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European Journal of Medicinal Chemistry 42 (2007) 1317–1324
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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Original article

1-Aryl-4,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(1*H*)-ones: A new class of antiproliferative agents with selectivity for human leukemia and breast cancer cell lines

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Received 11 October 2006; received in revised form 1 February 2007; accepted 15 February 2007
Available online 1 March 2007

Abstract

The synthesis of 1-aryl-4,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(1*H*)-ones by cyclization of 4-[(dimethylamino)methylidene]-3,4-dihydro-1*H*-[1]benzazepine-2,5-dione with arylhydrazines is reported. When tested on a panel of human cancer cell lines, the title compounds showed antiproliferative activity and a characteristic selectivity pattern of growth inhibition. Although structurally akin to established kinase inhibitors, the new compounds did not exhibit noteworthy inhibitory activity when tested on an array of cancer-related kinases.

Keywords: Antiproliferative activity; COMPARE; Cyclization; Kinase

1. Introduction

The paullones (general formula **1**, Fig. 1) constitute a class of cyclin-dependent kinase inhibitors for which antiproliferative activity against the tumor cell lines of the in vitro cell line screening project (IVCLSP) of the National Cancer Institute (NCI) has been demonstrated [1]. Paullones have also been reported to inhibit glycogen synthase kinase-3 (GSK-3) [2,3] and mitochondrial malate dehydrogenase (mMDH) [4], although it is currently not clear to what extent the distinct enzyme inhibiting properties contribute to the growth inhibitory activity of the compound family [5–7]. A crystal structure analysis of the complex of GSK-3 and alsterpaullone (**1**, R = NO_2) revealed that paullones are accommodated within the ATP binding pocket of the kinase and are held in position through a pair of hydrogen bonds between the lactam function of the paullone and Val135 of the hinge region of the protein [8]. With a view to identifying novel antiproliferative kinase inhibitors, 1-aryl-4,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(1*H*)-ones of the general formula **2** were designed in which the [1]benzazepinone substructure of the paullones is combined with an 1-arylpyrazolo annelated ring. Apparently the only published examples of this basic structure are four derivatives with the general formula **3** bearing a 3-methyl substituent, which were synthesized in a multi-step procedure with closure of the seven-membered lactam ring as final step [9]. Although these compounds were designed as anthramycin analogs with potential antitumor activity, biological test results with **3** have apparently not been published. A more general synthetic procedure for the preparation of **2** is reported here. The new compounds **2** were tested both for antiproliferative properties in the IVCLSP and on an array of kinases related to cancer.

2. Chemistry

For a generally applicable synthesis of **2** the enaminone **6** [10] was reacted with appropriately substituted phenylhydrazines in

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doi:10.1016/j.ejmech.2007.02.007

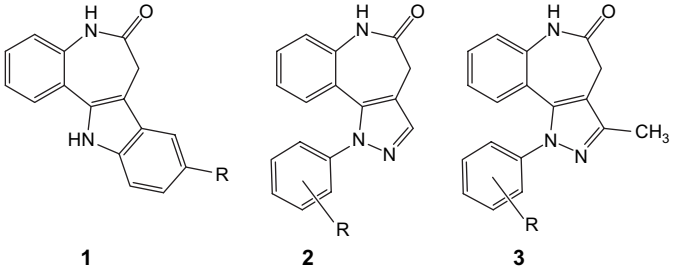


Fig. 1. Structures of paullones **1** and structurally related 1-aryl-4,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(1*H*)-ones **2**. The 3-methyl derivatives **3** have been described before [9].

glacial acetic acid at 70 °C to furnish the title compounds **2** in moderate to excellent yields.

Dependent on the direction of the initial nucleophilic attack by the phenylhydrazine, both **2** and the isomeric 2-aryl-2,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(4*H*)-ones **4** are conceivable as reaction products. According to examples from the literature, the formation of type **2** cyclization products was expected [11–17]. Because an unambiguous distinction between **2** and **4** based on spectral data was difficult, the structure assignment was supported by a single crystal structure analysis of the parent compound **2a** (Fig. 2, Table 1).

Compound **2a** crystallizes with two independent molecules in the asymmetric unit, which are, however, closely similar; a least-squares fit of all non-H atoms gives an r.m.s. deviation of 0.10 Å, which is reduced to 0.04 Å if the phenyl substituent

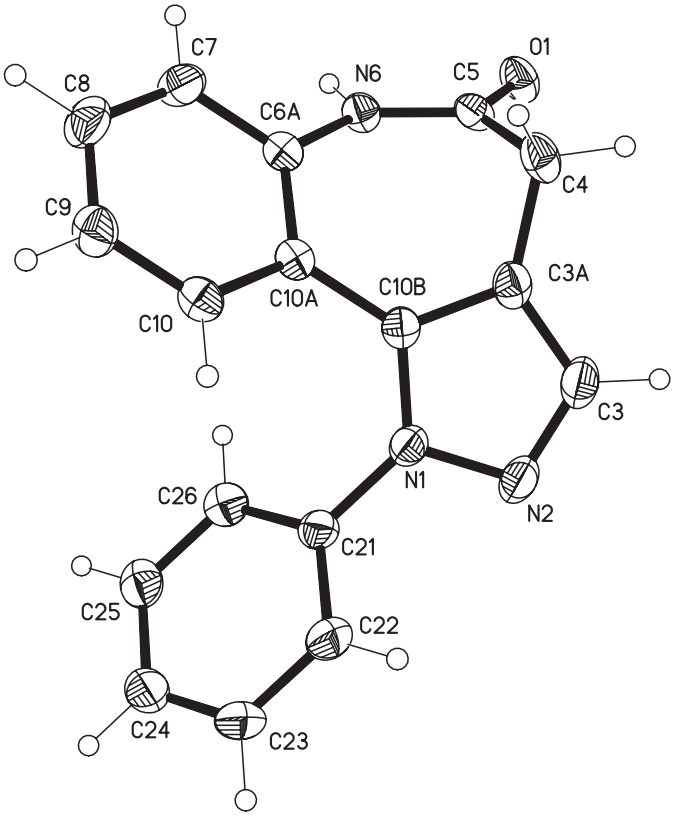


Fig. 2. X-ray structure of one of the two independent molecules of compound **2a**. Ellipsoids correspond to 50% probability levels.

