European Journal of Medicinal Chemistry 57 (2012) 323–328

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of novel pyrazolo[3,4-*d*]pyrimidine derivatives as potential anti-breast cancer agents

Mohammed K. Abd El Hamid [a], Marko D. Mihovilovic [b], Hala B. El-Nassan [a,*]

[a] *Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, 33 Kasr El-Aini Street, Cairo 11562, Egypt*
[b] *Institute of Applied Synthetic Chemistry, Vienna University of Technology, Vienna, Austria*

ARTICLE INFO

Article history:
Received 29 June 2012
Received in revised form
21 September 2012
Accepted 24 September 2012
Available online 29 September 2012

Keywords:
Pyrazolo[3,4-*d*]pyrimidine
Antitumor activity
MCF7

ABSTRACT

A series of new 1-aryl-4-benzylidenehydrazinyl-3-methylsulphanyl-pyrazolo[3,4-*d*]pyrimidines **6a**–**p** was synthesized. The cytotoxic activity of the newly synthesized compounds against human breast cancer cell line, MCF7 was investigated. Most of the test compounds showed potent antitumor activity comparable to that of doxorubicin. The 1-phenyl series (**6a**–**i**) exhibited better antitumor activity than 1-(4-methoxyphenyl) series (**6j**–**p**). 4-[2-(4-Fluorobenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**6d**) was the most active compound in this study with IC_{50} equal to 7.5 nM.

1. Introduction

Cancer is considered as the most serious health problem all over the world. Although, the development of novel chemotherapeutic agents has significantly progressed within the last 60 years, success in developing non-cytotoxic "targeted" drugs with fewer side effects has occurred only in the last decade. Despite these advances, the treatment of most types of solid tumors (e.g. breast and ovarian) is still a problem and survival rates remain significantly low [1]. Therefore, the discovery of new potent, safe and selective antitumor agents is strongly needed.

The pyrazolo[3,4-*d*]pyrimidine nucleus is considered as an isostere to the purine nucleus and hence exhibits promising antitumor activity by acting as ATP competitive inhibitor for many kinase enzymes. Indeed, many pyrazolo[3,4-*d*]pyrimidines were reported to exhibit potent anti-tumor activity [2–6]. Their cytotoxic activities might be attributed to inhibition of several enzymes such as Src kinase [5], tyrosine kinase [7,8], mammalian target of rapamycin (mTOR) [9], cyclin dependent kinase (CDK) [10–12] and glycogen synthase kinase (GSK) [13–15].

Hydrazinyl derivatives have been claimed to exhibit antitumor effect especially against breast cancer cell lines [16–20]. Recently, a number of publications had emerged describing the GSK inhibitory activity of 4-benzylidenehydrazinylpyrazolo[3,4-*d*]pyrimidines [13–15]. Although, the SAR of these derivatives as GSK inhibitors was fully investigated, none of these publications described the antitumor activity of such a nucleus.

On the other hand, the presence of a methylsulphanyl group at position 3 of the pyrazolo[3,4-*d*]pyrimidine nucleus was reported to enhance the antitumor activity of the nucleus [4,11,21].

Prompted by these claims, we assumed that incorporating these potent pharmacophores together may result in strong anticancer agents that act on breast cancer as an example of the solid tumors. In the present work, new 4-benzylidenehydrazinyl-pyrazolo[3,4-*d*]pyrimidine derivatives **6a**–**p** were synthesized, incorporating the 3-methylsulphanyl group and varying the substitution on the phenyl ring at position 1 [H or 4-CH_3O] and the phenyl ring at benzylidenehydrazinyl group in order to study the effect of the substitution at these two positions on the antitumor activity of the nucleus against MCF7 cell line.

2. Results and discussion

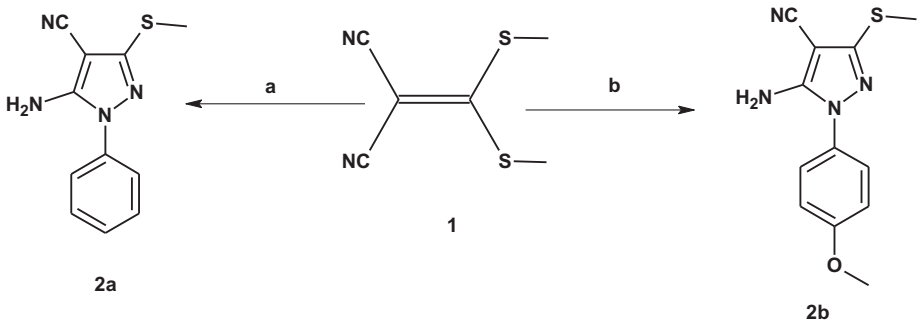
2.1. Chemistry

Schemes 1 and 2 outline the synthesis of the target compounds.

The synthesis of the starting pyrazole derivatives **2a** and **2b** was accomplished *via* reacting bis(methylsulphanyl)methylenemalononitrile (**1**) with phenylhydrazine in absolute ethanol [22] or with 4-methoxyphenylhydrazine HCl in ethanol and triethylamine. The formation of compound **2b** was confirmed by IR that showed CN band at 2202 cm^{-1} as well as NH_2 bands at 3410, 3325 cm^{-1}. ^1H NMR

* Corresponding author. Tel.: +20 223632245; fax: +20 223635140.
E-mail address: hala_bakr@hotmail.com (H.B. El-Nassan).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.09.031



Reagents: a) $C_6H_5NHNH_2$, C_2H_5OH; b) 4-$CH_3OC_6H_4NHNH_2$.HCl, $(C_2H_5)_3N$, C_2H_5OH

