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Synthesis and Biological Evaluation of some bis-pyrazole derivatives as Anticancer Agents

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Abstract

Cyclocondensation of chalcones **1a-f** with the appropriate hydrazine afforded the corresponding 1,4-Bis(1,3-disubstituted-4,5-dihydro-1H-pyrazol-5-yl)benzene. Mild oxidation of these pyrazolines with bromine water yielded the corresponding pyrazoles derivatives. Biological screening of the prepared compounds revealed weak anticancer activity of these derivatives.

Keywords:Chalcones, Pyrazole, Anticancer activity.

1.Introduction

Cancer has become most challenging problem and leading cause of death worldwide. Geographically, Asia is the continent with highest cancer cases, followed by Europe. [1,2] The World Health Organization reported that worldwide total morbidity and mortality was 6.2 million in 1997, 7.4 million in 2004, and 7.6 million in 2008, [1–3] It means 13% of all deaths were due to cancer and that the global cancer rate could increase by 50% to 15 million new cases by 2030.[3,4] According to the World Health Organization more than 70% of all cancer deaths occur in low- and middle-income countries.[1] Worldwide, the most prominent cancer types in men

are lung, bronchus, prostate, colon, and rectum and in women are lung, bronchus, breast, colon, and rectum.[1,2] Various synthetic and certainly naturally occurring compounds have been

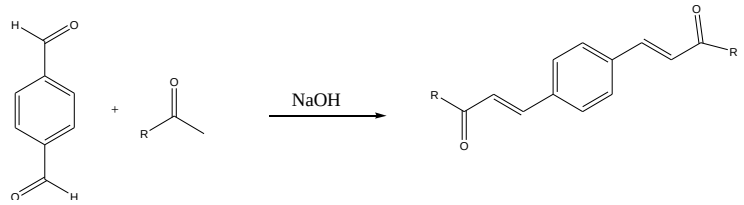
reported for their anticancer activity. The heterocyclic aromatic compound is in almost all anticancer agents. Pyrazole, a five-member, two-nitrogen-containing heterocyclic ring with two

endocyclic double bonds and is basic in nature. Most widely used methods for the synthesis of substituted pyrazoles are based either on the condensations of substituted hydrazines with dicarbonyl or on intermolecular cycloaddition reaction of alkynes 1 to 3-dipoles.[5,6] Pyrazole gives many reactions with electrophilic reagent such as addition at nitrogen,[7] alkylation at nitrogen,[8] acylation at nitrogen,[9–11] substitution at carbon,[12–14] halogination, [15,16] acylation,[17,18] reaction with bases[19] (deprotonation of pyrazole C-hydrogen, deprotonation of pyrazole N-hydrogen), reaction of N-metallated pyrazoles,[20–22] reaction of C-metallated pyrazoles,[23–25] and reaction with radicals.[26] In the past few decades extensive research has been carried out on the pyrazole nucleus and its anticancer activity. [27] Despite tremendous progress in the chemistry of pyrazole, no compound containing pyrazole showed potential to be a strong candidate for cancer treatment.

2. Results and Discussion

2.1*Chemistry*

The starting chalcones **1a-f** were obtained by condensing equimolar amounts of benzene-1,4-dicarbaldehyde with appropriate ketone, in an aqueous ethanolic solution of sodium hydroxide (20%) scheme 1.



1a-f

R= a) 4-BrC_6H_4, b) C_6H_5 CH_2, c) 3, 4-$(CH_3O)_2C_6H_3$,

d) 2-Theinyl, e) 5-Methyl-2-furyl, f) 2-pyridyl

Scheme 1

The IR spectra of the prepared chalcones, **1a-f** showed a carbonyl absorption in the region 1644-1654 cm^{-1} which is the characteristic of the α, β-unsaturated carbonyl group as well as an olefinic C=C bond in the region 1610-1618 cm^{-1}. Their ^1H NMR spectra showed the olefinic protons, H-α and H-β as two doublets (J = 14Hz) at 7.20-7.47 and 7.77-7.95 respectively. The structures of the above chalcones were further confirmed from their ^{13}C NMR data which showed the expected number of aliphatic and aromatic carbons signals**)**.The x-ray crystallography of chalcone **1d** was further support its structure (**Fig. I**).