Table 1
Crystal data and structure refinement of compound **2a**

Identification code	kofi	
Empirical formula	$C_{17}H_{13}N_3O$	
Formula weight	275.30	
Temperature	133(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/n$	
Unit cell dimensions	$a = 19.2027(15)$ Å	$\alpha = 90°$
	$b = 7.5016(6)$ Å	$\beta = 98.540(4)°$
	$c = 19.3614(15)$ Å	$\gamma = 90°$
Volume	2758.1(4) Å^3	
Z	8	
Density (calculated)	1.326 Mg/m3	
Absorption coefficient	0.086 mm^{-1}	
$F(000)$	1152	
Crystal habit, size	Colorless tablet, $0.45 \times 0.40 \times 0.10$ mm^3	
Theta range for data collection	1.39–30.04°	
Reflections collected	30 572	
Independent reflections	8038 [R(int) = 0.0365]	
Data/parameters	8038/387	
Goodness-of-fit on F^2	1.027	
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0468$, $wR2 = 0.1093$	
R Indices (all data)	$R1 = 0.0712$, $wR2 = 0.1185$	
Largest diff. peak and hole	0.307 and −0.259 e Å^{-3}	

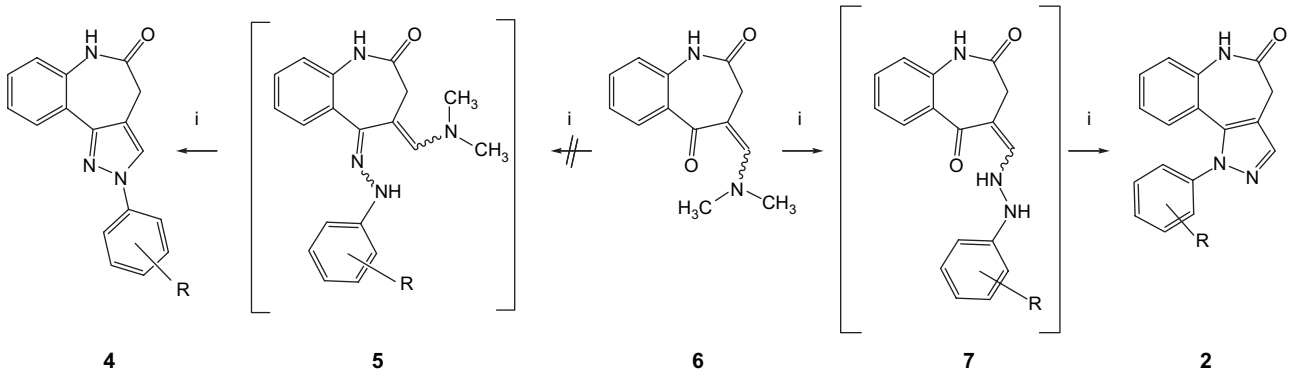
is excluded. The conformation of the seven-membered rings is such that atoms C5, N6, C3a and C10b are approximately coplanar, with the other three atoms lying ca. 0.6 Å to the same side of the plane thus defined (approximate mirror symmetry about C4 and the midpoint of C6a–C10a). Each independent molecule forms chains parallel to the *y* axis by hydrogen bonding of the form N6–H···O1; neighbouring molecules are related by the 2_1 axis.

In two cases the intermediates **7h** and **7m** of the reaction were isolated upon reacting **6** with the appropriate phenylhydrazines at room temperature. The structures **7** could be distinguished unequivocally from the hypothetical intermediates **5**. Cyclization of the structures **7h** and **7m** at 70 °C led to the 1-aryl-4,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(1*H*)-ones **2h** and **2m**, which were identical to the products obtained from the direct condensation of **6** with phenylhydrazines (Scheme 1).

3. Biological evaluation and discussion

For an assessment of the antiproliferative activity, the title compounds **2a–2o** were evaluated in the vitro cell line screening project (IVCLSP) of the American National Cancer Institute (NCI). In this screening setting, the growth inhibition of three cell lines, namely MCF7 cells (breast cancer), NCI-H460 (non-small cell lung cancer, NSCLC), and SF-268 (cancer of the central nervous system; CNS cancer) by 100 μM concentration of the title compounds is determined as initial parameter.

The results given in Table 2 revealed that while the SF-268 cells were apparently not sensitive to the test compounds, both the breast and the lung cancer cell lines were inhibited. Six of the compounds from Table 2 (**2b**, **2e–2i**) inhibited one of the



Scheme 1. Cyclization of enaminone **6** with phenylhydrazines. Reagents and conditions: (i) appropriately substituted phenylhydrazine hydrochloride, sodium acetate, glacial acetic acid, 70 °C, and 1 h.

cell lines to 32% growth or less and thus fulfilled a criterion fixed by the NCI for a further evaluation. These six derivatives were subsequently evaluated in the full 60 cell line screening panel of the NCI. Among other data, for a test compound the GI_{50} value (concentration that inhibits 50% net cell growth) is determined for each of the 60 cancer cell lines in the panel. As a general parameter for in vitro antiproliferative activity, an averaged value (Meangraph Midpoint, MG-MID) is calculated from the results of the distinct cell lines. The selectivity patterns or "fingerprints" found for distinct compounds over the whole cell line panel are characteristic for individual molecular mechanisms underlying the growth inhibition. The comparison of selectivity patterns is a suitable method for the determination of antiproliferative agents acting by similar molecular mechanisms. The bioinformatic tool COMPARE can be used by the Internet for the comparison of selectivity patterns included in the NCI database of test compound patterns [18,19]. For example, the paullones **1** were identified using a similarity search in the NCI database with the selectivity profile of the CDK inhibitor flavopiridol as a seed pattern [20].

