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Synthesis of new phenolic compounds and biological evaluation as antiproliferative agents

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Abstract

New series of phenolic azomethine compounds in addition to 5-arylidene thiazolidinones are synthesized and screened for their anticancer activity against the brain cancer cell line SNB-75 and non-small lung cancer cells HOP-92. The azomethine derivative **12b** is the most active compound against SNB-75 displaying an IC_{50} value of 0.14 μM. Compounds **7b**, **16a** and **27d** display submicromolar activity against the HOP-92 cell line with IC_{50} values of 0.73, 0.74 and 0.81 μM, respectively. Moreover, studying the cytotoxic effects of the most active compounds against normal lung cells WI-38 revealed that compounds **7b**, **16a** and **27d** showed high safety profiles as anticancer agents.

Keywords

anticancer, azomethines, phenolic compounds, synthesis, thiazolidinones

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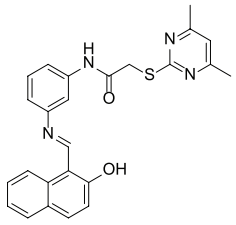
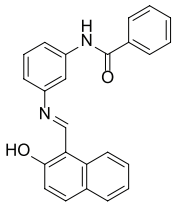


12b

SNB-75; IC_{50} = 0.14 μM

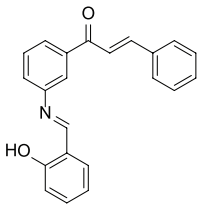
Normal WI-38; IC_{50} = 21.88 μM



7b

HOP-92; IC_{50} = 0.73 μM

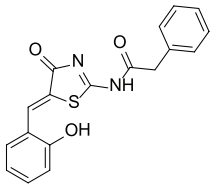
Normal WI-38; IC_{50} = 30.82 μM



16a

HOP-92; IC_{50} = 0.74 μM

Normal WI-38; IC_{50} = 29.90 μM



27d

HOP-92; IC_{50} = 0.81 μM

Normal WI-38; IC_{50} = 24.55 μM

Introduction

Cancer is one of the most feared diseases worldwide affecting around 14 million people every year.[1] Specifically, lung cancer is a major cause of death with a survival rate of only about 18%.[2] Although, chemotherapy remains one of the most effective strategies among all cancer treatments, resistance to chemotherapeutic agents is the major challenge.[3] Thus, the discovery of new antitumor agents with promising biological activity and safety profiles remains a necessity for scientific research.

In addition, it is well established that phenolic azomethine derivatives display biological activity as anticancer, antibacterial and anti-inflammatory agents.[4,5] In this context, many phenolic azomethine–based molecules featured with different aryl/heteroaryl moieties were reported to exert antitumor effects.[4] Luo and co-workers reported the synthesis of salicylaldehyde-*o*-phenylenediamine Schiff base **I**, which displayed antitumor activity against the leukaemia cell lines K562 and HEL with IC_{50} values of 11.95 and 9.75 μM, respectively (Figure 1).[5]Moreover, the phenolic azomethine derivatives **II** and **III** exhibited good anticancer activity against the colon cancer cell line HCT-116.[6,7]

