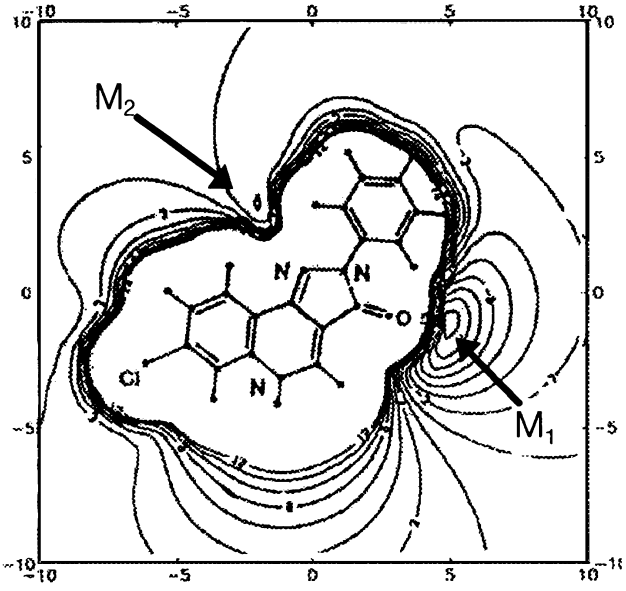


Fig. 3. MEP distribution in 7-chloro-2-phenylpyrazolo[4,3-*c*]quinolin-3-one (**4c**). M_1 and M_2 denote two MEP minima.

All the synthesized compounds (cf. Table 1) were subject to molecular modeling, geometry optimization, and calculation of the molecular electrostatic potential (MEP) distribution. An illustrative example of MEP distribution (compound **4c**) is shown in Fig. 3. In every case, a deep MEP minimum, marked as M_1, was observed in the vicinity of the carbonyl oxygen atom which is one of the potential acceptors of hydrogen from the receptor protein. Another minimum (M_2) is associated with the N(1) atom; it is much shallower and in some cases accompanied by an additional M_3 minimum. The M_1 minimum is located almost exactly in the plane of the tricyclic core, whereas M_2 and M_3 are to be found above or below this plane but always in close vicinity of the nitrogen atom. A slight deflection of the aryl substituent from the plane of the core seems to be the most plausible explanation of the observed splitting of the M_2 minimum into the M_2–M_3 pair. The distance between the M_1 and M_2 minima, which amounts to approximately 7.5 Å, is an almost perfect match for that between their equivalents (points marked H_1 and H_2) in the known models of the benzodiazepine receptor [9,10].

The results of calculations considering the depths of the M_1 MEP minimum are given in Table 2. The M_2 and M_3 minima were considered of no importance to the qualitative discussion and consequently not included in this table.

Determination of binding with BzR was carried out in vitro by a standard procedure using [^{3}H]flunitrazepam as the labeled ligand [20,21]. The results, expressed as % competitive inhibition (I) of [^{3}H]flunitrazepam (1.5 nM) binding by the investigated compounds (1×10^{-7} M concentration), are given in Table 2.

Correlation of the binding capacity (I) with the calculated electronic parameter revealed a highly significant dependence on the depth of the M_1 MEP minimum (kcal/mol) as shown by the 2nd order equation (Eq. (1)).

$$\log I = 0.0055M_1^2 - 0.2202M_1 - 0.3089 = \log I_{PC} \qquad (1)$$

$$n = 15; \quad r = 0.922; \quad F = 6.93; \quad p = 0.01; \quad s = 0.0483$$

The log I values calculated with the aid of this equation for each individual compound of the series investigated are

Table 2
Calculated electronic parameters and partition coefficients used in the correlation regressions and experimental data of affinity for the benzodiazepine receptor confronted with the calculated values