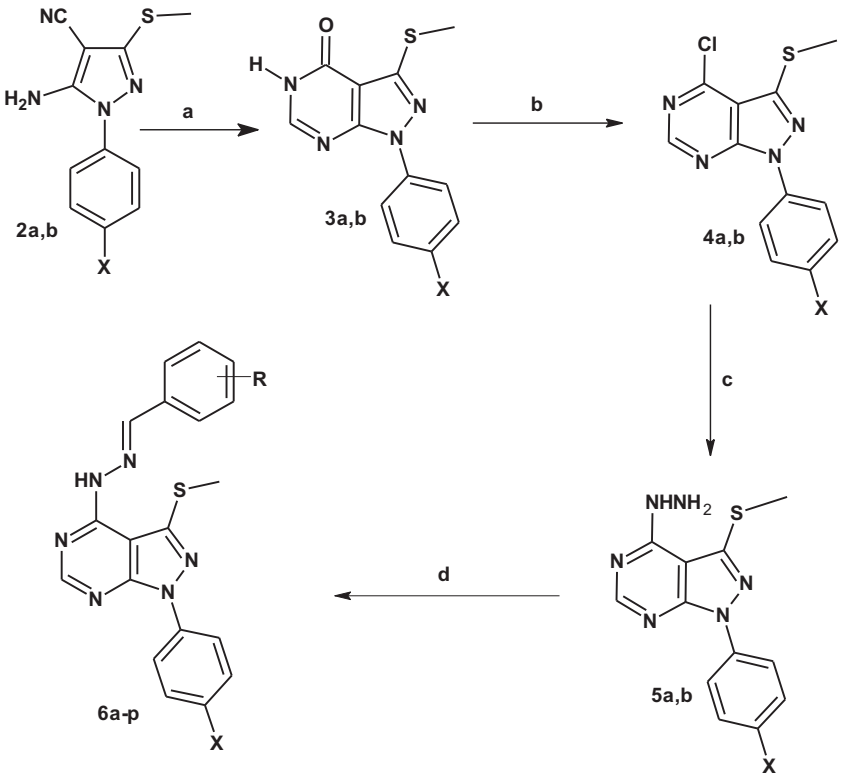
Scheme 1. Preparation of the starting pyrazole derivatives.

spectrum of compound **2b** showed an exchangeable singlet signal at δ 6.91 ppm corresponding to the NH_2 protons. Also, the mass spectrum of compound **2b** showed a molecular ion peak at *m*/*z* 260.

Cyclization of pyrazole derivatives **2a**,**b** with formic acid afforded pyrazolo[3,4-*d*]pyrimidin-4-ones **3a** [22] and **3b** in high yields. The disappearance of the CN and NH_2 bands in the IR spectrum of compound **3b**, together with the appearance of a single NH band at 3437 cm^{-1} and C=O band at 1681 cm^{-1} provided proof for the formation of **3b**. ^{1}H NMR spectrum of compound **3b** revealed the disappearance of the NH_2 characteristic signal and the appearance of an exchangeable singlet signal at δ 12.39 ppm corresponding to the NH proton. Besides, a singlet signal appeared at δ 8.13 ppm corresponding to the H-6 proton of the pyrimidine ring. The mass spectrum of compound **3b** showed a molecular ion peak at *m*/*z* 288.

Chlorination of pyrazolo[3,4-*d*]pyrimidin-4-one derivatives **3a**,**b** with $POCl_3$ gave 4-chloro derivatives **4a**,**b**. The latter compounds were reacted with hydrazine hydrate to give the corresponding 4-hydrazinyl derivatives **5a**,**b**.

The target compounds were obtained by reacting 4-hydrazinyl derivatives **5a**,**b** with the appropriate aromatic aldehyde in ethanol and glacial acetic acid. The formation of compounds **6a**–**p** was confirmed by ^{1}H NMR spectra that demonstrated the appearance of a singlet signal at δ 8.2–8.6 ppm corresponding to the N=CH proton as well as an exchangeable singlet signal at δ 11–12 ppm



For 6a-i: X=H, R= 4-NH_2, 4-Br, 4-Cl, 4-F, 2-OH, 3-OH, 4-OH, 3-CH_3O, 2-Cl-4-NO_2

For 6j-p: X=CH_3O, R= 4-Br, 4-Cl, 4-F, 2-OH, 3-OH, 4-OH, 4-CH_3O

Reagents: a) HCOOH; b) $POCl_3$; c) N_2H_4, C_2H_5OH; d) RC_6H_4CHO, C_2H_5OH

Scheme 2. Synthesis of the target compounds **6a**–**p**.

corresponding to the NH proton. ^{13}C NMR spectra of compounds **6a,b,e,h,i,j** and **6p** showed N=CH carbon signal at δ 99–104 ppm. Besides, the mass spectra of **6a**–**p** showed the corresponding molecular ion peaks and peaks due to loss of [RC_6H_4CH=N] and/or loss of [RC_6H_4C=N].

2.2. In vitro anticancer screening

The newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line (MCF7) using Doxorubicin as the reference drug.

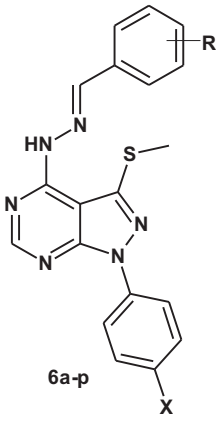
The relation between surviving fraction and drug concentrations was plotted to obtain the survival curve of MCF7 tumor cell line after addition of the specified compound. The parameter used here is IC_{50}, which corresponds to the concentration required for 50% inhibition of cell viability.

The IC_{50} of the synthesized compounds compared to the reference drug are shown in Table 1 and the results are represented graphically in Fig. 1.

From the results in Table 1, it was found that most of the test compounds showed potent antitumor activity comparable to that of doxorubicin.

Structurally, the test compounds belong to two series: 1-phenyl series and 1-(4-methoxyphenyl) series. In general, the 1-phenyl series (**6a**–**i**) exhibited better antitumor activity than 1-(4-methoxyphenyl) series (**6j**–**p**).

Table 1
Results of *in vitro* cytotoxic activity of the synthesized compounds on human breast adenocarcinoma cell line (MCF7).



Compound no.	X	R	IC_{50} in nM[a]
Doxorubicin	–	–	5.4
6a	H	4-NH_2	12
6b	H	4-Br	10.9
6c	H	4-Cl	12.1
6d	H	4-F	7.5
6e	H	2-OH	10.7
6f	H	3-OH	10.7
6g	H	4-OH	10.3
6h	H	3-CH_3O	11.1
6i	H	2-Cl–4-NO_2	15.7
6j	CH_3O	4-Br	23
6k	CH_3O	4-Cl	31.1
6l	CH_3O	4-F	12.1
6m	CH_3O	2-OH	13.3
6n	CH_3O	3-OH	9.9
6o	CH_3O	4-OH	34.4
6p	CH_3O	4-CH_3O	35

[a] The values given are means of three experiments.