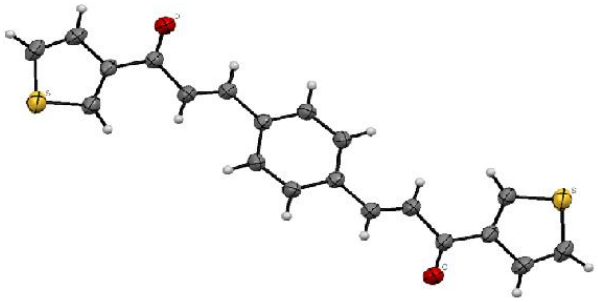
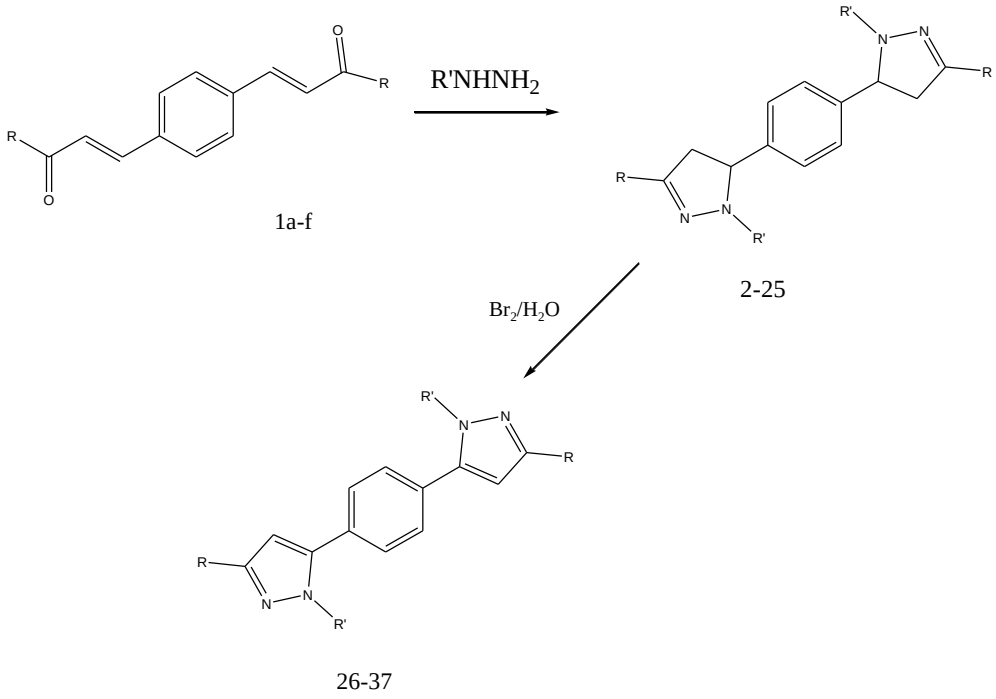


Fig. I. ***(2E,2'E)-1,1'-(1,4-phenylene)bis[3-(thiophen-2-yl)prop-2-en-1-one] 1d***

Cyclocondensation of chalcones **1a-f** with the appropriate hydrazine in boiling ethanol afforded the corresponding 1,4-Bis(1,3-disubstituted-4,5-dihydro-1H-pyrazol-5-yl)benzene **2-25 .** The IR spectra of the pyrazoline derivatives **2-7** revealed NH absorption at 3354-3378 cm^{-1}. In agreement with the suggested structures, the ^1H NMR spectra of the pyrazoline derivatives **2-25** exhibited beside the aromatic protons, three multiples (each of one proton intensity) at 5.38 -5.38 and 3.14-3.18 and 3.87-3.98. The low field multiplet is assigned to H-5 of the pyrazoline while the other two multiples are attributed to H-4. The structures of the above compounds **2-25** were further confirmed from their ^{13}C NMR data which showed the expected number of aromatic carbons signals as well as two other signals at 43.28 -43.78 and 57.30-62.42 for (*C*-4) and (*C-5*) respectively (see experimental section). Mild oxidation of the

appropriate pyrazoline derivatives with bromine water (5%) at room temperature yielded the corresponding pyrazoles **26-37** scheme 2. The IR spectra of these pyrazole derivatives **26-28** revealed weak band at 3374-3389 cm^{-1} due to NH group. Moreover, the ^{1}H NMR spectra of the pyrazole derivatives **26-37** displayed the signals attributed to aromatic protons at but lacked signals characteristic of H-4 and H-5 of the corresponding pyrazolines. The structures of the above compounds **26-37** were further confirmed from their ^{13}C NMR data (see experimental section).



Scheme 2

2.2. *In vitro MTT cytotoxicity assay*

Twenty one analogs namely **2,4,5,6,7,8,10,11,13,14,16,17, 26,27,28,29,30,31,32,33** and **34**were selected to be evaluated for their *in vitro* cytotoxic effect via the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method[28,29] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HePG2 and colon carcinoma HT29. Unfortunately, the results revealed that most of the compounds showed weak anticancer activity. However, in general the pyrazole derivatives were found to be more active than the pyrazoline analogues.

3. Experimental

3.1 *Chemistry*

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *m*: multiplet; *q*: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within

±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

General procedure for the preparation of (2E,2'E)-3,3'-(1,4-phenylene)bis(1-phenylprop-2-en-1-one) (Chalcones) (1a-f)

A solution of benzene-1,4-dicarbaldehyde (1.34g,10 mmol) in ethanol (20 ml) was added to a stirred solution of appropriate ketone (10 mmol) in ethanolic potassium hydroxide (20 ml, 20%) and stirring was maintained for 6-8 h at room temperature. The reaction mixture was then poured onto water (200 ml) and set aside for an overnight. The precipitated solid product was collected by filtration, washed with water, dried and recrystallized from proper.

