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Original article

Anti-breast cancer activity of some novel 1,2-dihydropyridine, thiophene and thiazole derivatives

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ABSTRACT

A variety of novel 1,2-dihydropyridines **10**–**17**, thiophenes **18**–**21** and thiazole **22** having a biologically active sulfone moiety were obtained *via* the reaction of 2-cyano-N′-[1-(4-(piperidin-1-ylsulfonyl) phenyl) ethylidene] acetohydrazide **3** with a variety of reagents. The structures of the newly synthesized compounds were confirmed by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the newly synthesized compounds were evaluated for their *in-vitro* anticancer activity against human breast cancer cell line (MCF7). Compounds **15** and **11** with IC_{50} values (20.6, 25.5 μM) exhibited better activity than Doxorubicin as a reference drug with IC_{50} value (32.02 μM), while compound **14** is nearly as active as Doxorubicin as positive control.

1. Introduction

Pyridine derivatives are currently an important group of organic compounds that are used as bactericides [1], fungicides [2], anticancer agents [3–6]. In addition thiophene and thiazole derivatives are known to possess interesting biological properties that show anticancer [7–9], and antimicrobial activities [10–12]., in previous work [13–22], we studied the anticancer activity of sulfonamides containing compounds, where the nitrogen of $-SO_2NH-$group is either free or substituted and these compounds proved a significant activity, in this study, we employed the sulfonamide group but in this case the nitrogen is emerged in piperidine ring to study the effect of this cyclized form on activity, furthermore, different substituents were introduced on the benzene ring such as cyanoacetohydrazide and hydrazide bearing either pyridine, thiazole, thiophene or benzothiophene moieties due to the well-documented anticancer activity of these moieties to study their SAR and their anti-breast cancer activity.

2. Results and discussion

2.1. Chemistry

The present work was to design and synthesis of some new 1,2-dihydropyridine, thiophene and thiazole derivatives carrying biologically active sulfone moiety expected to have anticancer activity. 2-Cyanoacetohydrazide **2** reacts with 1-[4-(piperidin-1-ylsulfonyl) phenyl] ethanone **1** [23], to give hydrazide hydrazone derivative **3**. The structure of compound **3** was proved on the basis of analytical and spectral data. Thus, IR spectrum showed bands at 3190 cm^{-1} (NH), 2264 cm^{-1} (C≡N), 1683 cm^{-1} (C=O) and 1384,1141 (SO_2). ^{1}H NMR spectrum revealed the presence of a singlet at δ 1.22 ppm corresponding to CH_3 group, a singlet at δ 4.24 ppm for CH_2 group, two duplets at δ 7.71,8.02 ppm for the aromatic protons and a singlet at 11.22 ppm for an (NH) group. Moreover, the ^{13}C NMR data exhibited the presence of δ 13.79 (CH_3), 24.88 (CH_2), 166.02 (C=O). Further evidence for the structure of **3** was obtained through studying its chemical reactivity *via* some chemical reagents. Thus, the reaction of compound **3** with cinnamonitrile derivatives was studied. Thus, the reaction of **3** with ethyl α-cyanocinnamate furnished the pyridine derivatives **10**–**15**, respectively. The reaction took place through the intermediate formation of **4**–**9**. Compounds **10**–**15** were confirmed from their

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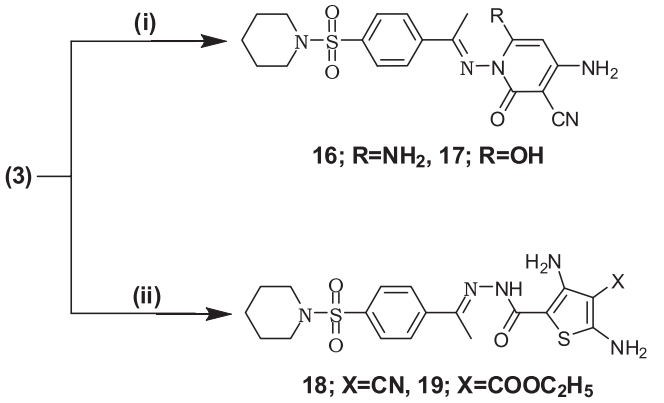
microanalytical and spectral data. IR spectra showed bands at 3392, 3115 cm^{-1} (NH_2), 2218-2208 cm^{-1} (C≡N), and 1690-1670 cm^{-1} (C=O) of ester group. ^1H NMR spectrum of **10**, revealed a triplet at δ 0.71 ppm for CH_3 of ethyl group, a quartet at δ 4.34 ppm due to CH_2 of ethyl group and a singlet at δ 7.11 ppm for NH_2 group, (Scheme 1).

