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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, and biological evaluation of certain 1,3-thiazolone derivatives bearing pyrazoline moiety as potential anti-breast cancer agents

Nadia A. Khalil · Eman M. Ahmed · Hala B. El-Nassan

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Abstract A series of 5-arylidene-2-(3,5-diaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)-ones were synthesized and screened for their in vitro antitumor activity against human breast adenocarcinoma cell line (MCF-7). Five of the test compounds exhibited good antitumor activity superior to the reference drug, doxorubicin, with IC_{50} range 1.4–2.3 μM. Among the test compounds, 2-[3,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-5-(2-methoxybenzylidene)-1,3-thiazol-4(5*H*)-one (**3i**) was found to show the most potent anticancer activity.

Keywords Pyrazoline · 1,3-Thiazolone · Antitumor activity · MCF-7

Introduction

Breast cancer remains the most commonly diagnosed cancer among women (Smith *et al.*, 2009). The overall survival rate of breast cancer patients has substantially increased during the last decades due to early tumor detection (Shien *et al.*, 2009; Lewis *et al.*, 2010). However, the low recovery rate of advanced breast cancer by currently available treatment modalities may be attributed to the development of resistance against the existing anticancer drugs (Higa, 2009). Besides, most of chemotherapeutics cause general toxicity to any proliferating cells, which can severely limit the therapeutic value of these drugs (Chari, 2008). Therefore, the identification of novel structures that can be potentially useful in designing new, potent, selective, and less toxic anticancer agents is still a major challenge to medicinal chemistry researchers.

The increasing diversity of small molecule libraries is an important source for the discovery of new drug candidates. In terms of this trend, many pyrazoline derivatives have been reported to possess antitumor and antiproliferative potential (Johnson *et al.*, 2007; Shaharyar *et al.*, 2010; Congiu *et al.*, 2010; Bashir *et al.*, 2011; Lv *et al.*, 2011; Wang *et al.*, 2011; Havrylyuk *et al.*, 2011).

4-Thiazolidinone derivatives represent another class of anticancer drugs that have attracted a special attention of medicinal chemists (Hafez and El-Gazzar, 2009; Havrylyuk *et al.*, 2010; Lv *et al.*, 2010). Their antineoplastic properties may be attributed to their affinity to anticancer biotargets such as JNK-stimulating phosphatase-1 (JSP-1) (Cutshall *et al.*, 2005), tumor necrosis factor TNFa (Carter *et al.*, 2001), anti-apoptotic biocomplex Bcl-XL-BH_3 (Degterev *et al.*, 2001), and integrin avb3 receptor (Dayam *et al.*, 2006).

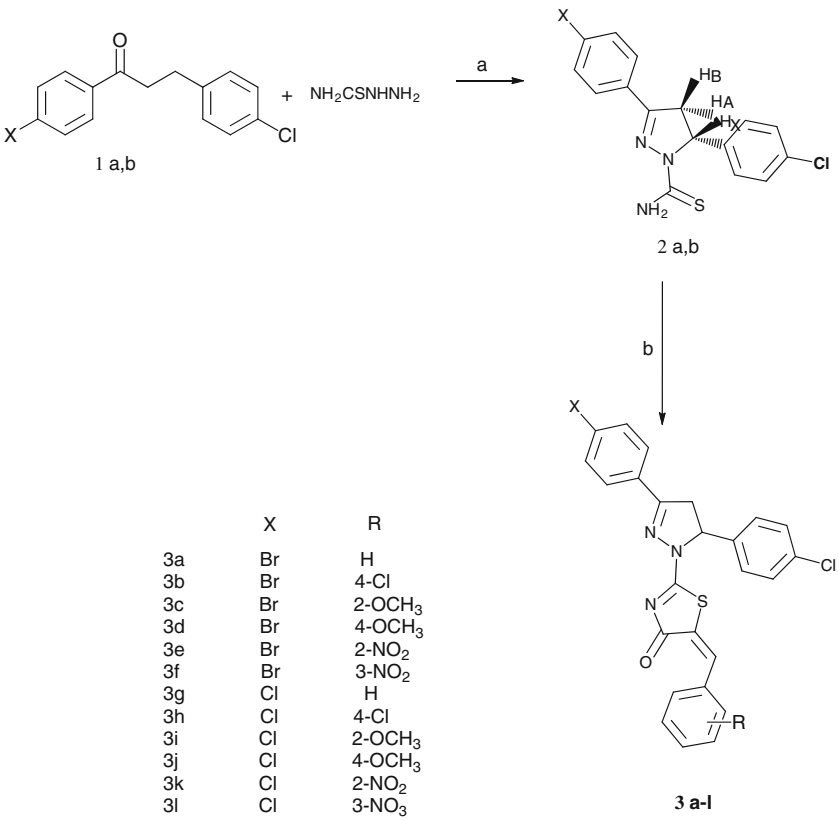
As a part of our ongoing research in discovery of new active anticancer compounds (El-Nassan, 2011), in this study, we try to study the influence of pyrazoline moiety and thiazolone scaffold combination on the anti-breast cancer activity and SAR analysis within these series. The structural variations were explored by placing the pyrazoline moiety in position 2 of 1,3-thiazolone ring and introduction of different arylidene substituents in position 5 of 1,3-thiazolone function.