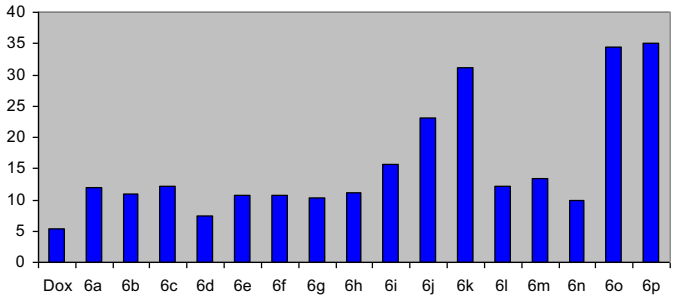


Fig. 1. IC_{50} in nM of the synthesized compounds and doxorubicin against human breast adenocarcinoma cell line (MCF7).

Careful examination of the influence of the substituents on the benzylidenehydrazinyl group (R) on the antitumor activity showed the following:

- In both series, the influence of 4-halide substituents on the antitumor activity was in the order F > Br > Cl
- In case of 1-phenyl series, no difference was observed with the hydroxy group at different position of the ring (*ortho*, *meta* and *para*). Whilst, in 1-(4-methoxyphenyl) series, the order of antitumor activity was 3-OH > 2-OH > 4-OH.

Compound **6d** (X = H, R = 4-F) was the most active compound in this study with IC_{50} equal to 7.5 nM.

3. Conclusion

In summary, a series of new 1-aryl-4-benzylidenehydrazinyl-3-methylsulphanyl-pyrazolo[3,4-*d*]pyrimidines **6a**–**p** was synthesized. The cytotoxic activity of the newly synthesized compounds against human breast cancer cell line (MCF7) was investigated. The 1-phenyl series (**6a**–**i**) exhibited better antitumor activity than 1-(4-methoxyphenyl) series (**6j**–**p**). Most of the test compounds showed potent antitumor activity comparable to that of doxorubicin, especially, compound (**6d**) which displayed the highest activity among the test compounds with IC_{50} equal to 7.5 nM. Further studies are still needed to determine the exact mechanism of the antitumor action as well as to explore the SAR of other positions of the nucleus.

4. Experimental part

4.1. General

Melting points were determined using a Griffin apparatus and were uncorrected. IR spectra were recorded on Shimadzu IR 435 spectrophotometer and values were represented in cm^{-1}. ^1H NMR were carried out on Varian Gemini 300 MHz spectrophotometer, Main Defense Chemical Laboratory, Cairo, Egypt and Bruker AC 200 (200 MHz) spectrometer, Institute for synthetic chemistry, Vienna University of Technology, Vienna, Austria. TMS was used as an internal standard and chemical shifts were recorded in ppm on δ scale and coupling constants (J) are given in Hz. ^{13}C NMR were carried out on Bruker AC 200 (200 MHz) spectrometer, Institute for synthetic chemistry, Vienna University of Technology, Vienna, Austria. The electron impact (EI) mass spectra were recorded on Hewlett Packard 5988 spectrometer, Microanalytical center, Cairo University, Cairo, Egypt. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques.

5-Amino-3-methylsulphanyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**2a**) [22] and 3-methylsulphanyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(*5H*)-one (**3a**) [22] were prepared according to the published method.

*4.1.1. 5-Amino-3-methylsulphanyl-1-(4-methoxyphenyl)-1H-pyrazole-4-carbonitrile (**2b**)*

A solution of bis(methylsulphanyl)methylenemalononitrile (**1**) (1.70 g, 10 mmol), 4-methoxyphenylhydrazine HCl (1.75 g, 10 mmol) and triethylamine (2 mL) in absolute ethanol (20 mL) was heated under reflux for 5 h. The reaction mixture was cooled and the precipitate formed was filtered, dried and crystallized from ethanol. Yield: 57%; mp: 128–129 °C; IR (cm^{-1}): 3410, 3325 (NH_2), 2202 (CN), 2927, 2850 (CH-aliphatic); ^1H NMR (300 MHz, DMSO-d_6) δ ppm 2.50 (s, 3H, SCH_3), 3.78 (s, 3H, OCH_3), 6.91 (s, 2H, NH_2, D_2O exchangeable), 7.03 (d, 2H, J = 8.7 Hz, Ar–H), 7.36 (d, 2H, J = 8.7 Hz, Ar–H); MS *m*/*z*: 260 [M^+, 100%].

*4.1.2. 3-Methylsulphanyl-1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d] pyrimidin-4(5H)-one (**3b**)*

A mixture of pyrazole derivative **2b** (2.78 g, 10 mmol) and formic acid (85%, 40 mL) was heated under reflux for 8 h. The reaction was cooled, and the separated solid was filtered, dried and crystallized from formic acid. Yield: 92%; mp: 268–269 °C; IR (cm^{-1}): 3437 (NH), 2920, 2850 (CH-aliphatic), 1681 (CO); ^1H NMR (300 MHz, DMSO-d_6) δ ppm 2.59 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 7.08 (d, 2H, J = 8.7 Hz, Ar–H), 7.84 (d, 2H, J = 8.7 Hz, Ar–H), 8.13 (s, 1H, Ar–H), 12.39 (s, 1H, NH, D_2O exchangeable); MS *m*/*z*: 288 [M^+, 100%], 287 [$(M-1)^+$, 63.6%].

*4.1.3. 1-Aryl-4-chloro-3-methylsulphanyl-pyrazolo[3,4-d] pyrimidines (**4a**,**b**)*

A suspension of the pyrazolo[3,4-*d*]pyrimidin-4-ones **3a**,**b** (10 mmol) in phosphorus oxychloride (80 mL) was heated under reflux for 8 h. The reaction was cooled, poured onto ice-cold water (200 mL) and the precipitate was filtered, dried and crystallized from ethanol.

*4.1.3.1. 4-Chloro-3-methylsulphanyl-1-phenyl-pyrazolo[3,4-d]pyrimidine (**4a**).* Yield: 60%; mp: 90–91 °C; IR (cm^{-1}): 2924, 2854 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.60 (s, 3H, SCH_3), 7.30–8.90 (m, 6H, Ar–H), MS *m*/*z*: 278 [$(M+2)^+$, 0.1%], 276 [M^+, 0.3%], 80 [100%], 77 [$C_6H_5^+$, 22.3%].