1a: Rrecrystallized from DMF as needles. (4.7 g , 96 %). m.p.244-246 °C. $\nu_{max.}$ (cm^{-1},KBr): 1644(C=O). ^{1}H NMR (δ/ppm,DMSO-d_6): 7.33(*d*, J=14Hz, 2H, 2*H*-α)7.79 (*d*, J=14Hz, 2H, 2*H*-β), 6.59-7.26&7.39-7.65 (2*m*,12H, Ar H). ^{13}C NMR (δ/ppm, DMSO-d_6): 121.3 (*C*- α), 145.1 (*C*- β), 128.9, 129.2, 130.9, 132.1, 136.9, 134.9 (Ar-*C*), 189.70 (*C*=O). Anal.% Calcd for $C_{24}H_{16}Br_2O_2$: C, 58.09; H, 3.25. Found: C, 58.12; H, 3.36.
1b*:* Rrecrystallized from ethanol as needles.(3.4 g,94%).m.p.196-198°C.$\nu_{max.}$(cm-1,KBr):1649(C=O). ^{1}H NMR (δ/ppm,DMSO-d_6): δ4.20(s,4H $2CH_2$) 7.20(*d*, J=14Hz, 2H, 2*H*-α)7.77 (*d*, J=14Hz, 2H, 2*H*-β), 6.59-7.23&7.39-7.65 (2*m*,14H, Ar H). ^{13}C NMR (δ/ppm, DMSO-d_6): 65.0 ($2CH_2$), 112.6, 116.2, 117.5, 120.9, 130.4 (*C*- α), 142.68, 146.55 (*C*- β), 153.68,163.29,164.96 (Ar-*C*),196.50 (*C*=O). Anal.% Calcd for $C_{26}H_{22}O_2$: C, 85.22; H, 6.05. Found: C, 85.42; H, 6.12.
1c: Rrecrystallized from ethanol/DMF as needles.(4.2g,92%).m.p.210-12°C.$\nu_{max.}$(cm^{-1},KBr):1651(C=O). ^{1}H NMR (δ/ppm,DMSO-d_6): δ2.44(s,6H $2CH_3$) 7.31(*d*, J=14Hz, 2H, 2*H*-α)7.93 (*d*, J=14Hz, 2H, 2*H*-β), 7.09-7.26&7.45-7.78 (2*m*,8H, Ar H). ^{13}C NMR (δ/ppm, DMSO-d_6): 21.71($2CH_3$), 116.19, 121.79, 128.65, 129.38 (*C*- α), 130.34, 135.56,143.09 (*C*- β), 143.76,163.18,164.85 (Ar-*C*),189.80 (*C*=O). Anal.% Calcd for $C_{28}H_{26}O_6$:C, 73.35; H, 5.72. Found: C, 73.44; H, 5.83.
1d: Rrecrystallized from ethanol as needles.(3.1g,88%).m.p.202-204°C.$\nu_{max.}$(cm^{-1}, KBr):1654(C=O). ^{1}H NMR (δ/ppm,DMSO-d_6): 7.33(*d*, J=14Hz, 2H, 2*H*-α)7.82 (*d*, J=14Hz, 2H, 2*H*-β), 6.59-7.26&7.39-7.65 (2*m*,10H, Ar H). ^{13}C NMR (δ/ppm, DMSO-d_6): 112.62, 116.22, 117.54,120.90, 130.49 (*C*- α), 142.68, 146.55 (*C*- β), 153.68, 163.29,164.96 (Ar-*C*),177.86 (*C*=O). Anal.% Calcd for $C_{20}H_{14}O_2S_2$: C, 68.54; H, 4.03. Found: C, 68.65; H, 4.12.
1e : Rrecrystallized from ethanol as needles. (2.9g, 85%).m.p.247-249°C.$\nu_{max.}$(cm^{-1}, Br): 1650(C=O).^{1}H NMR (δ/ppm,DMSO-d_6): δ2.44(s,6H $2CH_3$) 7.31(*d*, J=14Hz, 2H, 2*H*-α)7.93 (*d*, J=14Hz, 2H, 2*H*-β), 7.09-7.26&7.45-7.78 (2*m*,8H, Ar H). ^{13}C NMR (δ/ppm, DMSO-d_6): 21.71($2CH_3$), 116.19, 121.79, 128.65, 129.38 (*C*- α), 130.34, 135.56,143.09 (*C*- β), 143.76,163.18,164.85 (Ar-*C*),189.80 (*C*=O). Anal.% Calcd for $C_{22}H_{18}O_4$:C, 76.29; H, 5.24. Found: C, 76.19; H, 5.28.
1f : Rrecrystallized from ethanol as needles. (3.1g, 90%).m.p.170-172°C.$\nu_{max.}$(cm^{-1},KBr): 1650(C=O). ^{1}H NMR (δ/ppm,DMSO-d_6): 6.24(*d*, J=14Hz, 2H, 2*H*-α)7.77(*d*, J=14Hz, 2H, 2*H*-β), 7.33-7.90&7.97-8.85 (2*m*,12H, Ar H). ^{13}C NMR (δ/ppm, DMSO-d_6): 121.30 (*C*- α), 145.1 (*C*- β), 120.7, 129.0, 137.3, 149.7, 153.30 (Ar-*C*),187.00 (*C*=O). Anal.% Calcd for $C_{22}H_{16}N_2O_2$:C, 77.63; H, 4.74 ; N, 8. 23. Found: C, 77.74; H, 4.82 N, 8.32.