Results and discussion

Chemistry

The synthesis of the target compounds is outlined in Scheme 1. The chalcone derivatives **1a** and **1b** were

N. A. Khalil · E. M. Ahmed (✉) · H. B. El-Nassan
Pharmaceutical Organic Chemistry Department,
Faculty of Pharmacy, Cairo University,
Cairo 11562, Egypt
e-mail: dr_eman2001@hotmail.com



Scheme 1 Reagents and conditions: **a** NaOH/ethanol/ reflux 8 h, **b** apporpriate aromic aldehyde/$ClCH_2COOH$/glacial acetic acid, reflux 15 h

	X	R
3a	Br	H
3b	Br	4-Cl
3c	Br	2-OCH_3
3d	Br	4-OCH_3
3e	Br	2-NO_2
3f	Br	3-NO_2
3g	Cl	H
3h	Cl	4-Cl
3i	Cl	2-OCH_3
3j	Cl	4-OCH_3
3k	Cl	2-NO_2
3l	Cl	3-NO_3

synthesized via reacting 4-bromo- or 4-chloroacetophenone with 4-chlorobenzaldehyde (Davey and Gwilt, 1953; Straus and Ackermann 1909). Cyclization of **1a** or **1b** with thiosemicarbazide afforded 3,5-diaryl-4,5-dihydropyrazole-1-carbothioamides **2a** and **2b**, respectively (El-Enany *et al.*, 2010; Chimenti *et al.*, 2010). The target compounds **3a–3l** were prepared in 32–74 % yields through the reaction of the pyrazoline derivatives **2a** or **2b** with chloroacetic acid and the respective aromatic aldehyde in glacial acetic acid.

The formation of the target compounds **3a–3l** was confirmed by spectral data. The IR spectra of compounds **3a–3l** revealed the disappearance of NH_2 characteristic bands at 3,300–3,200 cm^{-1} and appearance of C=O band in the range of 1,681–1,716 cm^{-1}. The ^{1}H-NMR spectra showed the characteristic ABX system (Bashir *et al.,* 2011), which is attributed to geminal–vicinal coupling between the two protons at position 4 of pyrazoline ring (H_A and H_B) and the proton at position 5 of the same ring (H_X). The proton H_A appeared as doublet of doublets at δ 3.40–3.57 ppm indicating *trans* configuration to H_X and geminal to H_B (J_{AB} = 18–19 Hz, J_{AX} = 3.9–4.5 Hz). The proton H_B exhibited doublet of doublets at δ 4.01–4.17 ppm indicating *cis* configuration and vicinal to H_X. Besides, the doublet of doublets around δ 5.87–5.92 ppm (J_{BX} = 9.4–13.3 Hz) was assignable to Hx proton. On the other hand, the methine proton of thiazolone ring was observed as a singlet signal in the range of 7.8–8.4 ppm. Mass spectra of the compounds **3a–3l** are in accordance with their structure. All compounds showed molecular ion peaks corresponding to M, M+2, M+4 (and M+6 in the spectra of compounds **3b** and **3h**). The possible fragmentation pattern of these molecular ions is suggested to takes place by two pathways (a) and (b) (Fig. 1). Pathway (a) involves the production of prominent fragments due to loss of 4-bromo- or 4-chlorobenzonitrile [XC_6H_4CN], giving rise to the corresponding aziridine radical cation. On the other hand, fission by pathway (b) results in the formation of *m*/*z* fragments corresponding to [RC_6H_4CHCS]. Compound **3g** was chosen as a representative for characteristic fragmentation pattern, and is presented in Fig. 1.