The reaction of **3** with either malononitrile or ethyl cyanoacetate furnished the pyridine-2-one derivatives **16** and **17**, respectively. Analytical and spectral data are consistent with the proposed structures. The reaction of **3** with either malononitrile or ethyl cyanoacetate and elemental sulfur in the presence of triethylamine gave the corresponding thiophene derivatives **18** and **19**, respectively. The reaction goes in parallel to the reported Gewald's thiophene synthesis [24], (Scheme 2).

Similarly, the reaction of **3** with either cyclopentanone or cyclohexanone and elemental sulfur yielded the corresponding thiophene derivatives **20** and **21**, respectively. Formation of **20** and **21** took place according the similar reported reactions of cyclohexanone with methylene reagents and elemental sulfur [25]. On the other hand, the reaction of **3** with elemental sulfur and phenyl isothiocyanate afforded the thiazole derivative **22**. The formation of the latter product took place in parallel to the reported Hanzesch reported reaction [26], (Scheme 3). Structures of compound **20**, **21** and **22** were based on analytical and spectral data.



Scheme 2. Reagent and conditions: i, malononitrile or ethyl cyanoacetate/1,4-dioxane/TEA; ii, malononitrile or ethyl cyanoacetate and sulfur/ethanol/TEA.

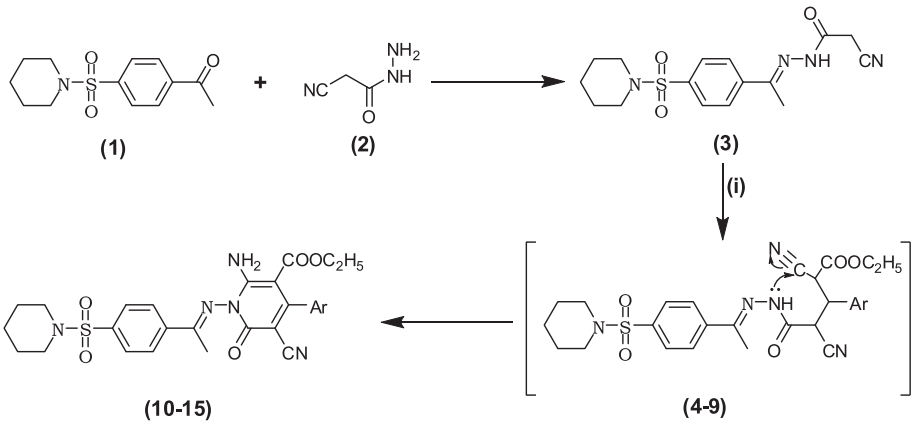
2.2. In-vitro anticancer screening

The newly synthesized compounds were evaluated for their *in-vitro* cytotoxic activity against human breast cancer cell line (MCF7). Doxorubicin HCl is one of the most effective anticancer agents was used as a reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. (Table 1), shows the *in-vitro* cytotoxic activity of the newly synthesized compounds where some compounds revealed significant activity compared to Doxorubicin as a reference drug. It was observed from the results obtained from Table 1 that most of the tested compounds showed significant anti-breast cancer activity, some of them were more potent and the others were found to equipotent than the reference drug (IC_{50} = 32.02 μM), considering the SAR for the tested compounds, it was found that introduction of the 1,2-dihydropyridine moiety resulted in an increase in the activity when compared to the starting material **3** (IC_{50} = 44.2 μM) especially for the 4-hydroxyphenyl derivative **11** (IC_{50} = 25.5 μM) and the 4-chlorophenyl derivative **15** (IC_{50} = 20.6 μM) which were the most potent compounds in this screening and they showed higher activity than doxorubicin, then compounds **10** (IC_{50} = 37.9 μM), **13** (IC_{50} = 38.7 μM), **14** (IC_{50} = 34.4 μM) and **16** (IC_{50} = 36.4 μM) showed nearly similar activity than doxorubicin. Introduction of thiophene, benzothiophene or thiazole moieties in compounds **18**-**22** showed a decrease in the activity with IC_{50} values ranging from 49.3 to 87.8 μM.

These preliminary results of biologically screening of the tested compounds give an idea about the possible importance of the 1,2-dihydropyridine moiety in the compounds acting as anti-breast cancer and give an encouraging framework in this field that may lead to the discovery of potent anticancer agent.