1,4-Bis(1,3-disubstituted-4,5-dihydro-1H-pyrazol-5-yl)benzene (2-31)

A solution of the appropriate chalcone (0.0 2 mol) in ethanol (25mL) was refluxed with the corresponding hydrazine (4.9 g, 0.022 mol) for 3 h. The reaction mixture was concentrated, and the separated product was filtered, washed with cold ethanol and recrystallized from the appropriate solvent.

2:Rrecrystallized from DMF as needles. (2.6g, 70%) . m.p. up300 °C.$\nu_{max.}$(cm^{-1} ,KBr): 3292 (NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 3.69,3.94 (2dd, 4H, pyrazoline 2*H-4*), 3.90(m, 2H, pyrazoline 2*H-5*),7.24-7.72(*m*,14H, Ar-H& NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 42.60 (*C*-4), 51.10 (*C- 5*),125.40,128.60,131.70,135.40,141.50, 151.70 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{24}H_{20}Br_2N_4$: C, 54.98; H, 3.85; N, 10.69. Found: C, 55.20; H,3.92; N, 10.73.

3:Rrecrystallized from DMF as needles. (2.2g, 62%) . m.p. up300 °C.$\nu_{max.}$(cm^{-1} ,KBr): 3285 (NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 2.66(s,4H 2CH_2) 2.90,3.15 (2dd, 4H, pyrazoline 2*H-4*), 3.90(m, 2H, pyrazoline 2*H-5*),7.23-7.33(*m*,16H, Ar-H& NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 42.60 (*C*-4), 51.10 (*C*-5), 35.7 (CH_2), 125.2, 125.7, 128.6, 137.4, 141.5, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{26}H_{26}N_4$: C, 79.16; H, 6.64; N, 14.20. Found: C, 79.20; H,6.72; N, 14.31.

4:Rrecrystallized from DMF as needles. (4.6g, 32%) . m.p. up300 °C.$\nu_{max.}$(cm^{-1} ,KBr): 3284 (NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 3.81(s,6H, 2OCH_3), 3.82(s,6H, 2OCH_3), 3.69,3.94 (2dd, 4H, pyrazoline 2*H-4*), 3.90(m, 2H, pyrazoline 2*H-5*),6.97-7.47(*m*,12H, Ar-H& NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 42.60 (*C*-4), 51.10 (*C*-5), 56.10 (OCH_3), 56.12 (OCH_3), 111.9, 114.3, 125.2, 127.3, 141.50, 151.7, 152.1 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{28}H_{30}N_4O_4$: C, 69.12; H, 6.21; N, 11.51. Found: C, 69.20; H,6.32; N, 11.62.

5:Rrecrystallized from DMF as needles. (3.6g, 42%) . m.p. 164-167 °C.$\nu_{max.}$(cm^{-1} ,KBr): 3286 (NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 3.69,3.94 (2dd, 4H, pyrazoline 2*H-4*), 3.90(m, 2H, pyrazoline 2*H-5*),7.17-7.96(*m*,12H, Ar-H& NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 43.30 (*C*-4), 51.10 (*C- 5*),124.40,125.20,125.80,127.40,141.50, 155.60 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{20}H_{18}N_4S_4$: C, 63.46; H, 4.79; N, 14.80. Found: C, 63.57; H,4.88; N, 14.93.

6:Rrecrystallized from DMF as needles. (2.0g, 40%) . m.p. up300 °C.$\nu_{max.}$(cm^{-1} ,KBr): 3285 (NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 2.30(s,6H 2CH_3) 3.69,3.90 (2dd, 4H, pyrazoline 2*H-4*), 3.90(m, 2H, pyrazoline 2*H-5*),6.08-7.24(*m*,10H, Ar-H& NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.80 (*C*-4), 48.70 (*C*-5), 13.4 (CH_3), 106.7, 110.1, 125.2, 140.3, 141.5, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{22}H_{22}N_4O_2$: C, 70.57; H, 5.92; N, 14.96. Found: C, 70.68; H,6.12; N, 15.11.