Compound	Hydrophobic constants π for substituents			MEP min. M_1 (kcal/mol)	log P	Inhibition of [^{3}H]flunitrazepam (1.5 nM) binding (%)	
	R^1	R^2	R^3			Determined	Calculated[a]
CGS-8216	0	0	0	17.79	2.05	77.8	77.98
4a	0.38	0	0	19.46	2.46	72.9	77.09
4b	–0.02	0	0	18.14	2.00	79.3	77.98
4c	0.71	0	0	16.24	2.77	77.0	73.45
4d	0	0.71	0	17.80	2.68	81.4	80.72
4e	0	–0.02	0	19.05	2.13	80.3	77.09
4f	0.88	0	0	15.54	3.21	58.6	61.52
4g	0	0.56	0	19.26	2.61	78.8	79.62
4h	0.56	0	0	19.19	2.48	74.1	78.89
5b	–0.02	0	0.71	17.20	2.73	62.2	62.66
5c	0.71	0	0.71	14.44	3.50	58.9	46.99
5f	0.88	0	0.71	11.83	3.93	33.2	34.83
5h	0.56	0	0.71	15.81	3.21	48.4	54.96
6f	0.88	0	–0.02	15.07	3.16	78.7	63.24
7f	0.88	0	0.14	13.42	3.38	45.6	55.59

[a] Eq. (5) was used to calculate the inhibition data in this column.

used in the following correlation approaches as the principal component data. They are denoted there as log I_{PC}. Attempts at correlating the binding efficacy with the calculated HOMO and LUMO energies and with the dipole moment values were unsuccessful. Analogously, positive correlations with the Hammett and Swain–Lupton constants failed.

As shown in Table 2, the depth of the M_1 MEP minimum depends on the substituents and thus indirectly reflects their effects on the binding capacity (I or log I) of the compounds. A straightforward estimation of the substituent effects was obtained by using the classical Hansch method. In the first approximation, the subset of nine compounds with $R^3 = H$ ($\pi = 0$) was investigated. With the standard values [22,23] of the Hansch hydrophobic constants π (Table 2), the following equation was generated.

$$\log I = -0.0934\pi_{R^1} + 0.0035\pi_{R^2} + 1.9021 \quad (2)$$

$$n = 9; \quad r = 0.781; \quad F = 10.2; \quad p = 0.01; \quad s = 0.0315$$

This shows that the affinity of the investigated compounds depends on both R^1 and R^2 substituents, although R^2 with its positive weight has a more profound effect. This can be seen in Table 2 where **4e** is shown as more active than **4b**, **4d** than **4c**, and **4g** than **4h**. This conclusion is consistent with the literature [13].

A similar regression with all 15 compounds gave

$$\log I = -0.1403\pi_{R^1} - 0.0244\pi_{R^2} - 0.2241\pi_{R^3} + 1.9204 \quad (3)$$

$$n = 15; \quad r = 0.841; \quad F = 6.22; \quad p = 0.01; \quad s = 0.0703$$

This substantiates the conclusion derived from Eq. (2) that the effect of R^2 outweighs that of R^1. Since the R^3 component is negative in this equation, R^3 substituents with negative values of π (OCH_3) should be expected to improve affinity for the receptor. Our investigations involved compounds with four different R^3 and, in fact, their affinity decreased in the following order of R^3: $OCH_3 > H > F > Cl$. The affinity of compounds with $R^1 = CF_3$, which decreased in the order **6f** > **4f** > **7f** > **5f**, correlates therefore very well with increasing π values of their R^3 substituents: $-0.02 < 0 < 0.14 < 0.71$, respectively.

It is well known that the overall lipophilicity is one of the most important molecular parameters to be used in QSAR studies. The lipophilicity of the compounds investigated was calculated as the logarithm of the octanol–water partition coefficient (log P) by using the program Pallas [24]. The respective values are included in Table 2. Correlation of log P with the experimental binding data (I) resulted in the following equation

$$\log I = -0.1210 \log P^2 + 0.5324 \log P + 1.3114 \quad (4)$$

$$n = 15; \quad r = 0.833; \quad F = 693; \quad p = 0.01; \quad s = 0.0583$$

In accord with this correlation, log P of the compounds exhibiting highest affinity for the receptor ($\log I \cong 2$) should fall into the range of 2–3. The highest value of log P (3.93) was calculated for the compound with lowest affinity and with two highly lipophilic substituents ($R = CF_3$ and $R^1 = Cl$). In the subset with $R = CF_3$, all compounds are characterized by $\log P > 3$. Although acceptable from the point of view of statistics, the parabolic relationship expressed by Eq. (4) may be misleading since, among other reasons, compounds with a rather widely different log P (e.g., in the range between 2 and 3.5) may

be closely similar in activity (log *I*). The linear approximation is statistically much inferior, however.