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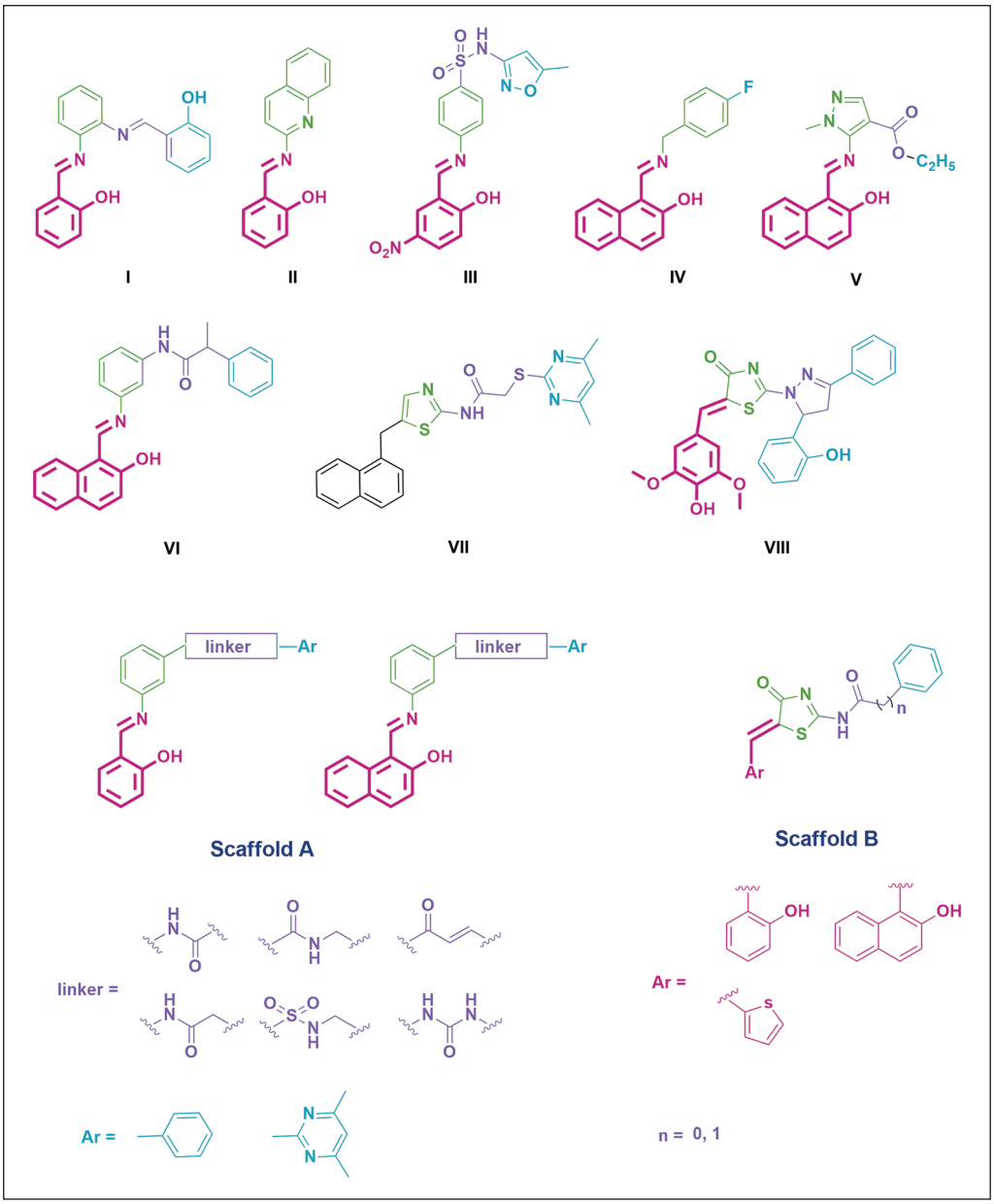


Figure 1. Structures of some reported anticancer compounds bearing phenolic/2-hydroxy-1-naphthyl azomethines, and the design of the proposed target compounds.

Furthermore, there is evidence that azomethine derivatives obtained from the condensation of 2-hydroxy-1-naphthaldehyde (fused phenolic azomethines) showed promising antitumor activity.[8,9] In 2018, 1-[(4-fluorobenzylimino)methyl]naphthalene-2-ol (**IV**) was described by Devi et al.[8] to exert antiproliferative activity against the alveolar adenocarcinoma A459, breast cancer MCF7 and prostate cancer DU145 cell lines (Figure 1). Moreover, cytotoxic studies showed that the phenolic azomethine derivative **V** elicited promising cytotoxic activity against five cell lines: HepG2, MGC80-3, T-24, SK-OV-3 and HL-7702.[9] Lara and co-workers[10] discovered the iminomethyl-2-hydroxy-1-naphthaldehyde derivative salermide **VI** that displayed anticancer activity against the leukaemia cell lines MOLT4 and KG1A.

Based on the aforementioned findings, the phenolic azomethine moiety was selected to build up the final target compounds. Therefore, two series with the phenolic azomethine moiety (scaffold A), namely, 2-hydroxy-1-phenylazomethines **3a**, **7a,c**, **12a**, **16a**, **20a** and **24a** and 2-hydroxy-1-naphthyl-azomethines **3b**, **7b,d**, **12b**, **16b**, **20b** and **24b**, were designed to compare the lipophilic effect on the obtained antiproliferative activity (Figure 1). The phenolic azomethine moiety was extended by a phenyl core linked to a pendent aryl group (either phenyl **3a,b**, **7a–d**, **16a,b**, **20a,b** and **24a,b** or 4,6-dimethylpyrimidine (**12a,b**)) through different linkers to ensure variable degrees of flexibility between the phenyl core and the side chain.