Testing the six title compounds **2b**, **2e**–**2i** in the 60-cell line screening indeed revealed a noteworthy antiproliferative activity of all compounds (Table 3). However, the 4′-methoxy derivative **2e** and the 3′,5′-dichloro derivative **2h** were clearly superior to the other derivatives, which showed MG-MID values in the two-digit micromolar concentration range. In contrast, **2e** and **2h** exhibited averaged growth inhibition in submicromolar concentrations, outperforming established antitumor drugs such as *cis*-platin and, with respect to distinct cell lines, even anticancer drugs with high potency such as adriamycin (Table 3). Furthermore, **2e** and **2h** show pronounced selectivity patterns with preference for cell lines from the leukemia and the breast cancer subpanel. Examples of selectively inhibited cell lines are K-562 (leukemia) and MDA-MB-435 (breast cancer) which are listed in Table 3.

Table 2
Results of the NCI prescreening using three cancer cell lines (cell growth [%] related to controls, test compound concentration = 100 μM)

	R	MCF7[a]	NCI-H460[b]	SF-268[c]
2a	4′-H	55	33	90
2b[d]	4′-Br	51	**32**	98
2c	4′-Cl	54	41	80
2d	2′-CH_3	64	49	98
2e[d]	4′-OCH_3	60	**26**	88
2f[d]	4′-CH_3	57	**29**	95
2g[d]	4′-CF_3	52	**28**	n.a.[e]
2h[d]	3′,5′-Di-Cl	40	**21**	62
2i[d]	4′-*tert*.-Butyl	130	**11**	n.a.[e]
2j	2′-Cl	66	53	71
2k	3′-Cl	64	39	101
2l	2′-F	66	49	98
2m	2′-Br	69	44	87
2n	2′,4′-Di-F	61	41	98
2o	4′-NO_2	55	62	62

[a] Breast cancer cell line.
[b] Non-small cell lung cancer cell line.
[c] CNS cancer cell line.
[d] Compound was selected for testing in the full IVCLSP panel of the NCI.
[e] n.a.: Not available.

Table 3
In vitro antitumor activity of the 1-aryl-4,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(1*H*)-ones **2b**, **2e**–**2i** compared with the standard antitumor agents *cis*-platin and adriamycin

	R	GI_{50} MG-MID[a]	GI_{50} K-562[b]	GI_{50} MDA-MB-435[c]
2b	4′-Br	14.8	4.07	1.91
2e	4′-OCH_3	1.82/0.37[d]	0.35/0.14[d]	0.025/0.16[d]
2f	4′-CH_3	12.3	1.78	2.09
2g	4′-CF_3	8.32	12.6	11.2
2h	3′,5′-Di-Cl	0.51/0.52[d]	<0.01/0.011[d]	<0.01/<0.01[d]
2i	4′-*tert*.-Butyl	22.4	31.6	18.6
cis-Platin (NSC 119875)[e]		10.3	12.6	12.6
Adriamycin (NSC 123127)[f]		0.11	0.10	0.20

Given are concentrations to reduce the cancer cell growth to 50% (GI_{50}) [μM].
[a] MG-MID = Meangraph Midpoint: averaged value of the concentrations needed for 50% growth inhibition over all cell lines in the IVCLSP. The highest test dose was 100 μM; the lowest test dose was 0.01 μM.
[b] Leukemia cell line. Bold numbers indicate values below the threshold for a further testing.
[c] Breast cancer cell testing.
[d] Repeated tests.
[e] Results from the NCI database, status of Sept. 2005, number of tests: 6.
[f] Results from the NCI database, status of Sept. 2005, number of tests: 2.

Compound **2h** inhibits these cancer cells in two-digit nanomolar concentrations and is in this respect far more potent than the standard agents included in Table 3.

In order to investigate whether the antiproliferative 1-aryl-4,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(1*H*)-ones **2b**, **2e**–**2i** act by a similar biological mechanism, a pairwise comparison of the selectivity patterns from the IVCLSP was performed by a matrix COMPARE analysis [18,19]. The resulting Pearson correlation coefficients (PCC) listed in Table 4 shows that several pronounced correlations indeed exist within the compound family. Although a single high correlation between two isolated compounds might be coincidental, the concentration of highly correlated patterns in this group of compounds is obvious and gives reason to suppose that **2** do not act by an undefined general toxicity but by a specific target or a specific target combination. Especially noteworthy are the correlations found between **2b** and **2f** (PCC = 0.853) and between the compounds showing the strongest antiproliferative activity, **2e** and **2h** (PCC = 0.723). In contrast, the low PCC values between **2b**, **2e**–**2i** on the one hand and alsterpaullone **1a** on the other hand lead to the hypothesis that the antiproliferative activity of the paullones and the novel compound family **2** is not based on identical mechanisms. However, given the close structural relationship between the paullones **1** and the title compounds **2**, a related mechanism of action appeared feasible, namely the inhibition of protein kinases.

In order to check this possibility, all novel derivatives **2a**–**2o** were tested in a proprietary protein kinase assay (^{33}PanQinase® Activity Assay) on an array of 23 cancer-related protein kinases, comprising the entities EGF-R, EphB4, ERBB2, FAK, IGF1-R, SRC, VEGFR-2, VEGFR-3, AKT1, Aurora A, Aurora B, CDK2/cyc A, CDK4/cyc D1, CK2-α1, PLK1, TIE2, FLT3, MET, PDGFR-β, ARK5, PAK4, PDK1 and SAK. For the initial test run, compounds were tested in a single concentration (10 μM). The results revealed that, with a few exceptions, the protein kinases were not inhibited by more than 40%. These exceptions included (inhibited kinases in brackets): **2c** (FLT3), **2k** (CDK4) and **2i** (VEGFR-3). Further investigations showed that **2c**, **2k** and **2i** did not inhibit any of the indicated kinases with IC_{50} values below 10 μM (ATP concentration = 1 μM; data not shown). Based on these results, the inhibition of one of the kinases from the screening array is unlikely as a mechanism underlying the antiproliferative activity of **2e** and **2h**. However, the inhibition of other kinases might play a role in the observed biological activity of **2**.