7:Rrecrystallized from DMF as needles. (2.3g, 38%) . m.p. up300 °C.$\nu_{max.}$(cm^{-1} ,KBr): 3279(NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 3.69,3.94 (2dd, 4H, pyrazoline 2*H-4*), 3.90(m, 2H, pyrazoline 2*H-5*),7.24-8.65(*m*,14H, Ar-H& NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 42.60 (*C*-4), 51.10 (*C- 5*),122.20,125.20,126.20,136.10,141.50, 149.10, 154.80, 155.60 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{22}H_{20}N_6$: C, 71.72; H, 5.47; N, 22.81. Found: C, 71.82; H,5.57; N, 22.93.

8:Rrecrystallized from DMF as needles. (2.1g, 40%) . m.p.253-255°C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.77-7.72(*m*,22H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C- 5*),116.7, 120.8, 125.2, 125.4, 128.6, 129.5, 131.4, 135.4, 141.5, 143.8, 151.7 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{36}H_{28}Br_2N_4$: C, 63.92; H, 4.17; N, 8.28. Found: C, 64.20; H, 4.28; N, 8.33.

9:Rrecrystallized from DMF as needles. (2.6g, 42%) . m.p. up300 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 2.66(s,4H 2CH_2) 2.88,3.13 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.77-7.33(*m*,24H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 37.5 (*C*-4), 60.8 (*C*-5), 36.0 (CH_2), 116.7, 120.8, 125.2, 125.7, 128.6, 129.0, 137.4, 141.5, 143.8, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{38}H_{34}N_4$: C, 83.48; H, 6.27; N, 10.25. Found: C, 83.57; H,6.42; N, 10.31.

10:Rrecrystallized from DMF as needles. (2.8g, 45%) . m.p. 216-218 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.81(s,6H 2OCH_3), 3.83(s,6H 2OCH_3), 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.97-7.47(*m*,20H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C*-5), 56.10 (OCH_3), 56.14 (OCH_3), 111.9, 114.3, 116.7, 120.8, 121.0, 125.2, 127.3, 129.5, 149.9, 151.7, 152.1 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{40}H_{38}N_4O_4$: C, 75.21; H, 6.00; N, 10.02. Found: C, 75.40; H,6.32; N, 10.12.

11:Rrecrystallized from DMF as needles. (2.8g, 40%) . m.p. 164-167 °C. ^{1}H NMR (δ/ppm, DMSO-d_6): 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.77-7.96 (*m*, 20H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.7 (*C*-4), 60.4 (*C- 5*),116.7, 120.8, 124.4, 125.2, 125.8, 127.2, 127.4, 129.5, 141.50, 143.8, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{32}H_{26}N_4S_2$: C, 72.42; H, 4.94; N, 10.56. Found: C, 72.57; H,5.12; N, 10.63.

12:Rrecrystallized from DMF as needles. (2.2g, 50%) . m.p.253-255 °C. ^{1}H NMR (δ/ppm, DMSO-d_6): 2.30(s,6H 2CH_3) 3.69,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.08-7.24(*m*,18H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 38.2 (*C*-4), 58.0 (*C*-5), 13.4 (CH_3), 106.7, 110.1, 116.7, 120.8, 129.5, 125.2, 140.3, 141.5, 143.8, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{34}H_{30}N_4O_2$: C, 77.54; H, 5.74; N, 10.64. Found: C, 77.68; H,5.82; N, 10.71.

13:Rrecrystallized from DMF as needles. 1.8g, 55%) . m.p. 211-215 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.15(m, 2H, pyrazoline 2*H-5*),6.83-8.65(*m*,22H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C- 5*), 116.7, 120.8, 122.2, 125.2, 126.2, 129.5, 136.1, 141.5, 143.8, 149.1, 154.8, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{34}H_{28}N_6$: C, 78.44; H, 5.42; N, 16.14. Found: C, 78.62; H,5.57; N, 16.24.

14:Rrecrystallized from DMF as needles. (2.3g, 40%) . m.p.214-215 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 2.34(s,6H 2CH_3) 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.48-7.72(*m*,20H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C*-5), 21.3 (CH_3), 113.4, 125.2, 125.4, 128.6, 129.6, 129.8, 131.7, 135.4, 141.5, 151.7 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{38}H_{32}Br_2N_4$: C, 64.78; H, 4.58; N, 7.95. Found: C, 64.88; H,4.82; N, 8.11.

15:Rrecrystallized from DMF as needles. (2.4g, 60%) . m.p.up 300 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 2.34(s,6H 2CH_3), 2.66(s,4H 2CH_2), 2.88,3.13 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.48-7.33(*m*,22H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 37.5 (*C*-4), 60.8 (*C*-5), 21.3 (CH_3), 36.0 (CH_2), 113.4, 125.2, 125.7, 128.6, 129.0, 129.6, 129.8, 137.4, 140.8, 141.5, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{40}H_{38}N_4$: C, 83.59; H, 6.66; N, 9.75. Found: C, 83.68; H,6.82; N, 9.81.