In vitro anticancer screening

All the synthesized compounds were tested for their possible in vitro cytotoxic activity against human breast carcinoma cell line, MCF-7. Doxorubicin was used as the positive control in this study.

The survival curve which presented the relationship between the percentage cell viability and drug concentration of breast cancer cell line (MCF-7) was plotted for all test compounds. The IC_{50} value, which corresponds to the concentration required for 50 % inhibition of cell viability, was determined for the test compounds and doxorubicin (Table 1; Fig. 2).

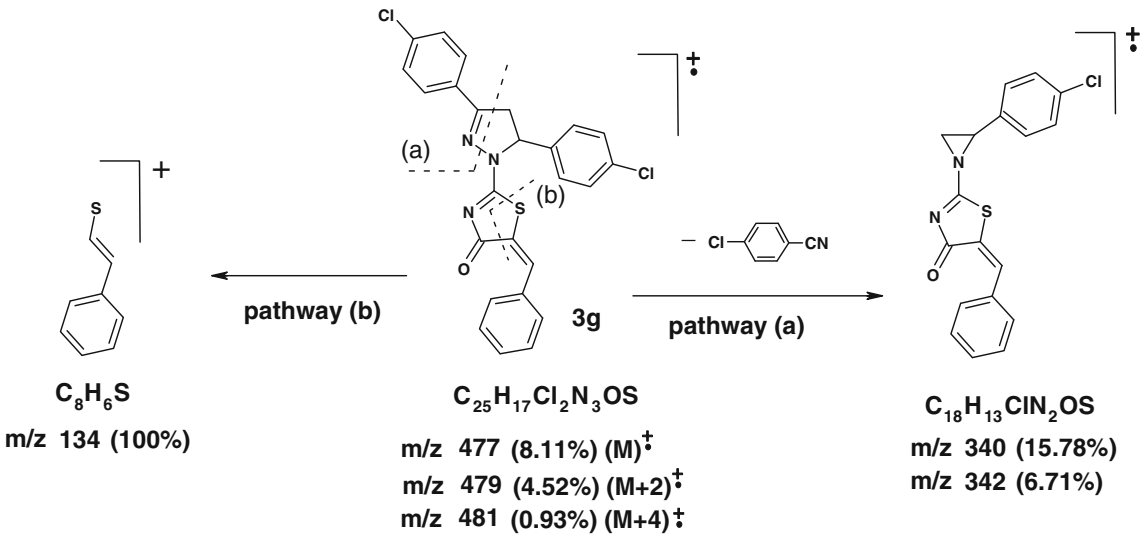


Fig. 1 The possible fragmentation pattern of compound **3g**

Table 1 Results of in vitro cytotoxic activity of the synthesized compounds and doxorubicin on human breast adenocarcinoma cell line (MCF-7)

Compd. no.	IC_{50} (μM)
3a	1.6
3b	2.3
3c	7.3
3d	1.7
3e	1.6
3f	13.6
3g	5.2
3h	17.3
3i	1.4
3j	5.3
3k	46.6
3l	5.3
Doxorubicin	2.9

In general, the test compounds **3a–3f** (X = Br) proved to be biologically more potent than the compounds **3g–3l** (X = Cl). Besides, compounds **3a**, **3b**, **3d**, **3e,** and **3i** exerted good antitumor activity superior to the reference drug, doxorubicin, with IC_{50} range between 1.4 and 2.3 μM. However, compounds **3c**, **3g**, and **3j** showed moderate cytotoxic activity with IC_{50} range between 5.2 and 7.3 μM. On the other hand, compounds **3f**, **3h**, **3k**, and **3l** showed weak cytotoxic activity with $IC_{50} > 13.6$ μM. Compound **3i** (R = 2-CH_3O, X = Cl) displayed the most potent cytotoxic effect among the test compounds with $IC_{50} = 1.4$ μM. In contrast, the lowest activity was observed with the test compound **3k** bearing 2-nitro group on the benzylidene function.