As a résumé, a simple synthesis for 1-aryl-4,6-dihydropyrazolo[4,3-*d*][1]benzazepin-5(1*H*)-ones **2** is reported, constituting a class of novel cancer cell growth inhibitors. Further investigations are required to determine the targets pivotal for the antiproliferative activity of **2**. Once identified, these targets might direct a further rational development of **2** as anticancer agents.

4. Experimental protocols

4.1. General remarks

Melting points (mp) were determined on an electric variable heater (Electrothermal 9100) and are not corrected. Infrared spectra were recorded on a Thermo Nicolet FT-IR 200. Nuclear magnetic resonance spectra were recorded at the NMR laboratories of the Chemical Institutes of the Technical University Braunschweig on a Bruker Avance DRX-400, using tetramethylsilane as internal standard. NMR signals are reported in parts per million on a δ scale. C, H, N analyses were performed with a CE Instruments FlashEA® 1112 Elementar Analyzer. Analyses indicated by the symbols of elements were within ±0.4% of the theoretical values. The purity of all compounds used for biological assays was >95% as determined by HPLC using a 100% method. The HPLC analyses were carried out using a Merck Hitachi LaChrom Elite system (pump: L-2130, DAD detector: L-2450; autosampler: L-2200; column: LiChrospher 100 RP-18 (5 μm); eluent: acetonitrile/water mixtures; elution rate 1.000 mL/min; detection wavelength: 254 nm and 280 nm; overall run time: 15 min).

4.2. General synthesis protocols

4.2.1. General method for the preparation of 1-aryl-4,6-dihydropyrazolo[4,3-d][1]benzazepinones (2a–o)

A mixture of 4-[(dimethylamino)methylene]-3,4-dihydro-1*H*-[1]benzazepine-2,5-dione (230 mg, 1 mmol) (**6**) and the appropriately substituted phenylhydrazine (either 1.5 mmol of the free base or 1.5 mmol of the phenylhydrazine hydrochloride and 1.5 mmol of sodium acetate) is stirred in glacial acetic acid (10 mL) at 70 °C for 1 h. After cooling to room temperature the mixture is poured into 5% aqueous sodium

Table 4
PCC values resulting from a matrix COMPARE analysis comprising the selectivity patterns of alsterpaullone (**1a**, R = NO_2) and pyrazolobenzazepinones **2b**, **2e**–**2i** [a,b]

	1a	2b	2e	2f	2g	2h
2i	0.069 (52)	0.284 (52)	0.246 (52)	**0.446** (52)	0.085 (49)	0.273 (52)
2h	0.202 (58)	**0.558** (55)	**0.723** (57)	**0.549** (55)	0.168 (52)	
2g	−0.420 (52)	**0.391** (52)	0.219 (52)	0.339 (52)		
2f	0.010 (55)	**0.853** (55)	**0.432** (55)			
2e	−0.052 (57)	**0.508** (55)				
2b	−0.131 (55)					

[a] Numbers of cell lines used for the comparison of selectivity patterns are given in brackets.
[b] PCC values above 0.350 are printed bold. Due to experiences in other compound classes this value can be used as a threshold for the discrimination of compound families [24].

acetate solution (20 mL). The resulting precipitate is collected, washed with water, dried and crystallized from ethanol.

4.2.2. General method for the preparation of 4-[(2-arylhydrazino)methylene]-3,4-dihydro-1H[1]benzazepine-2,5-diones (**7h** and **7m**)

A mixture of 4-[(dimethylamino)methylene]-3,4-dihydro-1*H*-[1]benzazepine-2,5-dione (230 mg, 1 mmol) (**6**) and the appropriately substituted phenylhydrazine (either 1.5 mmol of the free base or 1.5 mmol of the phenylhydrazine hydrochloride and 1.5 mmol of sodium acetate) is stirred in glacial acetic acid (15 mL) for 1 h at room temperature. Subsequently, the mixture is poured into 5% aqueous sodium acetate solution (30 mL). The resulting precipitate is collected, washed with water, dried and crystallized from ethanol (70%).

4.3. Synthesis of particular compounds

4.3.1. 1-Phenyl-4,6-dihydropyrazolo[4,3-d][1]benzazepin-5(1H)-one (**2a**)

Preparation according to the general method described in Section 4.2.1. yielded light yellow crystals (152 mg, 56%); mp.: 247–248 °C; IR (KBr): 3055 cm^{-1} (CH arom.), 2967 cm^{-1} and 2909 cm^{-1} (CH aliph.), 1682 cm^{-1} (C═O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 3.45 (s, 2H, CH_2), 6.95 (dd, 1H, J = 7.9/2.0 Hz, ArH), 6.99 (dt, 1H, J = 6.9/7.9/1.0 Hz, ArH), 7.21 (dd, 1H, J = 7.9/0.8 Hz, ArH), 7.30–7.42 (m, 6H, ArH), 7.67 (s, 1H, ArH), 8.59 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ (ppm) = 32.5 (CH_2); 123.2, 124.7, 125.3 (2C), 128.3, 129.60 (2C), 129.64, 129.66, 138.8 (*tert*. C); 118.0, 121.3, 135.5, 136.7, 140.3, 174.7 (quat. C). Anal. $C_{17}H_{13}N_3O$ (C, H, N).

4.3.2. 1-(4-Bromophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2b**)

Preparation according to the general method described in Section 4.2.1. yielded yellow needles (270 mg, 74%); mp.: 282 °C; IR (KBr): 3226 cm^{-1} (NH), 3123 cm^{-1} (CH arom.), 2956 cm^{-1} (CH aliph.), 1668 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.32 (s, 2H, CH_2), 6.88 (dd, 1H, J = 7.9/1.4 Hz, ArH), 7.03 (dt, 1H, J = 8.4/8.0/1.2 Hz, ArH), 7.25–7.30 (m, 3H, ArH), 7.36 (dt, 1H, J = 7.3/8.3/1.5 Hz, ArH), 7.64–7.67 (m, 2H, ArH), 7.80 (s, 1H, ArH), 10.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.9 (CH_2); 123.1, 123.5, 126.6 (2C), 128.4, 129.1, 132.2 (2C), 138.9 (*tert*. C); 118.3, 119.6, 120.5, 136.0, 136.1, 138.7, 172.6 (quat. C). Anal. $C_{17}H_{12}BrN_3O$ (C, H, N).