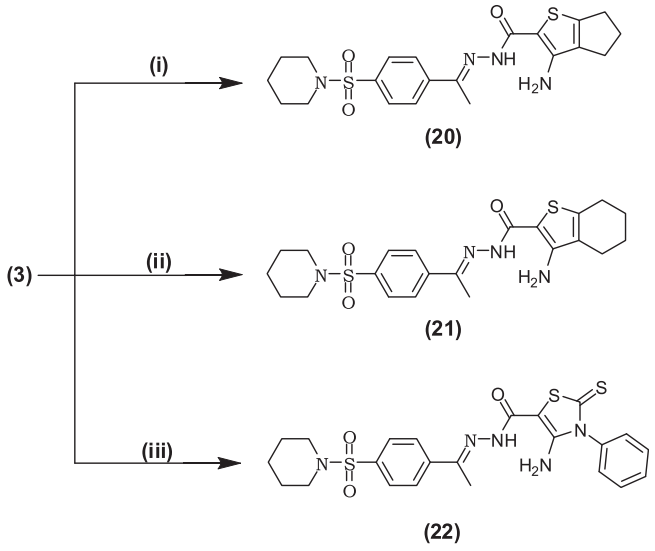
3. Conclusion

The synthesized compounds were evaluated for their *in-vitro* cytotoxicity against human breast cancer cell line (MCF7) and some



10; Ar=$C_6H_4CH_3$-4
11; Ar=C_6H_4OH-4
12; Ar=$C_6H_4OCH_3$-4
13; Ar=$C_6H_3OCH_3$-3, OH-4
14; Ar=$C_6H_4N(CH_3)_2$-4
15; Ar=C_6H_4Cl-4

Scheme 1. Reagent and conditions: i, ethyl α-cyanocinnamate derivatives/1,4-dioxane/TEA.



Scheme 3. Reagent and conditions: i, cyclopentanone and sulfur/ethanol/TEA; ii, cyclohexanone and sulfur/ethanol/TEA; iii, phenyl isothiocyanate and sulfur/ethanol/TEA.

of the tested compounds were equipotent, while the others were more potent compared with Doxorubicin. Compounds **15** and **11** showed a significant cytotoxic activity which was even higher than that of reference drug Doxorubicin, while compound **14** is nearly as active as Doxorubicin. Compound **19** showed no activity.

4. Experimental

4.1. Chemistry

Melting points (°C, uncorrected) were determined in open capillaries on a GallenKemp melting point apparatus (Sanyo GallenKemp, Southborough, UK). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5:0.5 mL) mixture was used as a developing solvent system and the spots were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra (in DMSO-d_6) were recorded on Bruker Ac-300 ultra shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 300 MHz, using TMS as internal standard. Electron impact Mass Spectra were recorded on a Shimadzu Gc-Ms-Qp 5000 instrument (Shimadzu, Tokyo, Japan). Elemental analysis was performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All compounds were within ±0.4% of the theoretical values.

4.1.1. 2-Cyano-N'-(1-(4-(piperidin-1-ylsulfonyl) phenyl) ethylidene) acetohydr-azide (*3*)

To a solution of 2-cyanoacetohydrazide (1.0 g, 0.01 mol) in 1,4-dioxane (20 mL), 1-[4-(piperidine-1-ylsulfonyl) phenyl] ethanone **1** (2.67 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h. then left to cool. The solid product formed upon pouring onto ice/water was collected by filtration and recrystallized from ethanol to give **3**. Yield, 94%; m.p. 215–217 °C IR (KBr, cm^{-1}): 3190 (NH), 3095 (CH arom.), 2983,2943,2852 (CH aliph.), 2264 (C≡N), 1683 (C=O), 1616 (C=N), 1384,1141 (SO_2). ^{1}H NMR (DMSO-d_6): 1.22 [s, 3H, CH_3], 1.31–1.62 [m, 6H, 3CH_2 cyclo], 2.94 [s, 4H, CH_2–N–CH_2 cyclo], 4.24 [s, 2H, CH_2CN], 7.71, 8.02 [2d, 4H, Ar-H, AB system, J = 7.6 Hz], 11.22 [s, 1H, NH]. ^{13}C NMR (DMSO-d_6): 13.79, 22.80, 24.62, 24.88, 46.51 (2C), 116.04, 126.92, 127.12 (2C), 127.40 (2C), 141.81, 147.38, 159.61, 166.02 MS, m/z (%): 348 [M^+] (8.4), 83 (100). Anal. Calcd. For $C_{16}H_{20}N_4O_3S$: C, 55.16; H, 5.79; N, 16.08. Found: C, 55.50; H, 5.40; N, 16.30.

4.1.2. 6-Amino-2-oxo-1-(1-(4-(piperidin-1-ylsulfonly) phenyl) ethylidene-amino)-4-substituted-1,2-dihydropyridine (*10*–*15*)

General procedure: To a solution of compound **3** (3.4 g, 0.01 mol) in 1,4-dioxane (30 mL) containing triethylamine (1 mL), ethyl benzalacetate derivatives (0.01 mol) was added. The reaction mixture was heated under reflux for 4 h. then poured onto ice/water and the solid obtained was recrystallized from ethanol to give **10**–**15**, respectively.