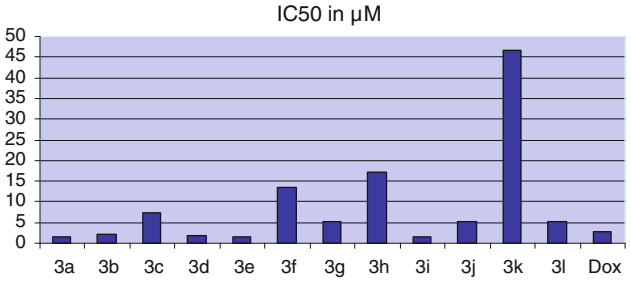


Fig. 2 IC_{50} in μM of the synthesized compounds and doxorubicin against human breast adenocarcinoma cell line (MCF-7)

Conclusion

In summary, 5-arylidene-2-(3,5-diaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)-ones were synthesized and evaluated for their cytotoxic activities against human breast adenocarcinoma cell line (MCF-7). The results revealed that the bromo-substituted derivatives **3a–3f** were generally more potent than their chloro analogs **3g–3l**. Compounds **3a**, **3d**, **3e,** and **3i** displayed excellent cytotoxic activity with IC_{50} ranging from 1.4 to 1.7 μM, superior to the reference drug, doxorubicin. Accordingly, this class of compounds could be considered as useful templates for future development and further derivatization or modification to obtain more potent and selective antitumor agents.

Experimental

Melting points were determined on a Griffin apparatus and were uncorrected. IR spectra were determined as KBr disks on Shimadzu IR 435 spectrophotometer and values were

represented in cm^{-1}. ^{1}H-NMR were carried out on Varian Gemini 300 MHz spectrophotometer, Cairo University, Cairo, Egypt, using TMS as an internal standard and chemical shifts were recorded in ppm on δ scale. Mass spectra were run on Hewlett Packard 5988 spectrometer, Microanalytical center, Cairo University, Cairo, Egypt. Elemental analyses were carried out at the Microanalytical center, Cairo University, Cairo, Egypt, and at the Microanalytical laboratory, National Research Center, Cairo, Egypt. Progress of the reactions was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel Merck 60 F 254 that were visualized using UV lamp.

1,3-Diaryl-2-propen-1-ones **1a** and **1b** (Davey and Gwilt, 1953; Straus and Ackermann 1909), 3-(4-bromophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (**2a**) (El-Enany *et al.*, 2010), and 3,5-bis(4-chlorophenyl)-4,5-dihydropyrazole-1-carbothioamide (**2b**) (Chimenti *et al.*, 2010) were prepared according to the literature procedures.

General procedure for the synthesis of 5-arylidene-2-(3,5-diaryl-4,5-dihydro-1H-pyrazol-1-yl)-1,3-thiazol-4(5H)-ones **3a–3l**

A mixture of the respective **2a** and **2b** (2 mmol), chloroacetic acid (0.19 g, 2 mmol), anhydrous sodium acetate (0.17 g, 2 mmol), and the appropriate aromatic aldehyde (2.4 mmol) in glacial acetic acid (10 mL) was heated under reflux for 15 h. The reaction mixture was cooled and poured gradually into crushed ice. The resulting solid product was filtered and crystallized from a suitable solvent.

5-Benzylidene-2-[3-(4-bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-1,3-thiazol-4(5H)-one (**3a**)

Yield 66 %; mp 288–289 °C (DMF); IR (cm^{-1}): 2951, 2850 (CH-aliphatic), 1712 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.46 (dd, 1H, H_A, J_{AB} = 18.3 Hz, J_{AX} = 3.9 Hz), 4.14 (dd, 1H, H_B, J_{AB} = 18.3 Hz, J_{BX} = 11.4 Hz), 5.90 (dd, 1H, H_x, J_{AX} = 3.9 Hz, J_{BX} = 11.4 Hz), 7.31–7.84 (m, 14H, Ar–H and =CH); MS *m/z* [%]: 525 [$(M+4)^+$, 20.39], 523 [$(M+2)^+$, 42.48], 521 [M^+, 46.72], 342 [M+2-$^{79}BrC_6H_4CN$, 39.42], 340 [M-$^{79}BrC_6H_4CN$, 100]; Anal. Calcd for $C_{25}H_{17}BrClN_3OS$: C, 57.43; H, 3.28; N, 8.04. Found: C, 57.02; H, 3.45; N, 8.41.