16:Rrecrystallized from DMF as needles. (1.6g, 57%) . m.p.215-217 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 2.34(s,6H, 2CH_3) , 3.80(s,6H 2OCH_3), 3.83(s,6H 2OCH_3), 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.48-7.47(*m*,18H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C*-5), 21.3(CH_3), 56.10 (OCH_3), 56.11 (OCH_3), 111.9, 113.4, 114.3, 121.0, 125.2, 127.3, 129.6, 129.8, 140.8, 141.5, 151.7, 152.1 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{42}H_{42}N_4O_4$: C, 75.65; H, 6.35; N, 8.40. Found: C, 75.78; H,6.42; N, 8.51.

17:Rrecrystallized from DMF as needles. (3.1g, 55%) . m.p.236-238 °C. ^{1}H NMR (δ/ppm, DMSO-d_6): 2.34(s,6H 2CH_3), 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 3.90(m, 2H, pyrazoline 2*H-5*),6.48-7.49(*m*,18H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.7 (*C*-4), 60.4 (*C*-5), 21.3 (CH_3), 113.4, 124.4, 125.2, 125.8, 129.6, 129.8, 140.8, 141.5, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{34}H_{30}N_4S_2$: C, 73.08; H, 5.41; N, 10.03. Found: C, 73.18; H,5.52; N, 10.11.

18:Rrecrystallized from DMF as needles. (2.6g, 45%) . m.p.up 300 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 2.30, (s,6H, 2CH_3), 2.34 (s,6H 2CH_3), 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.08-7.24(*m*,16H, Ar-H& NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 38.2 (*C*-4), 58.0 (*C*-5), 13.41(CH_3), 21.33 (CH_3), 106.7, 110.1, 113.4, 125.2, 129.6, 129.8, 140.3, 140.8, 141.5, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{36}H_{34}N_4O_2$: C, 77.95; H, 6.18; N, 10.10. Found: C, 78.22; H,6.32; N, 10.21.

19:Rrecrystallized from DMF as needles. (2.1g, 50%) . m.p.up 300 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 2.34 (s,6H 2CH_3), 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.48-8.65(*m*,20H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C*-5), 21.3 (CH_3), 113.4, 122.2, 125.2, 126.2, 129.6, 129.8,

136.1, 140.8, 141.5, 149.1, 154.8, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{36}H_{32}N_6$: C, 78.80; H, 5.88; N, 15.32. Found: C, 78.92; H,5.92; N, 15.41.

20:Rrecrystallized from DMF as needles. (2.6g, 45%) . m.p.193-195 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),7.17-8.04(*m*,20H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C*-5), 113.4, 124.7, 125.2, 125.4, 128.6, 131.7, 135.4, 136.3, 141.5, 149.9, 151.7 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{36}H_{26}Br_2N_6O_4$: C, 56.41; H, 3.42; N, 10.97. Found: C, 56.58; H,3.52; N, 11.11.

21:Rrecrystallized from DMF as needles. (3.2g, 55%) . m.p.215-218 °C. ^{1}H NMR (δ/ppm, DMSO-d_6): 2.66(s,4H 2CH_2), 2.88,3.13 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),7.17-8.04(*m*,22H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 37.5 (*C*-4), 60.8 (*C*-5), 36.0 (CH_2), 113.4, 124.7 125.2, 125.7, 128.6, 129.0, 136.3, 137.4, 141.5, 149.9, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{38}H_{32}N_6O_4$: C, 71.68; H, 5.07; N, 13.20. Found: C, 71.88; H,5.12; N, 13.31.

22:Rrecrystallized from DMF as needles. (3.6g, 60%) . m.p.199-200 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.82(s,6H, 2OCH_3), 3.83(s,6H, 2OCH_3), 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.95-8.04(*m*,18H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C*-5), 56.1 (CH_3), 111.9, 113.4, 114.3, 121.0, 124.7, 125.2, 127.3, 129.6, 136.3, 141.5, 149.9, 151.7, 152.1 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{40}H_{36}N_6O_8$: C, 65.93; H, 4.98; N, 11.53. Found: C, 66.18; H,5.422; N, 11.61.

23:Rrecrystallized from DMF as needles. (2.6g, 55%) . m.p.up300 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*), 7.17-8.04(*m*,18H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.7 (*C*-4), 60.4 (*C*-5), 113.4, 124.4, 124.7, 125.2, 125.8, 127.4, 136.3, 141.5, 149.9, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{32}H_{24}N_6O_4S_2$: C, 61.92; H, 3.90; N, 13.54. Found: C, 62.18; H,4.22; N, 13.61.

24:Rrecrystallized from DMF as needles. (2.9g, 77%) . m.p.177-181 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 2.30, (s,6H 2CH_3) 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),6.08-8.04(*m*,16H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 38.2 (*C*-4), 58.0 (*C*-5), 13.4, (CH_3), 106.7, 110.1, 111.3, 124.7, 125.2, 136.3, 140.3, 141.5, 149.9, 155.6 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{34}H_{28}N_6O_6$: C, 66.23; H, 4.58; N, 13.63. Found: C, 66.32; H,4.62; N, 13.71.