Literature reviews showed that compounds containing a thiazole core, for example, compound **VII**, or a thiazolidinone core, for example, compound **VIII**, displayed potent antitumor activity against several cell lines (Figure 1).[11,12] Consequently, a new approach was adopted through

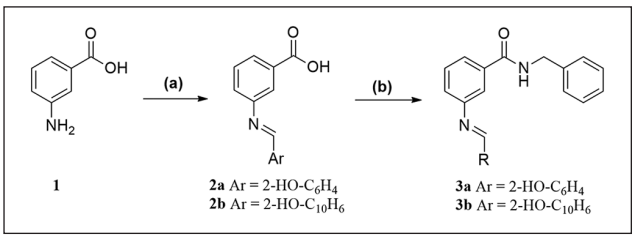
bioisosteric replacement of the azaphenyl core in **7a–d** with a thiazolidinone ring (scaffold B) as in compounds **27a,b,d,e**. Finally, the phenolic moiety in the latter congeners was replaced by a 2-thienyl ring in **27c,f** to prove the importance of the phenolic moiety on the cytotoxic activity.

The cytotoxic activity of all the target compounds was tested against the lung cancer cell line HOP-92 and the brain cancer cell line SNB-75. Subsequently, the growth inhibitory activity of the most active compounds against the human normal lung fibroblast cell line WI-38 was tested.

Results and discussion

Chemistry

The target compounds bearing a phenyl core **3a,b**, **7a–d**, **12a,b**, **16a,b**, **20a,b** and **24a,b** were synthesized adopting the chemical pathways outlined in Schemes 1–5. According to the reported procedures, 3-aminobenzoic acid **1** was employed as a suitable precursor for the synthesis of the intermediate azomethines **2a,b**.[13,14] The amide derivatives **3a,b** were prepared by reacting the 3-aminobenzoic acid derivatives **2a,b** with carbonyldiimidazole (CDI) in dry dichloromethane/dimethylformamide (DMF) at 0°C followed by reaction with benzylamine at room temperature (Scheme 1).