4.1.3. 2-Amino-5-cyano-6-[oxo-1-{1-[4-(piperidine-1-sulfonyl) phenyl]ethylidene-amino}-4-p-tolyl-1,6-dihydropyridine-3-carboxylic acid ethyl ester (*10*)

Yield, 87%; m.p. 115–117 °C; IR (KBr, cm^{-1}): 3414, 3329 (NH_2), 2980, 2939, 2852 (CH aliph.), 2214 (C≡N), 1710, 1662 (2C=O), 1598 (C=N), 1340, 1166 (SO_2). ^{1}H NMR (DMSO-d_6): 0.71 [t, 3H, CH_3 ethyl], 1.22 [s, 3H, CH_3], 1.33–1.52 [m, 6H, 3CH_2 cyclo], 2.30 [s, 3H, CH_3 tolyl], 2.93 [s, 4H, CH_2–N–CH_2 cyclo], 4.34 [q, 2H, CH_2 ethyl], 7.11 [s, 2H, NH_2 exchangeable with D_2O], 7.21–8.10 [m, 8H, Ar-H]. ^{13}C NMR

Table 1
In-vitro anticancer screening of the synthesized compounds against human breast cell line (MCF7).

Cpd. No.	Compound concentration (μM)				IC_{50} (μM)
	10 (μM)	25 (μM)	50 (μM)	100 (μM)	
	Surviving fraction (mean ± SE)[a]				
DOX	0.721 ± 0.020	0.546 ± 0.020	0.461 ± 0.010	0.494 ± 0.030	32.02
3	0.944 ± 0.002	0.666 ± 0.009	0.440 ± 0.028	0.511 ± 0.047	44.2
10	0.911 ± 0.058	0.677 ± 0.026	0.652 ± 0.030	0.581 ± 0.022	37.9
11	0.925 ± 0.032	0.647 ± 0.024	0.681 ± 0.023	0.641 ± 0.015	25.5
12	0.972 ± 0.085	0.657 ± 0.016	0.452 ± 0.022	0.375 ± 0.043	51.2
13	0.921 ± 0.008	0.694 ± 0.033	0.621 ± 0.032	0.478 ± 0.081	38.7
14	0.861 ± 0.017	0.745 ± 0.015	0.557 ± 0.003	0.407 ± 0.027	34.4
15	0.818 ± 0.044	0.723 ± 0.012	0.622 ± 0.014	0.553 ± 0.065	20.6
16	0.371 ± 0.074	0.298 ± 0.033	0.416 ± 0.004	0.394 ± 0.021	36.4
17	0.748 ± 0.025	0.540 ± 0.003	0.433 ± 0.076	0.437 ± 0.003	74.6
18	0.588 ± 0.021	0.507 ± 0.058	0.451 ± 0.004	0.406 ± 0.016	49.3
19	1.925 ± 0.032	0.947 ± 0.024	0.981 ± 0.023	0.841 ± 0.015	NA
20	0.942 ± 0.062	0.857 ± 0.016	0.552 ± 0.022	0.375 ± 0.043	75.3
21	0.971 ± 0.008	0.994 ± 0.033	0.621 ± 0.032	0.558 ± 0.081	87.8
22	0.861 ± 0.017	0.745 ± 0.015	0.557 ± 0.003	0.407 ± 0.027	60.1

NA: Compound having IC_{50} value > 100 μM.
[a] Each value is the mean of three experiments ± standard error.

(DMSO-d_6): 12.72, 13.88, 22.80, 24.64 (3C), 25.71, 46.51 (2C), 62.22, 80.24, 115.66, 117.02, 126.89, 127.61 (2C), 129.21 (2C), 130.92 (2C), 137.70 (2C), 139.74, 144.31, 154.82, 156.01, 161.94, 163.82, 165.81. MS *m/z* (%): 561 [M^+] (0.7), 172 (100). Anal. Calcd. for $C_{29}H_{31}N_5O_5S$: C, 62.02; H, 5.56; N, 12.47. Found: C, 62.40; H, 5.20; N, 12.10.

*4.1.4. 2-Amino-5-cyano-4-(4-hydroxyphenyl)-6-oxo-1-{1-[4-(piperidine-1-sulf-onyl)phenyl]ethylideneamino}-1,6-dihydropyridine-3-carboxylic acid ethyl ester (**11**)*