2-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(4-chlorobenzylidene)-1,3-thiazol-4(5H)-one **(3b)**

Yield 43 %; mp 250–251 °C (DMF); IR (cm^{-1}): 2974, 2927 (CH-aliphatic), 1685 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.48 (dd, 1H, H_A, J_{AB} = 18.4 Hz, J_{AX} = 4.3 Hz), 4.12 (dd, 1H, H_B, J_{AB} = 18.4 Hz, J_{BX} = 11.2 Hz), 5.91 (dd, 1H, H_x, J_{AX} = 4.3 Hz, J_{BX} = 11.2 Hz), 7.31–7.94 (m, 12H, Ar–H), 8.18 (s, 1H, =CH); MS *m/z* [%]: 561 [$(M+6)^+$, 1.69], 559 [$(M+4)^+$, 7.47], 557 [$(M+2)^+$, 12.82], 555 [M^+, 8.84], 183 [$^{81}BrC_6H_4CN$, 4.83], 181 [$^{79}BrC_6H_4CN$, 4.83], 170 [$^{37}ClC_6H_4CHCS$, 36.46], 168 [$^{35}ClC_6H_4CHCS$, 100]; Anal. Calcd for $C_{25}H_{16}BrCl_2N_3OS$: C, 53.88; H, 2.89; N, 7.54. Found: C, 53.72; H, 2.45; N, 7.41.

2-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(2-methoxybenzylidene)-1,3-thiazol-4(5H)-one **(3c)**

Yield 36 %; mp 290–291 °C (DMF); IR (cm^{-1}): 2931, 2831 (CH-aliphatic), 1681 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.45 (dd, 1H, H_A, J_{AB} = 18.3 Hz, J_{AX} = 4.5 Hz), 3.87 (s, 3H, OCH_3), 4.09 (dd, 1H, H_B, J_{AB} = 18.3 Hz, J_{BX} = 11.4 Hz), 5.87 (dd, 1H, H_x, J_{AX} = 4.5 Hz, J_{BX} = 11.4 Hz), 7.10–7.81 (m, 12H, Ar–H), 7.89 (s, 1H, =CH); MS *m/z* [%]: 555 [$(M+4)^+$, 2.46], 553 [$(M+2)^+$, 7.87], 551 [M^+, 5.41], 524 [M+4-CH_3O, 31.49], 522 [M+2-CH_3O, 100], 520 [M-CH_3O, 73.83], 183 [$^{81}BrC_6H_4CN$, 4.36], 181 [$^{79}BrC_6H_4CN$, 5.08], 164 [$CH_3OC_6H_4CHCS$, 94.43]; Anal. Calcd for $C_{26}H_{19}BrClN_3O_2S$: C, 56.48; H, 3.46; N, 7.60. Found: C, 56.63; H, 3.69; N, 7.29.

2-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(4-methoxybenzylidene)-1,3-thiazol-4(5H)-one **(3d)**

Yield 56 %; mp 246–247 °C (AcOH); IR (cm^{-1}): 2974, 2931 (CH-aliphatic), 1716 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.44 (dd, 1H, H_A, J_{AB} = 18.0 Hz, J_{AX} = 4.0 Hz), 3.80 (s, 3H, OCH_3), 4.01 (dd, 1H, H_B, J_{AB} = 18.0 Hz, J_{BX} = 11.0 Hz), 5.92 (dd, 1H, H_x, J_{AX} = 4.0 Hz, J_{BX} = 11.0 Hz), 7.11–7.80 (m, 12H, Ar–H), 7.90 (s, 1H, =CH); MS *m/z* [%]: 555 [$(M+4)^+$, 23.49], 553 [$(M+2)^+$, 78.68], 551 [M^+, 55.99], 164 [$CH_3OC_6H_4CHCS$, 100]; Anal. Calcd for $C_{26}H_{19}BrClN_3O_2S$: C, 56.48; H, 3.46; N, 7.60. Found: C, 56.12; H, 3.40; N, 7.81.