25:Rrecrystallized from DMF as needles. (1.6g, 40%) . m.p.up 300 °C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.65,3.90 (2dd, 4H, pyrazoline 2*H-4*), 5.19(m, 2H, pyrazoline 2*H-5*),7.17-8.65(*m*,20H, Ar-H). ^{13}C NMR (δ/ppm, DMSO-d_6): 40.0 (*C*-4), 60.4 (*C*-5), 113.4, 122.2, 124.7,125.2, 136.3, 141.5, 149.1, 149.9, 155.6, 155.8 (Ar-&Pyrazole-*C*). Anal.% Calcd for $C_{34}H_{26}N_8O_4$: C, 66.88; H, 4.29; N, 18.35. Found: C, 66.95; H,4.32; N, 158.41.

1,4-Bis(1,3-disubstituted-4,5-dihydro-1H-pyrazol-5-yl)benzene (26-37)

To a stirred suspension of the appropriate pyrazoline derivative (0.01 mol) in water (10 mL), bromine water (5%, 15 mL) was gradually added over a period of 30 min. at 25^{0}C. After stirring for 3 h at room temperature, the pyrazole derivatives thus formed, were collected by filtration, thoroughly washed with water and dried. They were recrystallized from proper solvent.

26 : Rrecrystallized from ethanol as needles. (5.5g, 76%). m.p.230-232°C. ν_{max} (cm^{-1} ,KBr): 3277 (NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 6.81-12.62 (m,16H, Ar-H+ pyrazole H-4+ NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 99.7, 123.1, 128.0, 128.3, 132.0,132.1, 133.0,147.7 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{24}H_{16}Br_2N_4$: C, 55.41; H, 3.10; N, 10.77. Found: C, 55.53; H, 3.21; N, 10.88.

27 : Rrecrystallized from ethanol as needles. (5.7g, 78%). m.p.241-242°C. ν_{max} (cm^{-1} ,KBr): 3277 (NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 3.81(s,6H, 2OCH_3), 3.83(s,6H, 2OCH_3), 6.81-12.62 (m,14H, Ar-H+ pyrazole H-4+

NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 56.09(OCH_3), 56.11(OCH_3), 99.7, 111.0, 120.8, 128.0, 128.8, 133.0, 147.7, 149.8, 150.0 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{28}H_{26}N_4O_4$: C, 69.70; H, 5.43; N, 11.61. Found: C, 69.82; H, 5.56; N, 11.72.

28 : Rrecrystallized from ethanol as needles. (5.2g, 73%). m.p.258-260°C. ν_{max} (cm^{-1} ,KBr): 3277 (NH). ^{1}H NMR (δ/ppm,DMSO-d_6): 6.76-12.62 (m,14H, Ar-H+ pyrazole H-4+ NH). ^{13}C NMR (δ/ppm, DMSO-d_6): 100.7, 128.0, 128.6, 131.9, 137.7, 139.9, 131.0, 146.6, 145.4 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{20}H_{14}N_4S_2$: C, 64.15; H, 3.77; N, 14.96. Found: C, 64.22; H, 3.82; N, 14.99.

29 : Rrecrystallized from ethanol as needles. (5.1g, 76%). m.p.257-259°C. ^{1}H NMR (δ/ppm,DMSO-d_6): 6.77-7.94 (m,24H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 116.10, 116.24, 121.35, 128.29, 130.39, 130.44, 130.98, 131.00, 131.82, 133.98, 142.78, 145.45, 163.28, 164.95(Ar-&Pyrazole-C). Anal.% Calcd for $C_{36}H_{24}$ Br_2N_4: C, 64.30; H, 3.60; N, 8.33. Found: C, 64.23; H, 3.49; N, 8.28.

30 : Rrecrystallized from ethanol as needles. (5.2g, 72%). m.p.248-250°C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.80(s,6H, $2OCH_3$), 3.81(s,6H, $2OCH_3$),6.77-7.94 (m,22H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 56.10(OCH_3), 56.11(OCH_3), 56.10, 106.20, 108.40, 111.00, 120.80, 124.50, 126.20, 128.00, 128.80, 129.30, 133.00, 139.70, 144.30, 149.80, 150.30, 151.10 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{40}H_{34}N_4O_4$: C, 75.69; H, 5.40; N, 8.83. Found: C, 75.72; H, 5.52; N, 8.93.

31 : Rrecrystallized from ethanol as needles. (5.5g, 77%). m.p.240-242°C. ^{1}H NMR (δ/ppm,DMSO-d_6): 7.05-8.30 (m,22H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 107.2, 124.5, 126.2, 128.6, 129.3, 131.9, 133.0, 137.2, 139.7, 139.9, 143.2, 145.4 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{32}H_{22}$ N_4S_2: C, 72.98; H, 4.21; N, 10.64. Found: C, 72.23; H, 4.32; N, 10.73.