Yield: 73%; m.p. 85–87 °C; IR (KBr, cm^{-1}): 3460 (OH), 3288, 3255 (NH_2), 3072 (CH arom.), 2983, 2939, 2854 (CH aliph.) 2218 (C≡N), 1732, 1714 (2C=O), 1610 (C=N), 1396, 1168 (SO_2). ^{1}H NMR (DMSO-d_6): 1.11 [t, 3H, CH_3 ethyl], 1.21 [s, 3H, CH_3], 1.51–1.60 [m, 6H, $3CH_2$ cyclo], 2.84 [s, 4H, CH_2–N–CH_2 cyclo], 4.31 [q, 2H, CH_2 ethyl], 6.74 [s, 2H, NH_2 exchangeable with D_2O], 6.91–8.04 [m, 8H, Ar-H], 9.77 [s, 1H, OH]. ^{13}C NMR (DMSO-d_6): 13.71, 17.54, 24.02 (3C), 25.42, 46.51 (2C), 61.83, 96.90, 115.21, 115.71 (2C), 125.92, 127.62 (2C), 129.43 (2C), 130.01 (2C), 139.71, 141.74, 156.52, 157.92, 162.58, 163.00, 165.91, 166.32. MS *m/z* (%): 563 [M^+], (1.4), 42 (100). Anal. Calcd. For $C_{28}H_{29}N_5O_6S$: C, 59.67; H, 5.19; N, 12.43. Found: C, 59.30; H, 5.50; N, 12.10.

*4.1.5. 2-Amino-5-cyano-4-(4-methoxyphenyl)-6-oxo-1-{1-[4-(piperidine-1-sulf-onyl)phenyl]ethylideneamino}-1,6-dihydropyridine-3-carboxylic acid ethyl ester (**12**)*

Yield, 89%; m.p. 153–155 °C; IR (KBr, cm^{-1}): 3414, 3387 (NH_2), 2939, 2852 (CH aliph.), 2216 (C≡N), 1707, 1662 (2C=O), 1608 (C=N), 1340, 1166 (SO_2). ^{1}H NMR (DMSO-d_6): 1.21 [t, 3H, CH_3 ethyl], 1.30 [s, 3H, CH_3], 1.51–1.72 [m, 6H, $3CH_2$ cyclo], 2.94 [s, 4H, CH_2–N–CH_2 cyclo], 3.83 [s, 3H, OCH_3], 4.21 [q, 2H, CH_2 ethyl], 6.60 [s, 2H, NH_2 exchangeable with D_2O], 7.02–8.01 [m, 8H, Ar-H]. MS *m/z* (%): 577 [M^+] (1.2), 84 (100). Anal. Calcd. for $C_{29}H_{31}N_5O_6S$: C, 60.30; H, 5.41; N, 12.12. Found: C, 60.00; H, 5.10; N, 12.50.

*4.1.6. 2-Amino-5-cyano-4-(4-hydroxy-3-methoxyphenyl)-6-oxo-1-{1-[4-(piperidine-1-sulfonyl)phenyl]ethylideneamino}-1,6-dihydropyridine-3-carboxylic acid ethyl ester (**13**)*

Yield, 67% m.p. 132–134 °C; IR (KBr, cm^{-1}): 3275 (OH), 3199, 3113 (NH_2), 2980, 2923, 2843 (CH aliph.), 2227 (C≡N), 1718, 1689 (2C=O), 1577 (C=N), 1398, 1192 (SO_2). ^{1}H NMR (DMSO-d_6): 1.31 [t, 3H, CH_3 ethyl], 1.40 [s, 3H, CH_3], 1.51–1.72 [m, 6H, $3CH_2$ cyclo], 2.84 [s, 4H, CH_2–N–CH_2 cyclo], 3.82 [s, 3H, OCH_3], 4.21 [q, 2H, CH_2 ethyl], 6.82 [s, 2H, NH_2 exchangeable with D_2O], 7.51–8.10 [m, 7H, Ar-H], 11.22 [s, 1H, OH]. ^{13}C NMR (DMSO-d_6): 13.81, 14.18, 24.40, 24.92 (2C), 46.53 (2C), 55.51, 61.82, 96.61, 113.94, 115.03, 115.81, 116.61, 122.02; 126.87, 127.21 (2C), 128.90 (2C), 135.73, 141.71, 147.81, 153.02, 154.81, 159.62, 162.61, 164.22, 166.02. MS *m/z* (%): 593 [M^+] (3.2), 84 (100). Anal. Calcd. for $C_{29}H_{31}N_5O_7S$: C, 58.67; H, 5.26; N, 11.80. Found: C, 58.30; H, 5.50; N, 12.10.

*4.1.7. 2-Amino-5-cyano-4-(4-dimethylaminophenyl)-6-oxo-1-{1-[4-(piperidine-1-sulfonyl)phenyl]ethylideneamino}-1,6-dihydropyridine-3-carboxylic acid ethyl ester (**14**)*