The regression expressed by Eq. (3) was finally supplemented by including log *I* values calculated for each individual compound according to Eq. (1) and denoted in the following equation as log I_{PC}.

$$\log I = -0.022\pi_{R^1} + 0.021\pi_{R^2} - 0.097\pi_{R^3} + 0.832 \log I_{PC} + 0.451 \quad (5)$$

$n = 15; \quad r = 0.949; \quad F = 5.99; \quad s = 0.0428$

This final correlation combines with high statistical significance the hydrophobic constants π of all three variable substituents with the depth of the main molecular electrostatic potential minimum M_1.

The results of the present QSAR analysis indicate that the affinity of the pyrazoloquinolinones investigated for the benzodiazepine receptor depends not only on the total hydrophobicity of the substituents but also on their position in the bicyclic core. Thus, for the same substituent, the 8-substituted compound is more active than the 7-substituted. In compounds with the same nature and position of the core substituents, the affinity for the receptor is defined by the *para* substituent in the 2-phenyl group and decreases with increasing hydrophobicity of this substituent.

The overall effect of all substituents is most precisely illustrated by the distribution of the MEP (Fig. 3). The M_1 minimum of MEP close to the carbonyl oxygen atom acts as a proton acceptor to produce a hydrogen bond which anchors the ligand to the receptor protein. The depth of this minimum is highly representative of the degree of affinity.

4. Experimental

4.1. Chemistry

4.1.1. General methods

Melting points were determined in a Büchi apparatus and are reported uncorrected. The ^{1}H and ^{13}C NMR spectra were taken with Varian 200 and 300 MHz and Brucker 400 MHz instruments with TMS as internal standard. Microanalyses were carried out by Mrs E. Godzisz, Warsaw University of Technology, on a Perkin Elmer C-H-N analyzer and were within ± 0.4% of the calculated values. Merck DC-Plastikfolien with Kieselgel 60 were used in the purity checking; the $CHCl_3$/MeOH/AcOEt/satd.aq. NH_3 (3:2:1:0.1) developing system was used. The reported melting points and elemental analyses refer to recrystallized, chromatographically homogeneous compounds.

4.1.2. Diethyl 2-[(arylamino)methylene]malonates (*1*)

The general procedure was as follows. The appropriate aniline derivative (0.05 mol) and diethyl ethoxymethylenemalonate (10.8 g, 0.05 mol) were heated for 2 h at 110–115°C in an open flask. The slightly yellowish mixture was treated with charcoal and filtered while hot. The filtrate was left standing for several days at room temperature and slowly crystallized to yield the crude product which was purified by recrystallization from light petroleum (a 45–60°C fraction) or hexane.

Diethyl 2-[3-ethoxyphenylamino)methylene]malonate (**1a**), m.p. 39–40°C (light petroleum), yield 84%. *Anal.*: Calc. For $C_{16}H_{21}NO_5$: C, 62.53; H, 6.89; N, 4.56. Found: C, 62.32; H, 6.78; N, 4.47%.

Diethyl 2-[(3-methoxyphenylamino)methylene]malonate (**1b**), m.p. 43–45°C (light petroleum), yield 79%. *Anal.*: Calc. for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.32; H, 6.50; N, 4.73%.