4.3.3. 1-(4-Chlorophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2c**)

Preparation according to the general method described in Section 4.2.1 yielded colorless crystals (240 mg; 73%); mp.: 273–274 °C; IR (KBr): 3178 cm^{-1} (NH), 3062 cm^{-1} (CH arom.), 2977 cm^{-1} (CH aliph.), 1691 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.32 (s, 2H, CH_2), 6.87 (dd, 1H, J = 7.9/1.4 Hz, ArH), 7.03 (dt, 1H, J = 7.0/8.0/1.3 Hz, ArH), 7.28 (dd, 1H, J = 8.2/1.0 Hz, ArH), 7.33–7.37 (m, 3H, ArH), 7.51–7.54 (m, 2H, ArH), 7.80 (s, 1H, ArH), 10.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.9 (CH_2); 123.0, 123.5, 126.3 (2C), 128.4, 129.1, 129.3 (2C), 138.5 (*tert*. C); 118.2, 119.6, 132.1, 136.0, 138.6, 172.6 (quat. C, one signal missing due to peak overlapping). Anal. $C_{17}H_{12}ClN_3O$ (C, H, N).

4.3.4. 1-(2-Methylphenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2d**)

Preparation according to the general method described in Section 4.2.1 yielded colorless crystals (212 mg; 73%); mp.: 245 °C; IR (KBr): 3202 cm^{-1} (NH), 3065 cm^{-1} (CH arom.), 2980 and 2920 cm^{-1} (CH aliph.), 1689 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 1.81 (s, 3H, CH_3), 3.35 (s, 2H, CH_2), 6.71 (dd, 1H, J = 7.9/1.3 Hz, ArH), 6.89 (dt, 1H, J = 6.9/8.0/1.4 Hz, ArH), 7.23–7.36 (m, 5H, ArH), 7.40 (dt, 1H, J = 7.5/7.1/1.5 Hz, ArH), 7.76 (s, 1H, ArH), 10.07 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 16.8 (CH_3); 31.9 (CH_2); 122.7, 123.3, 126.9, 127.1, 127.7, 128.8, 129.1, 131.0, 137.7 (*tert*. C); 115.9, 119.7, 134.6, 135.9, 137.3, 139.0, 172.7 (quat. C). Anal. $C_{18}H_{15}N_3O$ (C, H, N).

4.3.5. 1-(4-Methoxyphenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2e**)

Preparation according to the general method described in Section 4.2.1 yielded light yellow crystals (274 mg, 89%); mp.: 275 °C; IR (KBr): 3224 cm^{-1} (NH), 3128 cm^{-1} (CH arom.), 1669 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.31 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3), 6.84 (dd, 1H, J = 7.9/1.3 Hz, ArH), 6.96–7.02 (m, 3H, ArH), 7.20–7.27 (m, 3H, ArH), 7.32 (dt, 1H, J = 7.5/7.7/1.5 Hz, ArH), 7.71 (s, 1H, ArH), 10.07 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.9 (CH_2); 55.4 (CH_3); 114.3 (2C), 122.9, 123.3, 126.3 (2C), 128.2, 128.7, 137.6 (*tert*. C); 117.2, 119.9, 132.8, 136.0, 158.6, 172.6 (quat. C, one signal missing due to peak overlapping). Anal. $C_{18}H_{15}N_3O_2$ (C, H, N).

4.3.6. 1-(4-Methylphenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2f**)

Preparation according to the general method described in Section 4.2.1 yielded a white powder (245 mg, 85%); mp.: 285–287 °C; IR (KBr): 3177 cm^{-1} (NH), 3062 cm^{-1} (CH arom.), 2976 cm^{-1} and 2924 cm^{-1} (CH aliph.), 1692 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 2.35 (s, 3H, CH_3), 3.31 (s, 2H, CH_2), 6.84 (dd, 1H, J = 7.9/1.3 Hz, ArH), 6.98 (dt, 1H, J = 8.1/8.3/1.3 Hz, ArH), 7.17–7.19 (m, 2H, ArH), 7.25–7.28 (m, 3H, ArH), 7.33 (dt, 1H, J = 8.1/8.3/1.5 Hz, ArH), 7.74 (s, 1H, ArH), 10.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 20.6 (CH_3); 31.9 (CH_2); 123.0, 123.3, 124.6 (2C), 128.3, 128.8, 129.7 (2C), 137.3 (*tert*. C); 117.6, 120.0, 135.88, 135.94, 137.3, 172.7 (quat. C, one signal missing due to peak overlapping). Anal. $C_{18}H_{15}N_3O$ (C, H, N).

*4.3.7. 1-(4-Trifluoromethylphenyl)-4,6-dihydropyrazolo[4,3-d][1]benzazepin-5(1H)-one (**2g**)*

Preparation according to the general method described in Section 4.2.1 yielded a white powder (257 mg, 75%); mp.: 245 °C; IR (KBr): 3172 cm^{-1} (NH), 3065 cm^{-1} (CH arom.), 2979 cm^{-1} and 2927 cm^{-1} (CH aliph.), 1692 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.34 (obscured by the water peak), 6.89 (dd, 1H, J = 7.9/1.4 Hz, ArH), 7.04 (dt, 1H, J = 8.0/8.4/1.3 Hz, ArH), 7.31 (dd, 1H, J = 8.2/1.0 Hz, ArH), 7.39 (dt, 1H, J = 8.3/8.5/1.5 Hz, ArH), 7.53–7.56 (m, 2H, ArH), 7.83–7.85 (m, 2H, ArH), 7.87 (s, 1H, ArH), 10.18 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.9 (CH_2); 123.1, 123.6, 124.9 (2C), 126.5, 128.5 (2C), 129.3, 139.3 (*tert*. C); 119.0, 119.5, 125.3, 136.0, 136.3, 142.7, 172.7 (quat. C, one signal missing due to peak overlapping). Anal. $C_{18}H_{12}F_3N_3O$ (C, H, N).