32: Rrecrystallized from ethanol as needles. (5.6g, 73%). m.p.236-238°C. 1H NMR (δ/ppm,DMSO-d_6): 2.34(s,6H $2CH_3$), 7.02-8.30 (m,22H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 21.3 (CH_3), 107.20, 124.50, 126.26, 128.60, 129.30, 131.90, 133.00, 137.20, 139.72, 139.90, 143.20, 145.45 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{38}H_{28}$ Br_2N_4: C, 65.16; H, 4.03; N, 8.00. Found: C, 65.22; H, 4.21; N, 8.21.

33 : Rrecrystallized from ethanol as needles. (5.9g, 78%). m.p.250-251°C. 1H NMR (δ/ppm,DMSO-d_6):): 2.34(s,6H $2CH_3$), 3.81(s,6H, $2OCH_3$), 3.82(s,6H, $2OCH_3$),7.02-8.30 (m,20H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 21.3(CH_3), 56.09(OCH_3), 56.10(OCH_3), 106.2, 108.4, 111.0, 120.8, 125.1, 128.3, 128.3, 129.6, 133.0, 135.9, 136.7, 144.3, 149.8, 150.3, 151.1 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{42}H_{38}N_4O_4$: C, 76.11; H, 5.78; N, 8.45. Found: C, 76.22; H, 5.82; N, 8.53.

34 : Rrecrystallized from ethanol as needles. (5.5g, 76%). m.p.238-240°C. 1H NMR (δ/ppm,DMSO-d_6): 2.34(s,6H $2CH_3$), 7.05-8.30 (m,20H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 21.3 (CH_3), 107.2, 125.1, 128.0, 128.6, 129.6, 131.9, 133.0, 135.9, 136.7, 137.2, 139.9, 143.2, (Ar-&Pyrazole-C). Anal.% Calcd for $C_{34}H_{26}$ N_4S_2: C, 73.62; H, 4.72; N, 10.10. Found: C, 73.82; H, 4.89; N, 10.28.

35 : Rrecrystallized from ethanol as needles. (5.2g, 74%). m.p.235-237°C. ^{1}H NMR (δ/ppm,DMSO-d_6): 7.02-8.43 (m,22H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 106.2, 121.1, 123.1, 124.5, 128.0, 128.3, 132.0, 132.1, 133.0, 144.3, 145.4, 145.8, 151.1 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{36}H_{22}$ Br_2N_6 O_4: C, 56.71; H, 2.91; N, 11.02. Found: C, 64.23; H, 2.99; N, 11.12.

36 : Rrecrystallized from ethanol as needles. (5.4, 74%). m.p.248-249°C. ^{1}H NMR (δ/ppm,DMSO-d_6): 3.79(s,6H, $2OCH_3$), 3.80(s,6H, $2OCH_3$),6.94-8.43 (m,20H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 56.11 (OCH_3), 56.12(OCH_3), 106.2, 108.4, 111.0, 120.8, 121.1, 124.5, 128.0, 128.3, 133.0, 144.3,

145.4, 149.8, 150.3, 151.1 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{40}H_{32}N_6O_8$: C, 66.29; H, 4.45; N, 11.60. Found: C, 66.32; H, 4.54; N, 11.72.

37 : Rrecrystallized from ethanol/DMF as needles. (5.2g, 73%). m.p.256-258°C. ^{1}H NMR (δ/ppm,DMSO-d_6): 7.705-8.43 (m,20H, Ar-H+ pyrazole H-4). ^{13}C NMR (δ/ppm, DMSO-d_6): 107.2, 121.1, 124.5, 128.0, 128.6, 131.9, 137.3, 139.9, 143.2, 145.4, 145.8 (Ar-&Pyrazole-C). Anal.% Calcd for $C_{32}H_{20}N_6O_4S_2$: C, 62.33; H, 3.27; N, 13.63. Found: C, 62.43; H, 3.32; N, 13.78.

3.2 *Biological activity*

Methodology of the In vitro MTT cytotoxicity assay

The synthesized compounds were investigated for their *in vitro* cytotoxic effect *via* the standard [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method (MTT)[28,29]against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2 and colon carcinoma HT29. The procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of (100 – 50 – 25 – 12.5 – 6.25 – 3.125 – 1.56 – 0.78 µg/mL). DMSO was employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HepG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL penicillin potassium, 10,000 µg/mL streptomycin sulphate and 25 µg/mL amphotericin B), and 1% L-glutamine in 96-well flat bottom microplate at 37°C under 5% CO_2. After 24h of incubation, the medium was aspirated, 40 µL of MTT salt (2.5 µg/mL) were added to each well and incubated for further 4h at 37°C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 µL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. The results are presented in Tables 1as LC_{50} (µg/mL) which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

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