2-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(2-nitrobenzylidene)-1,3-thiazol-4(5H)-one **(3e)**

Yield 60 %; mp 265–266 °C (DMF); IR (cm^{-1}): 2950, 2855 (CH-aliphatic), 1701 (C=O), 1539, 1334 (NO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.48 (dd, 1H, H_A, J_{AB} = 18.4 Hz, J_{AX} = 4.2 Hz), 4.12 (dd, 1H, H_B, J_{AB} = 18.4 Hz, J_{BX} = 11.8 Hz), 5.90 (dd, 1H, H_x,

J_{AX} = 4.2 Hz, J_{BX} = 11.8 Hz), 7.31–7.84 (m, 12H, Ar–H), 7.94 (s, 1H, =CH); MS *m/z* [%]: 570 [$(M+4)^+$, 2.34], 568 [$(M+2)^+$, 7.59], 566 [M^+, 8.17], 387 [M+2-$^{79}BrC_6H_4CN$, 9.13], 385 [M-$^{79}BrC_6H_4CN$, 44.75], 183 [$^{81}BrC_6H_4CN$, 6.25], 181 [$^{79}BrC_6H_4CN$, 7.56], 179 [$NO_2C_6H_4CHCS$, 40.64], 103 [100]; Anal. Calcd for $C_{25}H_{16}BrClN_4O_3S$: C, 52.88; H, 2.84; N, 9.87. Found: C, 52.62; H, 3.05; N, 9.75.

2-[3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(3-nitrobenzylidene)-1,3-thiazol-4(5H)-one **(3f)**

Yield 74 %; mp 294–295 °C (DMF); IR (cm^{-1}): 2939, 2830 (CH-aliphatic), 1693, (C=O), 1527, 1350 (NO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.49 (dd, 1H, H_A, J_{AB} = 18.6 Hz, J_{AX} = 4.2 Hz), 4.17 (dd, 1H, H_B, J_{AB} = 18.6 Hz, J_{BX} = 9.4 Hz), 5.91 (dd, 1H, H_x, J_{AX} = 4.2 Hz, J_{BX} = 9.4 Hz), 7.32–8.28 (m, 12H, Ar–H), 8.46 (s, 1H, =CH); MS *m/z* [%]: 570 [$(M+4)^+$, 5.98], 568 [$(M+2)^+$, 12.32], 566 [M^+, 7.95], 387 [M+2-$^{79}BrC_6H_4CN$, 24.38], 385 [M-$^{79}BrC_6H_4CN$, 82.01] 183 [$^{81}BrC_6H_4CN$, 17.22], 181 [$^{79}BrC_6H_4CN$, 18.82], 179 [$NO_2C_6H_4CHCS$, 100]; Anal. Calcd for $C_{25}H_{16}BrClN_4O_3S$: C, 52.88; H, 2.84; N, 9.87. Found: C, 52.47; H, 2.55; N, 9.60.

5-Benzylidene-2-[3,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-1,3-thiazol-4(5H)-one **(3g)**

Yield 32 %; mp 257–258 °C (AcOH); IR (cm^{-1}): 2954, 2931 (CH-aliphatic), 1693 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.48 (dd, 1H, H_A, J_{AB} = 18.5 Hz, J_{AX} = 4.0 Hz), 4.12 (dd, 1H, H_B, J_{AB} = 18.5 Hz, J_{BX} = 11.2 Hz), 5.91 (dd, 1H, H_x, J_{AX} = 4.0 Hz, J_{BX} = 11.2 Hz), 7.31–7.67 (m, 13H, Ar–H), 7.90 (s, 1H, =CH); MS *m/z* [%]: 481 [$(M+4)^+$, 0.93], 479 [$(M+2)^+$, 4.52], 477 [M^+, 8.11], 342 [M+2-$^{35}ClC_6H_4CN$, 6.71], 340 [M-$^{35}ClC_6H_4CN$, 15.78], 134 [C_6H_5CHCS, 100]; Anal. Calcd for $C_{25}H_{17}Cl_2N_3OS$: C, 62.77; H, 3.58; N, 8.78. Found: C, 62.56; H, 3.45; N, 8.64.