*4.3.8. 1-(3,5-Dichlorophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2h**)*

Preparation according to the general method described in Section 4.2.1 yielded yellow crystals (274 mg, 80%); mp.: 283–284 °C; IR (KBr): 3215 cm^{-1} (NH), 3144 cm^{-1} and 3098 cm^{-1} (CH arom.), 1690 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.34 (obscured by water signal), 6.96 (dd, 1H, J = 7.9/1.4 Hz, ArH), 7.08 (dt, 1H, J = 8.0/8.4/1.2 Hz, ArH), 7.31 (dd, 1H, J = 8.2/1.0 Hz, ArH), 7.38 (d, 2H, J = 1.9 Hz, ArH), 7.41 (dt, 1H, J = 8.3/8.5/1.5 Hz, ArH), 7.68 (t, 1H, J = 1.9 Hz, ArH), 7.85 (s, 1H, ArH), 10.15 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.8 (CH_2); 123.1, 123.2 (2C), 123.4, 127.3, 128.5, 129.4, 139.3 (*tert*. C); 118.8, 119.2, 134.3 (2C), 136.1, 136.4, 141.3, 172.4 (quat. C). Anal. $C_{17}H_{11}Cl_2N_3O$ (C, H, N).

The preparation of **2h** was also accomplished by stirring a slurry of **7h** (0.25 mmol, 91 mg) in glacial acetic acid (3 mL) for 1 h at 70 °C. After cooling, the mixture was poured into 5% aqueous sodium acetate solution (6 mL). The precipitate was collected, washed with water, and subsequently crystallized from ethanol (70%) to yield light yellow crystals (27 mg, 32%). According to mp. and spectral data the compound was identical with the material prepared by general method described in Section 4.2.1.

*4.3.9. 1-(4-tert-Butylphenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2i**)*

Preparation according to the general method described in Section 4.2.1 yielded colorless crystals (138 mg, 42%); mp.: 228–229 °C; IR (KBr): 3204 cm^{-1} (NH), 3134 cm^{-1}, 3099 cm^{-1} (CH arom.), 2963 cm^{-1}, 2914 cm^{-1}, 2870 cm^{-1} (CH aliph.), 1690 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 1.30 (s, 9H, CH_3), 3.31 (s, 2H, CH_2), 6.85 (dd, 1H, J = 7.9/1.3 Hz, ArH), 6.96 (dt, 1H, J = 8.1/8.3/1.4 Hz, ArH), 7.21–7.24 (m, 2H, ArH), 7.45–7.48 (m, 2H, ArH), 7.27 (dd, 1H, J = 8.1/1.2 Hz, ArH), 7.33 (dt, 1H, J = 8.3/8.4/1.5 Hz, ArH), 7.75 (s, 1H, ArH), 10.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.0 (3 × CH_3); 31.9 (CH_2); 123.0, 123.3, 124.3 (2C), 125.9 (2C), 128.3, 128.8, 138.0 (*tert*. C); 34.4, 117.7, 120.0, 135.90, 135.92, 137.3, 150.3, 172.7 (quat. C). Anal. $C_{21}H_{21}N_3O$ (C, H, N).

*4.3.10. 1-(2-Chlorophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2j**)*

Preparation according to the general method described in Section 4.2.1 yielded orange crystals (201 mg, 65%); mp.: 244–245 °C; IR (KBr): 3286 cm^{-1} (NH), 1687 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.38 (br s, 2H, CH_2), 6.74 (dd, 1H, J = 7.9/1.3 Hz, ArH), 6.93 (dt, 1H, J = 8.1/8.3/1.4 Hz, ArH), 7.24 (dd, 1H, J = 8.2/1.1 Hz, ArH), 7.31 (dt, 1H, J = 8.3/8.4/1.5 Hz, ArH), 7.50–7.63 (m, 4H, ArH), 7.80 (s, 1H, ArH), 10.08 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.8 (CH_2); 122.8, 123.4, 126.5, 128.4, 129.0, 130.0, 130.3, 130.9, 138.5 (*tert*. C); 116.1, 119.6, 130.5, 136.0, 137.4, 138.0, 172.5 (quat. C). Anal. $C_{17}H_{12}ClN_3O$ (C, H, N).

*4.3.11. 1-(3-Chlorophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2k**)*

Preparation according to the general method described in Section 4.2.1 yielded light yellow crystals (252 mg, 82%); mp.: 272–273 °C; IR (KBr): 3220 cm^{-1} (NH), 3144 cm^{-1} (CH arom.), 1684 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.33 (s, CH_2, obscured by water peak), 6.89 (dd, 1H, J = 7.9/1.4 Hz, ArH), 7.03 (dt, 1H, J = 8.0/8.4/1.3 Hz, ArH), 7.22 (dt, 1H, J = 7.1/2.0/2.2 Hz, ArH), 7.29 (dd, 1H, J = 8.2/1.0 Hz, ArH), 7.38 (dt, 1H, J = 8.3/8.5/1.5 Hz, ArH), 7.43–7.51 (m, 3H, ArH), 7.81 (s, 1H, ArH), 10.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.9 (CH_2); 123.1, 123.3, 123.4, 124.4, 127.7, 128.4, 129.2, 130.8, 138.8 (*tert*. C); 118.4, 119.5, 133.4, 136.0, 136.2, 140.8, 172.6 (quat. C). Anal $C_{17}H_{12}ClN_3O$ (C, H, N).