*4.1.3.2. 4-Chloro-1-(4-methoxyphenyl)-3-methylsulphanyl-pyrazolo [3,4-d]pyrimidine (**4b**).* Yield: 72%; mp: 157–158 °C; IR (cm^{-1}): 2900, 2800 (CH-aliphatic); ^1H NMR (300 MHz, DMSO-d_6) δ ppm 2.62 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 7.06–8.12 (m, 5H, Ar–H); MS *m*/*z*: 308 [$(M+2)^+$, 39.4%], 306 [M^+, 100%], 77 [$C_6H_5^+$, 25.4%], 76 [$C_6H_4^+$, 19.8%].

*4.1.4. 1-Aryl-4-hydrazinyl-3-methylsulphanyl-pyrazolo[3,4-d] pyrimidines (**5a**,**b**)*

A mixture of 4-chloropyrazolo[3,4-*d*]pyrimidine derivative **4a**,**b** (10 mmol) and hydrazine hydrate (99%, 2 mL, 40 mmol) in absolute ethanol (35 mL) was heated under reflux for 3 h. The reaction was cooled, and the separated solid was filtered, dried and crystallized from ethanol.

*4.1.4.1. 4-Hydrazinyl-3-(methylsulfanyl)-1-phenyl-pyrazolo[3,4-d] pyrimidine (**5a**).* Yield: 84%; mp: 191–92 °C; IR (cm^{-1}): 3325, 3201 (NH/NH_2), 2924, 2854 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.61 (s, 3H, SCH_3), 4.95 (br s, 2H, NH_2, D_2O exchangeable), 7.42–8.36 (m, 6H, Ar–H), 8.60 (br s, 1H, NH, D_2O exchangeable), MS *m*/*z*: 272 [M^+, 24.5%], 77 [$C_6H_5^+$, 47.4%], 76 [$C_6H_4^+$, 19.8%], 64 [100%].

*4.1.4.2. 4-Hydrazinyl-1-(4-methoxyphenyl)-3-(methylsulfanyl)-pyrazolo[3,4-d]pyrimidine (**5b**).* Yield: 80%; mp: 175–176 °C; IR (cm^{-1}): 3300, 3200 (NH/NH_2), 2924, 2854 (CH-aliphatic); ^1H NMR (300 MHz, DMSO-d_6) δ ppm δ 2.59 (s, 3H, SCH_3), 3.82 (s, 3H, OCH_3), 4.83 (br s, 2H, NH_2, D_2O exchangeable), 7.07 (d, 2H, J = 9.0 Hz, Ar–H), 7.96 (d, 2H, J = 9.0 Hz, Ar–H), 8.01 (s, 1H, Ar–H), 8.28 (s, 1H, NH, D_2O exchangeable), MS *m*/*z*: 302 [M^+, 37.5%], 287 [$(M-CH_3)^+$, 87.5%], 60 [100%].

*4.1.5. General procedure for the synthesis of 1-aryl-4-[-2-(substituted benzylidene)hydrazinyl]-3-(methylsulphanyl)-1H-pyrazolo[3,4-d]pyrimidines **6a**–**p***

A mixture of 4-hydrazinylpyrazolo[3,4-*d*]pyrimidines **5a**,**b** (1 mmol) and the appropriate aromatic aldehyde (1 mmol) in absolute ethanol (25 mL) and glacial acetic acid (2 mL) was heated under reflux for 4 h. The solvent was concentrated under reduced pressure, and the solid formed was filtered, dried and crystallized from acetic acid.

*4.1.5.1. 4-[2-(4-Aminobenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6a**).* Yield: 97%; mp: 140–141 °C; IR (cm^{-1}): 3320, 3200 (NH/NH_2), 2924, 2854 (CH-aliphatic); ^1H NMR (200 MHz, $CDCl_3$) δ ppm 2.54 (s, 3H, SCH_3), 5.16 (s, 2H, NH_2, D_2O exchangeable), 6.86–7.74 (m, 12H, Ar–H + NH); ^{13}C NMR (DMSO-d_6) δ ppm 14.1 (SCH_3), 103.2 (N=CH), 114.5, 116.4, 120.6, 120.9, 122.6, 122.9, 126.2, 129.0, 129.2, 129.3, 129.9, 138.3, 144.1 (aromatic carbons); MS *m*/*z*: 375 [M^+, 6.5%], 374 [$(M-1)^+$, 4.3%], 257 [$(M-H_2NC_6H_4C{=}N)^+$, 40.2%], 256 [$(M-H_2NC_6H_4CH{=}N)^+$, 27.2%], 224 [17.8%], 92 [$H_2NC_6H_4^+$, 25.6%], 77 [$C_6H_5^+$, 100%], 76 [$C_6H_4^+$, 12.8%].

*4.1.5.2. 4-[2-(4-Bromobenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6b**).* Yield: 71%; mp: 192–193 °C; IR (cm^{-1}): 3205 (NH), 2924, 2854 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6/CF_3COOD) δ ppm 2.66 (s, 3H, SCH_3), 7.29–8.44 (m, 10H, Ar–H), 8.67 (s, 1H, N=CH), 11.99 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 13.0 (SCH_3), 101.7 (N=CH), 120.7, 121.1, 123.1, 126.5, 129.6, 130.1, 131.4, 134.5, 138.1, 144.4, 147.2, 148.4, 152.2 (aromatic carbons); MS *m*/*z*: 440 [$(M+2)^+$, 15.6%], 438 [M^+, 20.7%], 283 [$(M-BrC_6H_4)^+$, 23.1%], 257 [$(M-BrC_6H_4C{=}N)^+$, 99.6%], 256 [$(M-BrC_6H_4CH{=}N)^+$, 51.8%], 224 [24.7%], 77 [$C_6H_5^+$, 100%], 76 [$C_6H_4^+$, 31.9%].

*4.1.5.3. 4-[2-(4-Chlorobenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6c**).* Yield: 73%; mp: 208–209 °C; IR (cm^{-1}): 3421 (NH), 2924, 2850 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.50 (s, 3H, SCH_3), 7.30–8.38 (m, 10H, Ar–H), 8.69 (s, 1H, N=CH), 12.04 (s, 1H, NH, D_2O exchangeable); MS *m*/*z*: 396 [$(M+2)^+$, 5.5%], 394 [M^+, 22.0%], 257 [$(M-ClC_6H_4C{=}N)^+$, 42.5%], 256 [$(M-ClC_6H_4CH{=}N)^+$, 26.8%], 224 [25.2%], 77 [$C_6H_5^+$, 100%], 76 [$C_6H_4^+$, 25.2%].