Compounds **1c–1h** were prepared analogously. Their melting points were consistent with the literature data. Representative NMR spectra are reported for **1a**. ^{1}H NMR (400 MHz, $CDCl_3$): 1.33 (t, 3H, $J = 7.1$ Hz, ether CH_3), 1.37 (t, 3H, $J = 7.1$ Hz, ester CH_3), 1.41 (t, 3H, $J = 7.1$ Hz, ester CH_3), 4.01 (q, 2H, $J = 7.1$ Hz, ether CH_2), 4.25 (q, 2H, $J = 7.1$ Hz, ester CH_2), 4.30 (q, 2H, $J = 7.1$ Hz, ester CH_2), 6.63–6.70 (m, 3H, arom. 4-, 5- and 6-CH), 7.21–7.25 (m, 1H, arom. 2-CH), 8.49 (d, 1H, $J = 13.6$ Hz, =CH), 10.96 (d, 1H, $J = 13.6$, NH). ^{13}C NMR (400 MHz, $CDCl_3$): 14.32 and 14.75 (ester CH_3), 14.46 (ether CH_3), 60.03 and 60.32 (ester CH_2), 63.63 (ether CH_2), 93.58 (=C<), 103.74, 109.29, and 110.75 (arom. 4-, 5- and 6-CH), 130.90 (arom. 2-CH), 140.45 (arom. CNH), 151.74 (=CH), 160.26 (arom. COEt), 165.65 and 168.98 (2 × C=O).

4.1.3. 7-(6)-Substituted ethyl 4-hydroxyquinoline-3-carboxylates (*2*)

The general procedure was as follows. Diethyl [(arylamino)methylene]malonate (0.03 mol) was rapidly added in one portion with stirring to 75 ml of Dowtherm A preheated in an open flask under an argon blanket to 250°C. The mixture was kept at this temperature for 20–30 min. Upon cooling, 100 ml of hexane were added to complete precipitation of the product. Filtration yielded a tan product which was purified by recrystallization from acetic acid or methoxyethanol to give an off-white amorphous solid.

Ethyl 7-ethoxy-4-hydroxyquinoline-3-carboxylate (**2a**), m.p. 280–282°C (AcOH), yield 78%. *Anal.*: Calc. for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.44; H, 5.70; N, 5.28%.

Compounds **2b–h** were prepared analogously. Their melting points were consistent with the literature data. Representative NMR spectra are reported for **2a**: ^{1}H NMR (300 MHz, CF_3COOD): 1.45 (t, 3H, $J = 7.1$ Hz, CH_3), 1.49 (t, 3H, $J = 7.0$ Hz, CH_3), 4.25 (q, 2H, $J = 7.0$ Hz, CH_2), 4.58 (q, 2H, $J = 7.1$ Hz, CH_2), 7.33 (d, 1H, $J = 2.3$ Hz, 8-CH), 7.46 (dd, 1H, $J_{6,8} = 2.3$ Hz, $J_{6,5} = 9.3$ Hz, 6-CH), 8.44 (d, 1H, $J = 9.3$ Hz, 5-CH), 9.10 (d, 1H, $J = 7.3$ Hz, 2-CH). ^{13}C NMR (400 MHz, CF_3COOH-external acetone-d_6 lock): 12.3 and 12.6 (2 × CH_3), 64.5 and 65.7 (2 × CH_2),

100.0 (8-C), 104.0, 114.2, 122.5 (6-C), 126.5 (5-C), 142.8 144.7 (2-C), 167.6, 168.1, 172.3; chemical shift data without assignments refer to quaternary aromatic carbon atoms.

*4.1.4. 7-(6)-Substituted ethyl 4-chloroquinoline-3-carboxylates (**3**)*

The general procedure was as follows. The appropriately 6- or 7-substituted ethyl 4-hydroxyquinoline-3-carboxylate (**2**) (0.025 mol) was cautiously added to ice-cooled phosphorus oxychloride (17.5 ml) and the mixture was refluxed under argon for 5 h. Upon cooling, the mixture was poured onto crushed ice, neutralized with ammonia, and extracted with methylene chloride as rapidly as possible. The organic layer was washed with saline, dried with $MgSO_4$, and evaporated to yield the crude product which was purified by recrystallization from petroleum ether or hexane.