*4.3.12. 1-(2-Fluorophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2l**)*

Preparation according to the general method described in Section 4.2.1 yielded yellow crystals (182 mg, 62%); mp.: 234–236 °C; IR (KBr): 3210 cm^{-1} (NH), 3140 cm^{-1}, 3100 cm^{-1} (CH arom.), 2972 cm^{-1}, 2916 cm^{-1} (CH aliph.), 1685 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.36 (s, 2H, CH_2), 6.82 (dd, 1H, J = 7.8/1.3 Hz, ArH), 6.96 (dt, 1H, J = 8.0/8.3/1.3 Hz, ArH), 7.26 (dd, 1H, J = 8.1/1.1 Hz, ArH), 7.31–7.41 (m, 3H, ArH), 7.52–7.58 (m, 1H, ArH), 7.62 (t, 1H, J = 7.3/7.6 Hz, ArH), 7.83 (s, 1H, ArH), 10.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.8 (CH_2); 116.7 (d, $^2J_{C,F}$ = 19.6 Hz), 122.9, 123.4, 125.4 (d, $^4J_{C,F}$ = 3.6 Hz), 126.4, 128.9, 129.0, 130.9 (d, $^3J_{C,F}$ = 8.1 Hz), 138.8 (*tert*. C); 119.6, 127.6, 127.7, 135.9, 137.9, 155.6 (d, $^1J_{C,F}$ = 155.6 Hz), 172.4 (quat. C). Anal. $C_{17}H_{12}FN_3O$ (C, H, N).

*4.3.13. 1-(2-Bromophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2m**)*

Preparation according to the general method described in Section 4.2.1 yielded orange crystals (206 mg, 57%); mp.: 248–249 °C; IR (KBr): 3281 cm^{-1} (NH), 3158 cm^{-1}, 3062 cm^{-1} (CH arom.), 1686 cm^{-1} (C═O); ^{1}H NMR

(DMSO-d_6, 400 MHz): δ (ppm) = 3.37 (br s, 2H, CH_2), 6.74 (dd, 1H, J = 7.8/1.5 Hz, ArH), 6.92 (dt, 1H, J = 8.1/8.3/1.4 Hz, ArH), 7.24 (dd, 1H, J = 8.1/1.3 Hz, ArH), 7.30 (dt, 1H, J = 8.3/8.6/1.5 Hz, ArH), 7.47 (ddd, 1H, J = 7.8/7.8/2.3 Hz, ArH), 7.53–7.59 (m, 2H, ArH), 7.77–7.78 (m, 2H, ArH), 10.07 (s, 1H, NH); ^{1}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.8 (CH_2); 122.8, 123.3, 126.7, 128.9 (2C), 130.2, 131.2, 133.4, 138.3 (*tert.* C); 116.0, 119.5, 121.1, 136.0, 137.7, 139.0, 172.5 (quat. C). Anal. $C_{17}H_{12}BrN_3O$ (C, H, N).

The preparation of **2m** was also accomplished by stirring a slurry of **7m** (0.25 mmol, 93 mg) in glacial acetic acid (3 mL) for 1 h at 70 °C. After cooling, the mixture was poured into 5% aqueous sodium acetate solution (6 mL). The precipitate was collected, washed with water, and subsequently crystallized from ethanol (70%) to yield orange crystals (49 mg, 54%). According to mp. and spectral data the compound was identical with the material prepared by general method described in Section 4.2.1.

4.3.14. 1-(2,4-Difluorophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2n**)

Preparation according to the general method described in Section 4.2.1 yielded colorless crystals (193 mg, 62%); mp.: 217–218 °C; IR (KBr): 3233 cm^{-1} (NH), 3126 cm^{-1}, 3088 cm^{-1} (CH arom.), 2981 cm^{-1}, 2896 cm^{-1} (CH aliph.), 1673 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.33 (s, CH_2 obscured by water signal), 6.85 (dd, 1H, J = 7.8/1.3 Hz, ArH), 7.00 (dt, 1H, J = 8.1/8.3/1.3 Hz, ArH), 7.26–7.37 (m, 3H, ArH), 7.47 (dt, 1H, J = 10.9/10.9/2.8 Hz, ArH), 7.71 (q, 1H, J = 8.6/6.3/8.3 Hz, ArH), 7.83 (s, 1H, ArH), 10.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.8 (CH_2); 105.3 (t, $^2J_{C,F}$ = 24.0/27.4 Hz), 112.6 (d, $^2J_{C,F}$ = 26.6 Hz), 122.9, 123.5, 126.5, 129.1, 130.3 (t, $^3J_{C,F}$ = 10.6 Hz), 139.0 (*tert.* C); 116.6, 119.4, 124.5, 136.0, 138.0, 172.4 (quat. C, two C not detected due to low intensity or peak overlapping). Anal. $C_{17}H_{11}F_2N_3O$ (C, H, N).

4.3.15. 1-(4-Nitrophenyl)-4,6-dihydropyrazolo-[4,3-d][1]benzazepin-5(1H)-one (**2o**)

Preparation according to the general method described in Section 4.2.1 (reflux instead of strirring at 70 °C yielded colorless crystals (214 mg, 66%); mp.: 264 °C; IR (KBr): 3196 cm^{-1} (NH), 3067 cm^{-1} (CH arom.), 2954, 2898 cm^{-1} (CH aliph.),1681 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.33 (s, CH_2 obscured by water peak), 6.94 (dd, 1H, J = 8.0/1.4 Hz, ArH), 7.05 (dt, 1H, J = 8.1/8.3/1.3 Hz, ArH), 7.32 (dd, 1H, J = 8.1/0.9 Hz, ArH), 7.41 (dt, 1H, J = 8.3/8.6/1.5 Hz, ArH), 7.57–7.61 (m, 2H, ArH), 7.91 (s, 1H, ArH), 8.30–8.33 (m, 2H, ArH), 10.21 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.9 (CH_2); 123.2, 123.6, 124.79 (2C), 124.82 (2C), 128.7, 129.4, 139.9 (*tert.* C); 119.4, 119.6, 136.1, 136.5, 144.4, 145.8, 172.6 (quat. C). Anal. $C_{17}H_{12}N_4O_3$ (C, H, N).