*4.1.5.4. 4-[2-(4-Fluorobenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6d**).* Yield: 71%; mp: 237–238 °C; IR (cm^{-1}): 3421 (NH), 2924, 2854 (CH-aliphatic); ^1H NMR (200 MHz, $CDCl_3$) δ ppm 2.64 (s, 3H, SCH_3), 7.31–8.09 (m, 10H, Ar–H), 8.30 (s, 1H, N=CH), 11.50 (s, 1H, NH, D_2O exchangeable); MS *m*/*z*: 378 [M^+, 30.0%], 377 [$(M-1)^+$, 13.4%], 283 [$(M-FC_6H_4)^+$, 13.4%], 257 [$(M-FC_6H_4C{=}N)^+$, 91.2%], 256 [$(M-FC_6H_4CH{=}N)^+$, 39.5%], 224 [30.1%], 121 [$(FC_6H_4C{=}N)^+$, 38.4%], 95 [$FC_6H_4^+$, 19.9%], 77 [$C_6H_5^+$, 100%].

*4.1.5.5. 4-[2-(2-Hydroxybenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6e**).* Yield: 96%; mp: 203–204 °C; IR (cm^{-1}): 3614 (OH), 3394 (NH), 2924, 2854 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.60 (s, 3H, SCH_3),

6.90–8.40 (m, 10H, Ar–H), 8.60 (s, 1H, N=CH), 10.20 (s, 1H, OH, D_2O exchangeable), 12.00 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 14.9 (SCH_3), 101.7 (N=CH), 116.0, 119.2, 120.6, 121.0, 126.4, 129.4, 131.2, 138.1, 138.5, 144.3, 145.5, 148.5, 150.2, 153.7, 156.9 (aromatic carbons); MS *m/z*: 376 [M^+, 9.4%], 375 [$(M - 1)^+$, 3.7%], 257 [$(M - HOC_6H_4C{=}N)^+$, 15.2%], 256 [$(M - HOC_6H_4CH{=}N)^+$, 6.2%], 240 [100%], 224 [19.4%], 121 [$(HOC_6H_4CH{=}NH)^+$, 67.1%], 120 [$(HOC_6H_4CH{=}N)^+$, 46.5%], 77 [$C_6H_5^+$, 46.8%].

*4.1.5.6. 4-[2-(3-Hydroxybenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6f**).* Yield: 39%; mp: 223–224 °C; IR (cm^{-1}): 3286 (NH/OH), 2924, 2850 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.60 (s, 3H, SCH_3), 7.20–8.40 (m, 10H, Ar–H), 8.50 (s, 1H, N=CH), 10.00 (s, 1H, OH, D_2O exchangeable), 12.00 (s, 1H, NH, D_2O exchangeable); MS *m/z*: 376 [M^+, 28.5%], 375 [$(M - 1)^+$, 21.6%], 283 [$(M - HOC_6H_4)^+$, 25.9%], 257 [$(M - HOC_6H_4C{=}N)^+$, 65.5%], 256 [$(M - HOC_6H_4CH{=}N)^+$, 44.8%], 224 [25.9%], 120 [$(HOC_6H_4CH{=}N)^+$, 21.1%], 77 [$C_6H_5^+$, 100%], 76 [$C_6H_4^+$, 17.0%].

*4.1.5.7. 4-[2-(4-Hydroxybenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6g**).* Yield: 55%; mp: 262–263 °C; IR (cm^{-1}): 3371 (NH/OH), 2924, 2854 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.58 (s, 3H, SCH_3), 6.81–8.09 (m, 10H, Ar–H), 8.27 (s, 1H, N=CH), 9.92 (s, 1H, OH, D_2O exchangeable), 11.84 (br s, 1H, NH, D_2O exchangeable); MS *m/z*: 376 [M^+, 10.4%], 375 [$(M - 1)^+$, 6.1%], 257 [$(M - HOC_6H_4C{=}N)^+$, 30.7%], 256 [$(M - HOC_6H_4CH{=}N)^+$, 15.3%], 224 [17.8%], 121 [$(HOC_6H_4CH{=}NH)^+$, 14.7%], 120 [$(HOC_6H_4CH{=}N)^+$, 16.6%], 77 [$C_6H_5^+$, 54.0%], 55 [100%].

*4.1.5.8. 4-[2-(3-Methoxybenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6h**).* Yield: 54%; mp: 238–239 °C; IR (cm^{-1}): 3205 (NH), 2924, 2835 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6/CF_3COOD) δ ppm 2.62 (s, 3H, SCH_3), 3.78 (s, 3H, OCH_3), 6.97–8.45 (m, 10H, Ar–H), 8.58 (s, 1H, N=CH), 11.02 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6/CF_3COOD) δ ppm 14.0 (SCH_3), 55.1 (OCH_3), 99.8 (N=CH), 112.1, 114.2, 117.8, 121.2, 126.2, 128.9, 129.9, 137.7, 144.3, 157.1, 157.9, 158.7, 159.4, 161.2, 161.6 (aromatic carbons); MS *m/z*: 390 [M^+, 22.2%], 389 [$(M - 1)^+$, 8.3%], 257 [$(M - CH_3OC_6H_4C{=}N)^+$, 100%], 256 [$(M - CH_3OC_6H_4CH{=}N)^+$, 39.8%], 224 [23.1%], 77 [$C_6H_5^+$, 83.0%], 76 [$C_6H_4^+$, 14.4%].