Yield, 76%; m.p. 205–207 °C; IR (KBr, cm^{-1}): 3197, 3111 (NH_2), 2922, 2841 (CH aliph.), 2209 (C≡N), 1707, 1689 (2C=O), 1610 (C=N), 1350, 1166 (SO_2). ^{1}H NMR (DMSO-d_6): 1.32 [t, 3H, CH_3 ethyl], 1.41 [s, 3H, CH_3], 1.51–1.62 [m, 6H, $3CH_2$ cyclo], 2.82 [s, 4H, CH_2–N–CH_2 cyclo], 3.31 [s, 6H, $N(CH_3)_2$], 4.20 [q, 2H, CH_2 ethyl], 6.82 [s, 2H, NH_2 exchangeable with D_2O], 7.21–8.20 [m, 8H, Ar-H]. ^{13}C NMR (DMSO-d_6): 13.81, 14.22, 24.21, 24.80 (2C), 39.88 (2C), 46.51 (2C), 61.41, 92.02, 111.61 (2C), 115.90, 117.41, 118.22, 126.90 (2C), 127.11 (2C), 127.32 (2C), 135.94, 141.71, 147.41, 151.22, 154.04, 159.61, 163.42, 166.01. MS *m/z* (%): 590 [M^+] (0.6), 83(100). Anal. Calcd. for $C_{30}H_{34}N_6O_5S$: C, 61.00; H, 5.80; N, 14.23. Found: C, 61.30; H, 5.75; N, 14.13.

*4.1.8. 2-Amino-4-(4-chlorophenyl)-5-cyano-6-oxo-1-{1-[4-(piperidine-1-sulf-onyl)phenyl]ethylideneamino}-1,6-dihydropyridine-3-carboxylic acid ethyl ester (**15**)*

Yield, 84%; m.p. 133–135 °C; IR [(KBr, cm^{-1}): 3431, 3327 (NH_2), 2980, 2939, 2852 (CH aliph.), 2214 (C≡N), 1700, 1676 (2C=O), 1595 (C=N), 1396, 1166 (SO_2), 715 (C-Cl). ^{1}H NMR (DMSO-d_6): 1.10 [t, 3H, CH_3 ethyl], 1.21 [s, 3H, CH_3], 1.31–1.62 [m, 6H, $3CH_2$ cyclo], 2.94 [s, 4H, CH_2–N–CH_2 cyclo], 4.21 [q, 2H, CH_2 ethyl], 7.22–8.01 [m, 10H, Ar-H + NH_2]. MS *m/z* (%): 581 [M^+] (0.6), 83 (100). Anal. Calcd. for $C_{28}H_{28}ClN_5O_5S$: C, 57.78; H, 4.85; N, 12.03. Found: C, 57.40; H, 4.50; N, 12.40.

*4.1.9. 4,6-Diamino-2-oxo-1-[1-(1-(4-(piperidin-1-ylsulfonyl)phenyl)ethylidene-amino]-1,2-dihydropyridine-3-carbonitrile (**16**), and 4-amino-6-hydroxy-2-oxo-1-[1-(4-(piperidin-1-ylsulfonyl)phenyl)ethylideneamino)-1,2-dihydropyridine-3-carbonitrile (**17**)*

Equimolecular amounts of compound **3** (3.4 g, 0.01 mol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g; 0.01 mol) in dioxane (20 mL) containing triethylamine (1 mL) was heated under reflux for 6 h. The reaction mixture was left to cool and evaporated under vacuum. The remaining product was triturated with ethanol and the obtained solid was recrystallized from dioxane to give **16** and **17**, respectively.

16: Yield, 81%; m.p. 295–297 °C; IR (KBr, cm^{-1}): 3412, 3334, 3248, 3178 ($2NH_2$), 2918, 2860 (CH aliph.), 2214 (C≡N), 1653 (C=O), 1579 (C=N), 1380, 1170 (SO_2). ^{1}H NMR (DMSO-d_6): 1.21 [s, 3H, CH_3], 1.31–1.52 [m, 6H, $3CH_2$ cyclo], 2.91 [s, 4H, CH_2–N–CH_2 cyclo], 4.10 [s, 3H, CH + NH_2], 7.01–8.02 [m, 6H, Ar-H + NH_2]. ^{13}C NMR (DMSO-d_6): 14.22, 24.64, 25.82 (2C), 46.51 (2C), 70.90, 81.31, 116.30, 127.01 (2C), 127.42 (2C), 137.81, 141.22, 143.11, 161.50, 186.81, 197.33. MS *m/z* (%): 414 [M^+] (5.5), 47(100). Anal. Calcd. for $C_{19}H_{22}N_6O_3S$: C, 55.06; H, 5.35; N, 20.28. Found: C, 55.40; H, 5.00; N, 20.50.