4.3.16. 4-{[2-(3,5-Dichlorophenyl)hydrazino]methylene}-3,4-dihydro-1H-[1]benzazepine-2,5-dione (**7h**)

Preparation according to the general method described in Section 4.2.2 yielded beige crystals (178 mg, 49%); mp.: 219–222 °C; IR (KBr): 3188 cm^{-1} (NH), 3070 cm^{-1} (CH arom.), 2965 cm^{-1} (CH aliph.), 1688 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 2.98 (s, 2H, CH_2), 6.82 (t, 1H, J = 1.8 Hz, ArH), 6.91 (d, 2H, J = 1.3 Hz, ArH), 7.15 (d, 1H, J = 8.1 Hz, ArH), 7.20 (dt, 1H, J = 7.2/8.1/1.0 Hz, ArH), 7.40 (dt, 1H, J = 6.9/8.3/1.4 Hz, ArH), 7.70 (dd, 1H, J = 8.1/1.3 Hz, ArH), 8.17 (s, 1H, ArH), 9.62 (s, 1H, NH), 10.19 (s, 1H, NH), 10.57 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.1 (CH_2); 109.7 (2C), 116.7, 121.3, 123.0, 127.8, 129.5, 138.7 (*tert.* C); 108.2, 127.4, 134.6 (2C), 136.6, 147.5, 151.2, 172.0 (quat. C). Anal. $C_{17}H_{13}Cl_2N_3O_2$ (H, N, C) calcd. 56.37; found 55.90.

4.3.17. 4-{[2-(2-Bromophenyl)hydrazino]methylene}-3,4-dihydro-1H-[1]benzazepine-2,5-dione (**7m**)

Preparation according to the general method described in Section 4.2.2 yielded brown crystals (223 mg; 60%); mp.: 212 °C (dec.); IR (KBr): 3430 cm^{-1}, 3324 cm^{-1}, 3198 cm^{-1} (NH), 3099 cm^{-1}, 3055 cm^{-1} (CH arom.), 2957 cm^{-1} (CH aliph.), 1647 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 2.99 (s, 2H, CH_2), 6.72 (dt, 1H, J = 7.2/7.9/1.6 Hz, ArH), 7.15 (d, 1H, J = 7.8 Hz, ArH), 7.20 (dt, 1H, J = 7.3/7.9/0.9 Hz, ArH), 7.30 (dt, 1H, J = 7.2/8.2/1.0 Hz, ArH), 7.37–7.42 (m, 2H, ArH), 7.47 (dd, 1H, J = 7.8/1.3 Hz, ArH), 7.72 (dd, 1H, J = 7.9/1.4 Hz, ArH), 8.45 (s, 1H, ArH), 9.48 (s, 1H, NH), 9.81 (s, 1H, NH), 10.18 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.8 (CH_2); 114.0, 119.9, 121.3, 123.0, 127.9, 128.5, 129.5, 132.5, 141.2 (*tert.* C); 106.0, 107.5, 127.7, 136.6, 142.6, 152.9, 172.1 (quat. C). Anal. $C_{17}H_{14}BrN_3O_2$ (C, H, N).

4.4. X-ray structure determination

Numerical details are summarised in Table 1. Data were recorded on a Bruker SMART 1000 CCD diffractometer. The structure was refined on F^2 using the program SHELXL-97 (G.M. Sheldrick, University of Göttingen, Germany). Hydrogens of NH groups were refined freely, others with a riding model.

Complete data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre under the number CCDC-623071. These data can be obtained free of charge from www.ccdc.cam.ac.uk/data_request/cif.

4.5. In vitro anticancer assays

4.5.1. Prescreening

At the National Cancer Institute, a 3-cell line panel primary anticancer assay is used, consisting of the MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) lines. For testing, each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at 10^{-4} M concentration and the culture was incubated for 48 h. End-point determinations were made using alamar blue as described previously [21]. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds that reduce the growth of at least

one of the cell lines to approximately 32% or less are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

4.5.2. Screening on 60 cell lines

Screening compounds are tested against 60 human tumor cell lines at five concentrations (10^{-8}–10^{-4} M). After 48 h of continuous drug exposure, a sulforhodamine B protein assay is used to determine cell viability or growth. The percentage of growth is calculated on the basis of the optical density of the treated cultures compared to untreated control cell lines. Details concerning the performance of this screening have been published [22,23].

4.6. Kinase assays

A proprietary protein kinase assay (^{33}PanQinase® Activity Assay) was used for measuring the kinase activity of the 23 protein kinases. All assays were performed with a Beckman-Coulter/Sagian robotic system and 96-well FlashPlates™ from Perkin Elmer/NEN (Boston, MA, USA) in a 50 μL reaction volume. The reaction cocktail was pipetted in 4 steps in the following order: 20 μL of assay buffer; 5 μL of ATP solution (in H_2O); 5 μL of test compound (in 10% DMSO); 10 μL of substrate/10 μL of enzyme solution (premixed). The assay for all enzymes contained 60 mM HEPES–NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 μM Na-orthovanadate, 1.2 mM DTT, 50 μg/mL $PEG_{20\,000}$, 1 μM [γ-^{33}P]-ATP (approx. 5×10^5 cpm per well). For the 23 kinases the following substrates were used [substrate amount in square brackets given in ng/50 μL]: poly(Glu,Tyr)$_{4:1}$ [125]: EGF-R, EPHB4, ERBB2, FAK, IGF1-R, SRC, VEGFR-2, VEGFR-3, TIE2; GSK-3(14-27) [1000]: AKT1; tetra(LRRWSLG) [500/250, respectively]: Aurora A, Aurora B; Histone H1 [125]: CDK2/CycA; Rb-CTF [500]: CDK4/CycD1; p53-CTM [125]: CK2-alpha1; casein [1000]: PLK1; poly(Ala,Glu,Lys,Tyr)$_{6:2:5:1}$ [125]: FLT3, MET, PDGFR-beta; tetra(LRRWSLG) [500]: PAK4, PDK1; autophosphorylation: ARK5, SAK. The reaction cocktails were incubated at 30 °C for 80 min. The reaction was stopped with 50 μL of 2% (v/v) H_3PO_4, plates were aspirated and washed two times with 200 μL of 0.9% (w/v) NaCl. Incorporation of $^{33}P_i$ was determined with a microplate scintillation counter (Microbeta Trilux, Wallac).

Acknowledgements

We are grateful to the American National Cancer Institute for performing the cell line screening. Funding of the project by the European Commission (Contract No LSHB-CT-2004-503467, to F.T., C.S., M.H. G.K., and C.K.) is gratefully acknowledged.

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