*4.1.5.9. 4-[2-(2-Chloro-4-nitrobenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (**6i**).* Yield: 45%; mp: 234–235 °C; IR (cm^{-1}): 3201 (NH), 2924, 2835 (CH-aliphatic), 1531, 1342 (NO_2); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.60 (s, 3H, SCH_3), 7.31–8.17 (m, 9H, Ar–H), 8.50 (s, 1H, N=CH), 12.17 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 12.9 (SCH_3), 101.7 (N=CH), 121.0, 123.2, 124.9, 126.5, 128.9, 131.7, 132.4, 135.9, 138.0, 144.5, 148.0, 148.1, 148.2, 149.9, 150.4 (aromatic carbons); MS *m/z*: 441 [$(M + 2)^+$, 10.0%], 439 [M^+, 28.4%], 283 [$(M - Cl(NO_2)C_6H_3)^+$, 34.0%], 257 [$(M - Cl(NO_2)C_6H_3C{=}N)^+$, 56.0%], 256 [$(M - Cl(NO_2)C_6H_3CH{=}N)^+$, 40.2%], 224 [24.5%], 185 [$(^{37}Cl(NO_2)C_6H_3CH{=}N)^+$, 4.2%], 183 [$(^{35}Cl(NO_2)C_6H_3CH{=}N)^+$, 12.0%], 77 [$C_6H_5^+$, 100%].

*4.1.5.10. 4-[2-(4-Bromobenzylidene)hydrazinyl]-1-(4-methoxyphenyl)-3-(methylsulphanyl)-1H-pyrazolo[3,4-d]pyrimidine (**6j**).* Yield: 52%; mp: 254–255 °C; IR (cm^{-1}): 3394 (NH), 2924, 2835 (CH-aliphatic); ^1H NMR (300 MHz, DMSO-d_6) δ ppm 2.50 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 6.94–8.35 (m, 9H, Ar–H), 8.42 (s, 1H, N=CH), 12.00 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6/CF_3COOD) δ ppm 14.3 (SCH_3), 55.8 (OCH_3), 103.5 (N=CH), 113.4, 115.9, 126.8, 131.8, 132.8, 133.6, 134.0, 145.9, 147.1, 148.8, 153.7, 156.0, 160.2 (aromatic carbons); MS *m/z*: 470 [$(M + 2)^+$, 19.3%], 468 [$(M)^+$, 19.3%], 287 [$(M - BrC_6H_4C{=}N)^+$, 28.1.6%], 286 [$(M - BrC_6H_4CH{=}N)^+$, 33.3%], 183 [$(^{81}BrC_6H_4C{=}N)^+$, 52.6%], 181 [$(^{79}BrC_6H_4C{=}N)^+$, 54.4%], 107 [$CH_3OC_6H_4^+$, 36.8%], 102 [$C_6H_4CN^+$, 100%], 76 [$C_6H_4^+$, 63.2%].

*4.1.5.11. 4-[2-(4-Chlorobenzylidene)hydrazinyl]-1-(4-methoxyphenyl)-3-(methylsulphanyl)-1H-pyrazolo[3,4-d]pyrimidine (**6k**).* Yield: 70%; mp: 238–239 °C; IR (cm^{-1}): 3209 (NH), 2924, 2835 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6/CF_3COOD) δ ppm 2.62 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 7.02 (d, 2H, J = 8.7 Hz, Ar–H), 7.50 (d, 2H, J = 8.8 Hz, Ar–H), 7.65 (d, 2H, J = 8.7 Hz, Ar–H), 7.95 (d, 2H, J = 8.8 Hz, Ar–*H*), 8.20 (s, 1H, Ar–H), 8.35 (s, 1H, N=CH), 11.87 (br s, 1H, NH, D_2O exchangeable); MS *m/z*: 287 [$(M - ClC_6H_4C{=}N)^+$, 17.2%], 286 [$(M - ClC_6H_4CH{=}N)^+$, 34.4%], 102 [$C_6H_4CN^+$, 42.2%], 76 [$C_6H_4^+$, 42.2%], 64 [100%].

*4.1.5.12. 4-[2-(4-Flourobenzylidene)hydrazinyl]-1-(4-methoxyphenyl)-3-(methylsulphanyl)-1H-pyrazolo[3,4-d]pyrimidine (**6l**).* Yield: 91%; mp: 258–259 °C; IR (cm^{-1}): 3209 (NH), 2924, 2850 (CH-aliphatic); ^1H NMR (300 MHz, DMSO-d_6) δ ppm 2.55 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 6.94–8.39 (m, 9H, Ar–H), 8.41 (s, 1H, N=CH), 11.98 (br s, 1H, NH, D_2O exchangeable); MS *m/z*: 408 [M^+, 16.7%], 407 [$(M - 1)^+$, 45.2%], 287 [$(M - FC_6H_4C{=}N)^+$, 16.7%], 286 [$(M - FC_6H_4CH{=}N)^+$, 66.7%], 122 [$(FC_6H_4CHN)^+$, 50.0%], 121 [$(FC_6H_4C{=}N)^+$, 100%], 107 [$(CH_3OC_6H_4)^+$, 47.6%], 95 [$FC_6H_4^+$, 45.2%], 76 [$C_6H_4^+$, 28.6%].

*4.1.5.13. 4-[2-(2-Hydroxybenzylidene)hydrazinyl]-1-(4-methoxyphenyl)-3-(methylsulphanyl)-1H-pyrazolo[3,4-d]pyrimidine (**6m**).* Yield: 48%; mp: 158–159 °C; IR (cm^{-1}): 3410, 3275 (NH/OH), 2924, 2835 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.89 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 6.84–8.17 (m, 9H, Ar–H), 8.50 (s, 1H, N=CH), 10.10 (br s, 1H, OH, D_2O exchangeable), 12.17 (br s, 1H, NH, D_2O exchangeable); MS *m/z*: 406 [M^+, 21.4%], 405 [$(M - 1)^+$, 28.6%], 286 [$(M - HOC_6H_4CH{=}N)^+$, 28.6%], 121 [$(HOC_6H_4CHNH)^+$, 100%], 120 [$(HOC_6H_4CH{=}N)^+$, 71.4%], 119 [$(HOC_6H_4C{=}N)^+$, 75.0%], 93 [$(HOC_6H_4)^+$, 60.7%], 76 [$C_6H_4^+$, 39.3%].

*4.1.5.14. 4-[2-(3-Hydroxybenzylidene)hydrazinyl]-1-(4-methoxyphenyl)-3-(methylsulphanyl)-1H-pyrazolo[3,4-d]pyrimidine (**6n**).* Yield: 69%; mp: 179–180 °C; IR (cm^{-1}): 3271, 3213 (NH/OH), 2931, 2835 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.62 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 6.82–8.17 (m, 9H, Ar–H), 8.50 (s, 1H, N=CH), 9.88 (br s, 1H, OH, D_2O exchangeable), 12.17 (br s, 1H, NH, D_2O exchangeable); MS *m/z*: 120 [$(HOC_6H_4CH{=}N)^+$, 21.2%], 107 [$(CH_3OC_6H_4)^+$, 18.2%], 106 [$(HOC_6H_4CH)^+$, 21.2%], 55 [100%].