Ethyl 4-chloro-7-ethoxyquinoline-3-carboxylate (**3a**), m.p. 120–122°C (petroleum ether, 45–60°C fraction), yield 60%. *Anal.*: Calc. for $C_{14}H_{14}ClNO_3$: C, 60.11; H, 5.04; N, 5.01. Found: C, 60.17; H, 4.92; N, 4.98%.

Ethyl 4-chloro-7-methoxyquinoline-3-carboxylate (**3b**), m.p. 130–132°C (hexane), yield 67%. *Anal.*: Calc. for ($C_{l3}H_{12}ClNO_3$): C, 58.77; H, 4.55; N, 5.27. Found: C, 58.85; H, 4.48; N, 5.20%.

Ethyl 4-chloro-6-methoxyquinoline-3-carboxylate (**3e**), m.p. 96–98°C (petroleum ether), yield 54%. *Anal.*: Calc. for ($C_{13}H_{12}ClNO_3$): C, 58.77; H, 4.55; N, 5.27. Found: C, 58.88; H, 4.39; N, 5.18%.

Ethyl 4-chloro-6-methylquinoline-3-carboxylate (**3g**), m.p. 64–66°C (petroleum ether), yield 71%. *Anal.*: Calc. for ($C_{13}H_{12}ClNO_2$): C, 62.53; H, 4.84; N, 5.61. Found: C, 62.44; H, 4.78; N, 5.58%.

Compounds **3c**, **3d**, **3f**, and **3h** were prepared analogously. Their melting points were consistent with the literature data.

Representative NMR spectra are reported for **3a**: ^{1}H NMR (400 MHz, $CDCl_3$): 1.46 (t, 3H, $J = 7.0$, ether CH_3), 1.52 (t, 3H, $J = 7.1$, ester CH_3), 4.22 (q, 2H, $J = 7.0$, ether CH_2), 4.48 (q, 2H, $J = 7.1$, ester CH_2), 7.32 (dd, 1H, $J_{6,8} = 2.3$ Hz, $J_{6,5} = 9.3$ Hz, 6-CH), 7.41 (d, 1H, $J = 2.3$ Hz, 8-CH), 8.29 (d, 1H, $J = 9.3$ Hz, 5-CH), 9.16 (s, 1H, 2-CH).

^{13}C NMR (400 MHz, $CDCl_3$): 14.6 and 14.3 (2 × CH_3), 64.2 and 61.8 (2 × CH_2), 108.1 (8-C), 121.1, 120.5, 121.8 (6-C), 126.7 (5-C), 143.5, 150.9 (2-C), 151.7, 162.0, 164.6 (C=O); chemical shift data without assignments refer to quaternary aromatic carbon atoms.

*4.1.5. 7-(6)-Substituted 2-phenylpyrazolo[3,2-c]quinolin-3-ones (**4**)*

The general procedure was as follows. The appropriately 6- or 7-substituted 4-chloroquinoline-3-carboxylate (**3**) (0.0036 mol) and 0.45 g (0.0045 mol) of phenylhydrazine was refluxed in 20 ml of xylene for 4 h. A bright yellow product quickly began to precipitate. Ethyl ether was added to the cooled mixture to complete precipitation and the solid was filtered, thoroughly washed with ether, and dissolved in 15 ml of 2 N NaOH. The solution was stirred for 1 h with ethyl ether, the ether solution was separated and discarded, whereas the aqueous solution was neutralized to pH 8–9 by adding solid NH_4Cl to precipitate the crude product. The final purification was done by recrystallization from ethanol.

Anal.: **4a**. Calc. for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76. Found: C, 70.62; H, 4.89; N, 13.80%. **4b**. Calc. for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 4.50; N, 14.42. Found: C, 70.23; H, 4.37; N, 14.38%. **4c**. Calc. for $C_{16}H_{10}ClN_3O$: C, 64.98; H, 3.41; N, 14.21. Found: C, 65.11; H, 3.28; N, 14.12%. **4f**. Calc. for $C_{17}H_{10}F_3N_3O$: C, 62.01; H, 3.06; N, 12.76. Found: C, 61.87; H, 2.96; N, 12.70%. **4g**. Calc. for $C_{17}H_{13}N_3O$: C, 74.17; H, 4.76; N, 15.26. Found: C, 74.05; H, 4.84; N, 15.19%. **4h**. $C_{17}H_{13}N_3O$: C, 74.17; H, 4.76; N, 15.26. Found: C, 74.27; H, 4.70; N, 15.24%.