17: Yield, 73%; m.p. 220–222 °C; IR (KBr, cm^{-1}): 3412 (OH), 3367, 3190 (NH_2), 3074 (CH arom.), 2943, 2843 (CH aliph.), 2264 (C≡N), 1685 (C=O), 1360, 1168 (SO_2). ^{1}H NMR (DMSO-d_6): 1.21 [s, 3H, CH_3], 1.30–1.54 [m, 6H, $3CH_2$ cyclo], 2.80 [s, 4H, CH_2–N–CH_2 cyclo], 4.21 [s, 2H, NH_2 exchangeable with D_2O], 4.40 [s, 1H, CH], 7.71,8.02 [2d, 4H, Ar-H, AB system, J = 7.1 Hz], 11.21 [s, 1H, OH]. ^{13}C NMR (DMSO-d_6): 14.20, 24.62, 24.91 (2C), 46.51 (2C), 66.33, 84.64, 116.01, 127.12 (2C), 127.42 (2C), 141.86, 147.42, 151.31, 159.62, 166.01, 174.62. MS *m/z* (%): 415 [M^+] (2.1), 78 (100). Anal. Calcd. for $C_{19}H_{21}N_5O_4S$: C, 54.93; H, 5.09; N, 16.86. Found: C, 54.60; H, 4.70; N, 16.50.

*4.1.10. 3,5-Diamino-4-cyano-N`-(1-(4-(piperidin-1-ylsulfonyl)phenyl)ethylidene] thiophene-2-carbohydrazide (**18**) and ethyl-2,4-diamino-5-[2-(1-(4-piperidin-1-ylsulfonyl)phenyl)ethylidene) hydrazinecarbonyl]thiophene-3-carboxylate (**19**)*

To a solution of compound **3** (3.4 g, 0.01 mol) in absolute ethanol (30 mL) containing triethylamine (1 mL) either malononitrile (0.66 g, 0.01 mol), or ethyl cyanoacetate (1.13 g, 0.01 mol) together with elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 1h. then poured onto ice/water and the solid obtained was recrystallized from dioxane to give **18** and **19**, respectively.

18: Yield, 88%; m.p. >300 °C; IR (KBr, cm^{-1}): 3446, 3385, 3346 (NH, NH_2), 2939, 2852 (CH aliph.), 2204 (C≡N), 1680 (C=O), 1610 (C=N), 1370, 1165 (SO_2). ^{1}H NMR (DMSO-d_6): 1.10 [s, 3H, CH_3], 1.31–1.52 [m, 6H, $3CH_2$ cyclo], 3.02 [s, 4H, CH_2–N–CH_2 cyclo], 6.41 [s, 2H, NH_2 exchangeable with D_2O], 7.01–8.02 [m, 6H, Ar-H + NH_2], 8.30 [s, 1H, NH]. MS *m/z* (%): 446 [M^+] (1.9), 84 (100). Anal. Calcd. for $C_{19}H_{22}N_6O_3S_2$: C, 51.10; H, 4.97; N, 18.82. Found: C, 51.40; H, 4.60; N, 18.50.

19: Yield, 69% m.p. 230–232 °C; IR (KBr, cm^{-1}): 3412, 3348, 3192 (NH, NH_2), 3095 (CH arom.), 2943, 2852 (CH aliph), 1700, 1647 (2C=O), 1616 (C=N), 1382, 1166 (SO_2). ^{1}H NMR (DMSO-d_6): 1.03 [t,

3H, CH_3 ethyl], 1.22 [s, 3H, CH_3], 1.31–1.50 [m, 6H, $3CH_2$ cyclo], 2.84 [s, 4H, CH_2–N–CH_2 cyclo], 4.02 [q, 2H, CH_2 ethyl], 4.41 [s, 2H, NH_2 exchangeable with D_2O], 7.42 [s, 2H, NH_2 exchangeable with D_2O], 7.71–8.02 [2d, 4H, Ar-H, AB system $J = 7.8$ Hz], 11.21 [s, 1H, NH]. ^{13}C NMR (DMSO-d_6): 13.83, 14.21, 24.64, 24.91 (2C), 46.52 (2C), 56.02, 126.86, 127.10 (2C), 127.41 (2C), 135.92, 141.71, 141.90, 147.44, 151.21, 159.62, 166.02, 173.41. MS *m/z* (%): 493 [M^+] (1.7), 83 (100). Anal. Calcd. for $C_{21}H_{27}N_5O_5S_2$: C, 51.10; H, 5.51; N, 14.19. Found: C, 51.40; H, 5.20; N, 14.50.

4.1.11. 3-Amino-N′-[1-(4-(piperidin-1-ylsulfonyl)phenyl) ethylidene]-5,6-dihy-dro-4H-cyclopenta[b]thiophene-2-carbohydrazide (20), and 3-amino-N′-[1-(4-(piperidin-1-ylsulfonyl) phenyl)ethylidene-4,5,6,7-tetrahydrobenzo[b]-thiophene-2-carbohydrazide (*21*)

To a solution of compound **3** (3.4 g, 0.01 mol) in absolute ethanol (30 mL) containing triethylamine (1 mL) either cyclopentanone or cyclohexanone (0.01 mol) together with elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was refluxed for 2 h. then poured onto ice/water and the solid obtained was recrystallized from dioxane to give **20** and **21**, respectively.