*4.1.5.15. 4-[2-(4-Hydroxybenzylidene)hydrazinyl]-1-(4-methoxyphenyl)-3-(methylsulphanyl)-1H-pyrazolo[3,4-d]pyrimidine (**6o**).* Yield: 30%; mp: 113–114 °C; IR (cm^{-1}): 3205, 3170 (NH/OH), 2966, 2835 (CH-aliphatic); ^1H NMR (200 MHz, DMSO-d_6) δ ppm 2.63 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 6.89 (d, 2H, J = 8.7 Hz, Ar–H), 7.40 (d, 2H, J = 9.0 Hz, Ar–H), 7.65 (d, 2H, J = 8.6 Hz, Ar–H), 7.85 (d, 2H, J = 9.0 Hz, Ar–H), 8.17 (s, 1H, Ar–H), 8.50 (s, 1H, N=CH), 10.15 (br s, 1H, OH, D_2O exchangeable), 12.17 (br s, 1H, NH, D_2O exchangeable); MS *m/z*: 404 [$(M - 2)^+$, 12.0%], 287 [$(M - HOC_6H_4C{=}N)^+$, 23.2%], 286 [$(M - HOC_6H_4CH{=}N)^+$, 24.0%], 120 [$(HOC_6H_4CH{=}N)^+$, 41.6%], 119 [$(HOC_6H_4C{=}N)^+$, 65.6%], 107 [$(CH_3OC_6H_4)^+$, 52.8%], 106 [$(HOC_6H_4CH)^+$, 27.2%], 93 [$(HOC_6H_4)^+$, 28.0%], 76 [$C_6H_4^+$, 20.8%], 55 [100%].

*4.1.5.16. 4-[2-(4-Methoxybenzylidene)hydrazinyl]-1-(4-methoxyphenyl)-3-(methylsulphanyl)-1H-pyrazolo[3,4-d]pyrimidine (**6p**).* Yield: 61%; mp: 228–229 °C; IR (cm^{-1}): 3205 (NH), 2927, 2835 (CH-aliphatic); ^1H NMR (300 MHz, DMSO-d_6) δ ppm 2.50 (s, 3H, SCH_3),

3.78 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.83–8.38 (m, 10H, Ar–H + N=CH), 11.89 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR (CDCl3) δ ppm 16.2 (SCH_3), 49.9, 51.2 (OCH_3), 104.3 (N=CH), 116.8, 126.8, 126.8, 126.9, 126.9, 127.5, 128.1, 128.4, 128.8, 128.9, 134.0, 134.8, 144.2 (aromatic carbons); MS *m/z*: 420 [M^+, 5.3%], 419 [$(M-1)^+$, 11.7%], 287 [$(M-CH_3OC_6H_4C{=}N)^+$, 14.6%], 286 [$(M-CH_3OC_6H_4CH{=}N)^+$, 28.7%], 134 [$(CH_3OC_6H_4CH{=}N)^+$, 83.0%], 133 [$(CH_3OC_6H_4C{=}N)^+$, 100%], 120 [$(CH_3OC_6H_4CH)^+$, 25.1%], 107 [$(CH_3OC_6H_4)^+$, 21.1%], 76 [$C_6H_4^+$, 33.3%].

4.2. Biological testing

4.2.1. Materials and methods

The human breast adenocarcinoma cell line (MCF7) was obtained as a gift from NCI, MD, USA.

All chemicals and solvents were purchased from Sigma–Aldrich.

4.2.1.1. Measurement of potential cytotoxicity. The cytotoxic activity of the newly synthesized compounds was measured *in vitro* on human breast adenocarcinoma cell line (MCF7) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan et al. [23].

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the test compounds to allow attachment of the cells to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compound (0, 5, 12.5, 25 and 50 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the test compound for 48 h at 37 °C in atmosphere of 5% CO_2. After 48 h, cells were fixed with trichloroacetic acid, washed with water and stained for 30 min with 0.4% (wt/vol) Sulforhodamine-B stain dissolved with 1% acetic acid. Excess stain was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in ELISA reader. The relation between surviving fraction and compound concentration was plotted and IC_{50} [the concentration required for 50% inhibition of cell viability] was calculated for each compound and results are given in Table 1 and represented graphically in Fig. 1.

Acknowledgments

We are grateful to all members of the Department of Cancer Biology, National Cancer Institute, Cairo, Egypt, for carrying out the cytotoxicity testing. The authors thank Dr. Michael Schnürch, Institute of Applied Synthetic Chemistry, Vienna University of Technology, Vienna, Austria for the help in performing the NMR spectra. Mohammed K. Abd El Hameid would like to thank OeAD, Austria for providing travel grant to support this work.