*4.1.6. 7-(6)-Substituted 2-arylpyrazolo[3,2-c]quinolin-3-ones (**5–7**)*

The general procedure was as follows. 4-Chlorophenylhydrazine hydrochloride (0.005 mol) was suspended in 60 ml of xylene and the mixture was stirred for 2 h with 5 ml of aqueous ammonia. The xylene layer was separated and distilled until moisture stopped passing with the distillate. The appropriately substituted ethyl 4-chloroquinoline-3-carboxylate (0.003 mol) was then added; the reaction continued as above to yield the corresponding **5**.

An analogous procedure starting with 4-methoxyphenylhydrazine and 4-fluorophenylhydrazine was used to prepare **6** and **7**, respectively.

Data for compounds **5–7** are given in Table 1.

The representative ^{1}H NMR spectrum is reported for **5a** (400 MHz, DMSO-d_6): 1.50 (t, 3H, CH_3), 4.17 (q, 2H, CH_2), 7.41 (dd, 1H, $J_{8,6} = 2.1$ Hz, $J_{8,9} = 9.4$ Hz, 8-CH), 7.5–7.8 (m, 6H), 8.17 (d, 1H, $J = 9.4$ Hz, 9-CH), 8.97 (s, 1H, 4-CH).

Anal.: **5b** Calc. for $C_{17}H_{12}ClN_3O_2$: C, 62.68; H, 3.71; N, 12.90. Found: C, 62.49; H, 3.67; N, 12.85%. **5c**. Calc. for $C_{16}H_9Cl_2N_3O$: C, 58.20; H, 2.75; N, 12.73. Found: C, 58.02; H, 2.81; N, 21.37%. **5f**. Calc. for $C_{17}H_9ClF_3N_3O$: C, 56.14; H, 2.49; N, 11.55. Found: C, 55.97; H, 2.38; N, 11.60%. **5h**. Calc. for $C_{17}H_{12}ClN_3O$: C, 65.92; H, 3.90; N, 13.57. Found: C, 66.09; H, 3.78; N, 13.48%. **6f**. Calc. for $C_{18}H_{12}F_3N_3O_2$: C, 60.17; H, 3.37; N, 11.69. Found: C, 59.94; H, 3.28; N, 11.65%. **7f**. Calc. for $C_{17}H_9F_4N_3O$: C, 58.80; H, 2.61; N, 12.10. Found: C, 58.64; H, 2.53; N, 12.19%.

4.2. Receptor tests

Rat brains were homogenized at 0°C in 20 vols. of 50 mM Tris–HCl (pH 7.4) and the homogenates were incubated for 1 h at 37°C and centrifuged at 20 000 × *g*. Samples of the homogenate (800 μl corresponding to 13.3 mg of the brain tissue) were mixed with 1000 μl of [^{3}H]flunitrazepam (specific activity 81 Ci/mM) and 100 μl of the tested compound (10^{-7} M). The mixture was incubated for 2 h

at 5°C and filtered through a glass fiber filter (Whatman GF/C). The filter was washed twice with 5-μl portions of Tris, dried, and immersed in 10 ml of the scintillation liquid (POPOP 50 mg, PPO 4 g, methanol 20 ml, and toluene 1000 ml). Radioactivity was measured in a Betamatic II Kontron β-scintillation counter and expressed as % inhibition of binding of the labeled flunitrazepam.

Acknowledgements

The research was carried out under grant nos. 4 4404 91 02 and 4 P05F 013 09 from the Committee of Scientific Research, Poland. This financial support is gratefully acknowledged.

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