20: Yield, 66%; m.p. 222–224 °C; IR (KBr, cm^{-1}): 3421, 3387, 3327 (NH, NH_2), 2937, 2850 (CH aliph.), 1676 (C=O), 1325, 1165 (SO_2). ^1H NMR (DMSO-d_6): 1.11 [s, 3H, CH_3], 1.30–1.52 [m, 6H, $3CH_2$ cyclo], 1.80–2.51 [m, 6H, $3CH_2$ cyclopenta], 2.94 [s, 4H, CH_2–N–CH_2 cyclo], 7.21–8.02 [m, 6H, Ar-H + NH_2], 9.41 [s, 1H, NH]. ^{13}C NMR (DMSO-d_6): 17.91, 22.82, 24.62, 27.04 (2C), 28.31, 37.51, 46.52 (2C), 126.74, 126.82 (2C), 127.41 (2C), 135.22, 138.91, 142.03, 142.31, 147.81, 166.92, 193.90. MS *m/z* (%): 446 [M^+] (6.2), 83 (100). Anal. Calcd. for $C_{21}H_{26}N_4O_3S_2$: C, 56.48; H, 5.87; N, 12.55. Found: 56.80; H, 5.50; N, 12.20.

21: Yield, 87%; m.p. 232–234 °C; IR (KBr, cm^{-1}): 3446, 3390, 3265 (NH, NH_2), 2927, 2854 (CH aliph.), 1662 (C=O), 1608 (C=N) 1350, 1175 (SO_2). ^1H NMR (DMSO-d_6): 0.81 [s, 3H, CH_3], 1.51–2.02 [m, 6H, $3CH_2$ cyclo], 2.11–2.53 [m, 8H, $4CH_2$ benzothiophene], 2.90 [s, 4H, CH_2–N–CH_2 cyclo], 6.61 [br, 2H, NH_2], 7.02–7.91 [m, 4H, Ar-H], 10.91 [s, 1H, NH]. MS *m/z* (%): 460 [M^+] (1.6), 84 (100). Anal. Calcd. for $C_{22}H_{28}N_4O_3S_2$: C, 57.37; H, 6.13; N, 12.16. Found: C, 57.60; H, 5.80; N, 12.40.

4.1.12. 4-Amino-3-phenyl-N′-(1-(4-(piperidin-1-ylsulfonyl)phenyl) ethylidene)-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (*22*)

A mixture of compound **3** (3.4 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol), phenyl isothiocyanate (1.3 g, 0.01 mol) in ethanol (50 mL) containing triethylamine (1 mL) was refluxed for 3h. then left to cool. The solid product formed upon pouring onto ice/water was recrystallized from ethanol to give **22**. Yield, 91%; m.p. 280–282 °C; IR (KBr, cm^{-1}): 3421, 3311, 3163 (NH, NH_2), 3100 (CH arom.), 2940, 2895 2837 (CH aliph), 1635 (C=O), 1585 (C=N), 1388, 1165 (SO_2), 1280 (C=S). ^1H NMR (DMSO-d_6): 1.20 [s, 3H, CH_3], 1.31–1.62 [m, 6H, $3CH_2$ cyclo], 2.90 [s, 4H, CH_2–N–CH_2 cyclo], 7.01–8.03 [m, 11H, Ar-H + NH_2], 10.82 [s, 1H, NH]. ^{13}C NMR (DMSO-d_6): 14.41, 22.82, 24.62 (2C), 46.53 (2C), 79.54, 125.01 (2C), 127.20, 127.52 (2C), 128.91 (2C), 130.11 (2C), 134.82, 135.74, 142.02, 154.41, 163.34, 164.92, 189.75. MS *m/z* (%): 515 [M^+] (2.8), 42 (100). Anal. Calcd. for $C_{23}H_{25}N_5O_3S_3$: C, 53.57; H, 4.89; N, 13.58. Found: C, 53.20; H, 4.50; N, 13.90.

4.2. In-vitro anticancer screening

Human tumor breast cancer cell line (MCF7) was used in this study. The cytotoxic activity was measured *in-vitro* for the newly synthesized compounds using the Sulforhodamine B stain (SRB) assay using the method of Skehan et al. [27]. The *in-vitro* anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University.

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h. before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in dimethyl sulfoxide. Different concentration of the compound under test (10, 25, 50 and 100 μM) were added to the cell monolayer. Triplicate wells were prepared for each individual concentration. Monolayer cells were incubated with the compound(s) for 48 h. at 37 °C and in atmosphere of 5% CO_2. After 48 h. cells were fixed, washed and stained for 30 min with 0.4% (W/V) SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time [27]. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared to the reference drug Doxorubicin (CAS, 25316-40-9). The surviving fractions were expressed as means ± standard error and the results are given in (Table 1).

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