References

[1] D.E. Thurston, Chemistry and Pharmacology of Anticancer Drugs, 1st ed., (2007). p.1.
[2] A.E. Rashad, A.E. Mahmoud, M.M. Ali, Synthesis and anticancer effects of some novel pyrazolo[3,4-d]pyrimidine derivatives by generating reactive oxygen species in human breast adenocarcinoma cells, Eur. J. Med. Chem. 46 (2011) 1019–1026.
[3] M.M. Ghorab, F.A. Ragab, S.I. Alqasoumi, A.M. Alafeefy, S.A. Aboulmagd, Synthesis of some new pyrazolo[3,4-d]pyrimidine derivatives of expected anticancer and radioprotective activity, Eur. J. Med. Chem. 45 (2010) 171–178.
[4] M.M. El-Enany, M.M. Kamel, O.M. Khalil, H.B. El-Nassan, Synthesis and antitumor activity of novel 6-aryl and 6-alkylpyrazolo[3,4-d]pyrimidin-4-one derivatives, Eur. J. Med. Chem. 45 (2010) 5286–5291.
[5] A. Kumar, I. Ahmad, B.S. Chhikara, R. Tiwari, D. Mandal, K. Parang, Synthesis of 3-phenylpyrazolopyrimidine-1,2,3-triazole conjugates and evaluation of their Src kinase inhibitory and anticancer activities, Bioorg. Med. Chem. Lett. 21 (2011) 1342–1346.
[6] G.S. Hassan, H.H. Kadry, S.M. Abou-Seri, M.M. Ali, A.E. Mahmoud, Synthesis and in vitro cytotoxic activity of novel pyrazolo[3,4-*d*]pyrimidines and related pyrazole hydrazones toward breast adenocarcinoma MCF-7 cell line, Bioorg. Med. Chem. 19 (2011) 6808–6817.
[7] S. Schenone, O. Bruno, F. Bondavalli, A. Ranise, L. Mosti, G. Menozzi, P. Fossa, S. Donnini, A. Santoro, M. Ziche, F. Manetti, M. Botta, Antiproliferative activity of new 1-aryl-4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives toward the human epidermoid carcinoma A431 cell line, Eur. J. Med. Chem. 39 (2004) 939–946.
[8] R. Ducray, P. Ballard, B.C. Barlaam, M.D. Hickinson, J.G. Kettle, D.J. Ogilvieb, C.B. Trigwell, Novel 3-alkoxy-1*H*-pyrazolo[3,4-*d*]pyrimidines as EGFR and erbB2 receptor tyrosine kinase inhibitors, Bioorg. Med. Chem. Lett. 18 (2008) 959–962.
[9] K.J. Curran, J.C. Verheijen, J. Kaplan, D.J. Richard, L. Toral-Barza, I. Hollander, J. Lucas, S. Ayral-Kaloustian, K. Yu, A. Zask, Pyrazolopyrimidines as highly potent and selective, ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR): optimization of the 1-substituent, Bioorg. Med. Chem. Lett. 20 (2010) 1440–1444.
[10] D.C. Kim, Y.R. Lee, B. Yang, K.J. Shin, D.J. Kim, B.Y. Chung, K.H. Yoo, Synthesis and biological evaluations of pyrazolo[3,4-*d*]pyrimidines as cyclin-dependent kinase 2 inhibitors, Eur. J. Med. Chem. 38 (2003) 525–532.
[11] J.A. Markwalder, M.R. Arnone, P.A. Benfield, M. Boisclair, C.R. Burton, C. Chang, S.S. Cox, P.M. Czerniak, C.L. Dean, D. Doleniak, R. Grafstrom, B.A. Harrison, R.F. Kaltenbach, D.A. Nugiel, K.A. Rossi, S.R. Sherk, L.M. Sisk, P. Stouten, G.L. Trainor, P. Worland, S.P. Seitz, Synthesis and biological evaluation of 1-Aryl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one inhibitors of cyclin-dependent kinases, J. Med. Chem. 47 (2004) 5894–5911.
[12] A.J. Peat, J.A. Boucheron, S.H. Dickerson, D. Garrido, W. Mills, J. Peckham, F. Preugschat, T. Smalley, S.L. Schweiker, J.R. Wilson, T.Y. Wang, H.Q. Zhou, S.A. Thomson, Novel pyrazolopyrimidine derivatives as GSK-3 inhibitors, Bioorg. Med. Chem. Lett. 14 (2004) 2121–2125.
[13] A.J. Peat, D. Garrido, J.A. Boucheron, S.L. Schweiker, S.H. Dickerson, J.R. Wilson, T.Y. Wang, S.A. Thomson, Novel GSK-3 inhibitors with improved cellular activity, Bioorg. Med. Chem. Lett. 14 (2004) 2127–2130.
[14] T.L. Smalley, A.J. Peat, J.A. Boucheron, S. Dickerson, D. Garrido, F. Preugschat, S.L. Schweiker, S.A. Thomson, T.Y. Wang, Synthesis and evaluation of novel heterocyclic inhibitors of GSK-3, Bioorg. Med. Chem. Lett. 16 (2006) 2091–2094.
[15] C. Luma, J. Kahl, L. Kessler, J. Kucharski, J. Lundstrm, S. Miller, H. Nakanishi, Y. Pei, K. Pryor, E. Roberts, L. Sebo, R. Sullivan, J. Urban, Z. Wang, 2,5-Diaminopyrimidines and 3,5-disubstituted azapurines as inhibitors of glycogen synthase kinase-3 (GSK-3), Bioorg. Med. Chem. Lett. 18 (2008) 3578–3581.
[16] J. Pandey, R. Pal, A. Dwivedi, K. Hajela, Synthesis of some new diaryl and triaryl hydrazine derivatives as possible estrogen receptor modulators, Arzneim. Forsch., Drug Res. 52 (2002) 39–44.
[17] H. Zhang, J. Drewe, B. Tseng, S. Kasibhatla, S.X. Cai, Discovery and SAR of indole-2-carboxylic acid benzylidenehydrazides as a new series of potent apoptosis inducers using a cell based HTS assay, Bioorg. Med. Chem. 12 (2004) 3649–3655.
[18] N. Demirbas, S. Karaoglu, A. Demirbas, K. Sancak, Synthesis and antimicrobial activities of some new 1-(5-phenylamino-[1,3,4]thiadiazol-2-yl)methyl-5-oxo-[1,2,4]triazole and 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-5-oxo-[1,2,4]triazole derivatives, Eur. J. Med. Chem. 39 (2004) 793–804.
[19] M.T. Cocco, C. Congiu, V. Lilliu, V. Onnis, Synthesis and in vitro antitumoral activity of new hydrazinopyrimidine-5-carbonitrile derivatives, Bioorg. Med. Chem. 14 (2005) 366–372.
[20] S. Rollas, Ş.G. . Küçükgüzel, Biological activities of hydrazone derivatives, Molecules 12 (2007) 1910–1939.
[21] O.A. Fathalla, N.A. Mohamed, E.M. Abbas, Sh. I. Abd-Elmoez, A.M. Soliman, Synthesis and evaluation of some new pyrazolopyrimidine and thiazolidin-4-one derivatives as antimicrobial and anticancer, World J. Chem. 4 (2009) 141–148.
[22] Y. Tominaga, Y. Honkawa, M. Hara, A. Hosomi, Synthesis of pyrazolo[3,4-*d*] pyrimidine derivatives using ketene dithioacetals, J. Heterocycl. Chem. 27 (1990) 775–785.
[23] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric assay for anticancer-drug screening, J. Natl. Cancer Inst. 82 (1990) 1107–1112.