2-[3,5-Bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(4-chlorobenzylidene)-1,3-thiazol-4(5H)-one **(3h)**

Yield 37 %; mp 289–290 °C (AcOH); IR (cm^{-1}): 2974, 2924 (CH-aliphatic), 1681 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.41 (dd, 1H, H_A, J_{AB} = 18.0 Hz, J_{AX} = 4.0 Hz), 4.10 (dd, 1H, H_B, J_{AB} = 18.0 Hz, J_{BX} = 11.0 Hz), 5.90 (dd, 1H, H_x, J_{AX} = 4.0 Hz, J_{BX} = 11.0 Hz), 7.30–7.88 (m, 12H, Ar–H), 7.91 (s, 1H, =CH); MS *m/z* [%]: 517 [$(M+6)^+$, 1.05], 515 [$(M+4)^+$, 4.08], 513 [$(M+2)^+$, 7.81], 511 [M^+, 10.08], 378 [M+4-$^{35}ClC_6H_4CN$, 2.49], 376 [M+2-$^{35}ClC_6H_4CN$, 14.73], 374 [M-$^{35}ClC_6H_4CN$, 23.30], 170 [$^{37}ClC_6H_4CHCS$, 42.44], 168 [$^{35}ClC_6H_4CHCS$, 100], 139 [$^{37}ClC_6H_4CN$, 3.77], 137 [$^{35}ClC_6H_4CN$, 8.69]; Anal. Calcd for $C_{25}H_{16}Cl_3N_3OS$: C, 58.55; H, 3.14; N, 8.19. Found: C, 58.36; H, 3.49; N, 8.57.

2-[3,5-Bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(2-methoxybenzylidene)-1,3-thiazol-4(5H)-one **(3i)**

Yield 58 %; mp 281–282 °C (AcOH); IR (cm^{-1}): 2974, 2835 (CH-aliphatic), 1685 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.42 (dd, 1H, H_A, J_{AB} = 18.6 Hz, J_{AX} = 4.0 Hz), 3.87 (s, 3H, OCH_3), 4.10 (dd, 1H, H_B, J_{AB} = 18.6 Hz, J_{BX} = 11.0 Hz), 5.90 (dd, 1H, H_x, J_{AX} = 4.0 Hz, J_{BX} = 11.0 Hz), 7.12–7.89 (m, 12H, Ar–H), 7.91 (s, 1H, =CH); MS *m/z* [%]: 511 [$(M+4)^+$, 0.6], 509 [$(M+2)^+$, 3.90], 507 [M^+, 5.02], 480 [M+4-CH_3O, 15.14], 478 [M+2-CH_3O, 70.36], 476 [M-CH_3O, 100], 164 [$CH_3OC_6H_4CHCS$, 60.69]; Anal. Calcd for $C_{26}H_{19}Cl_2N_3O_2S$: C, 61.42; H, 3.77; N, 8.26. Found: C, 61.32; H, 3.76; N, 8.53.

2-[3,5-Bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(4-methoxybenzylidene)-1,3-thiazol-4(5H)-one **(3j)**

Yield 43 %; mp 250–251 °C (AcOH); IR (cm^{-1}): 2974, 2927 (CH-aliphatic), 1701 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.40 (dd, 1H, H_A, J_{AB} = 18.0 Hz, J_{AX} = 4.0 Hz), 3.80 (s, 3H, OCH_3), 4.10 (dd, 1H, H_B, J_{AB} = 18.0 Hz, J_{BX} = 11.0 Hz), 5.90 (dd, 1H, H_x, J_{AX} = 4.0 Hz, J_{BX} = 11.0 Hz), 7.30–7.80 (m, 12H, Ar–H), 7.90 (s, 1H, =CH); MS *m/z* [%]: 511 [$(M+4)^+$, 5.99], 509 [$(M+2)^+$, 3.90], 507 [M^+, 0.6], 164 [$CH_3OC_6H_4CHCS$, 100]; Anal. Calcd for $C_{26}H_{19}Cl_2N_3O_2S$: C, 61.42; H, 3.77; N, 8.26. Found: C, 61.54; H, 3.85; N, 8.18.

2-[3,5-Bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(2-nitrobenzylidene)-1,3-thiazol-4(5H)-one **(3k)**

Yield 53 %; mp 255–256 °C (AcOH); IR (cm^{-1}): 2981, 2927 (CH-aliphatic), 1689 (C=O), 1543, 1338 (NO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.56 (dd, 1H, H_A, J_{AB} = 18.4 Hz, J_{AX} = 4.3 Hz), 4.15 (dd, 1H, H_B, J_{AB} = 18.4 Hz, J_{BX} = 11.2 Hz), 5.92 (dd, 1H, H_x, J_{AX} = 4.3 Hz, J_{BX} = 11.2 Hz), 7.30–8.16 (m, 12H, Ar–H), 8.19 (s, 1H, =CH); MS *m/z* [%]: 524 [$(M+2)^+$, 0.33], 522 [M^+, 0.46], 387 [M+2-$^{35}ClC_6H_4CN$, 8.13], 385 [M-$^{35}ClC_6H_4CN$, 16.87], 179 [$NO_2C_6H_4CHCS$, 35.48], 57 [100]; Anal. Calcd for $C_{25}H_{16}Cl_2N_4O_3S$: C, 57.37; H, 3.08; N, 10.70. Found: C, 57.04; H, 3.17; N, 10.41.

2-[3,5-Bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-(3-nitrobenzylidene)-1,3-thiazol-4(5H)-one **(3l)**

Yield 42 %; mp > 300 °C (DMF); IR (cm^{-1}): 2958, 2935 (CH-aliphatic), 1693 (C=O), 1527, 1350 (NO_2); ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 3.57 (dd, 1H, H_A, $J_{AB} = 18.6$ Hz, $J_{AX} = 4.3$ Hz), 4.14 (dd, 1H, H_B, $J_{AB} = 18.6$ Hz, $J_{BX} = 10.6$ Hz), 5.92 (dd, 1H, H_x, $J_{AX} = 4.3$ Hz, $J_{BX} = 10.6$ Hz), 7.33–8.29 (m, 12H, Ar–H), 8.47 (s,1H, = CH); MS *m/z* [%]: 526 $[(M+4)^+$, 3.08], 524 $[(M+2)^+$, 23.06], 522 $[M^+$, 31.30], 387 [M+2-$^{35}ClC_6H_4CN$, 41.44], 385 [M-$^{35}ClC_6H_4CN$, 82.78], 179 [$NO_2C_6H_4CHCS$, 100]; Anal. Calcd for $C_{25}H_{16}Cl_2N_4O_3S$: C, 57.37; H, 3.08; N, 10.70. Found: C, 57.56; H, 3.04; N, 11.01.

Cytotoxicity assessment against MCF-7 breast adenocarcinoma cell line

Methodology

The cytotoxicity of test compounds was determined against human breast adenocarcinoma cell line (MCF-7) by Sulforhodamine-B stain SRB assay (Skehan *et al.*, 1990). Exponentially growing cells were collected using 0.25 % Trypsin–EDTA and plated in 96-well plates at 1,000–2,000 cells/well. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compound (0.01, 0.1, 1, 10, and 100 μM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Cells were exposed to test compounds for 72 h and subsequently fixed with trichloroacetic acid (10 %) for 1 h at 4 °C. After several washings, cells were exposed to 0.4 % (w/v) SRB stain dissolved with 1 % acetic acid for 10 min in a dark place and subsequently washed with 1 % glacial acetic acid to remove excess stain. After drying overnight, Tris–HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm.

The relation between percentage cell viability and compound concentration was plotted, IC_{50} (the concentration required for 50 % inhibition of cell viability) was calculated for each compound and the results are given in Table 1 and Fig. 2.

Data analysis

The dose–response curve of compounds was analyzed using E_{max} model.

$$\%\ \text{Cell viability} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d^m + [D]^m}\right) + R$$

where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50 % reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce fluorescence to 50 % of that of the control (i.e., $K_d = IC_{50}$ when $R = 0$ and $E_{max} = 100 - R$) (Al-Abd *et al.*, 2008).

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