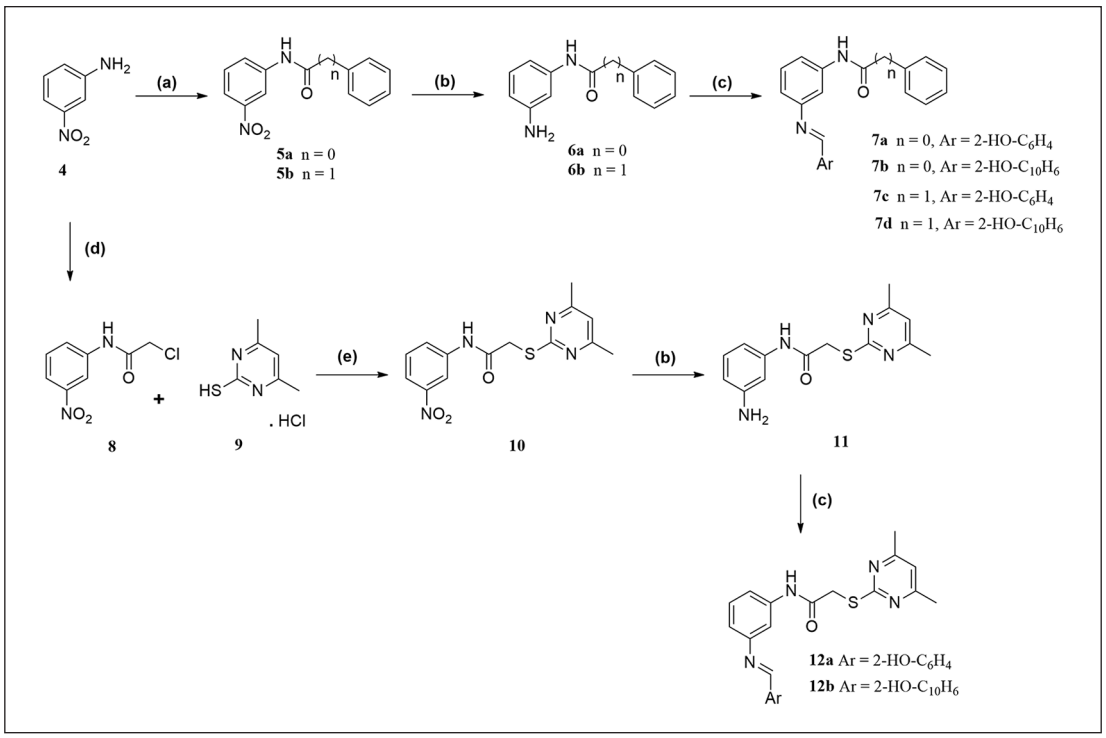


Scheme 1. Synthetic pathway to compounds **3a,b**. Reagents and conditions: (a) aldehyde, EtOH, glacial CH_3COOH, 70°C, 6 h, yield 77%–85%; (b) CDI, benzylamine, CH_2Cl_2/DMF, RT, 24 h, yield 52%–76%.

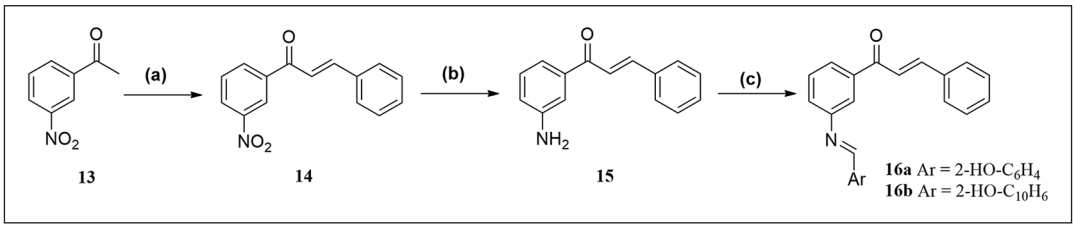
Starting from 3-nitroaniline (**4**), derivatives **6a,b** were synthesized adopting methods described in the previous literature.[15,16] Synthesis of the required Schiff bases **7a–d** was performed via reaction of the aryl amino intermediates **6a,b** with the appropriate aldehyde in refluxing ethanol in the presence of a catalytic amount of glacial acetic acid (Scheme 2). Meanwhile, intermediate **10** was synthesized by heating the 2-mercapto-4,6-dimethylpyrimidine hydrochloride (**9**) in ethanol in the presence of potassium carbonate to liberate the free base, followed by the addition of chloro-acylated compound **8**. Reduction of the nitro derivative **10** using $SnCl_2$/HCl afforded the corresponding amino counterpart **11**, which in turn reacted with the appropriate aldehyde to yield the desired Schiff bases **12a,b** (Scheme 2).

Moreover, derivatives **14** and **15** were synthesized from 3-nitroacetophenone (**13**) according to the reported methods.[16,17] The target azomethine derivatives **16a,b** were prepared via the reaction of the aryl amino intermediate **15** as mentioned previously (Scheme 3).

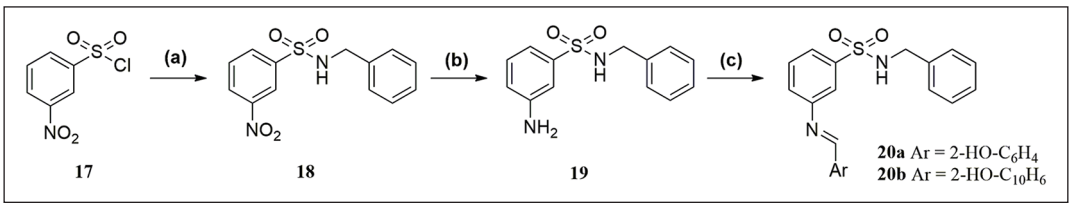
To afford the 3-nitrobenzenesulfonamide derivative **18**, 3-nitrobenzenesulfonyl chloride (**17**) was treated with benzylamine, followed by reduction with $SnCl_2$/HCl to yield the corresponding amino derivative **19**. The target final



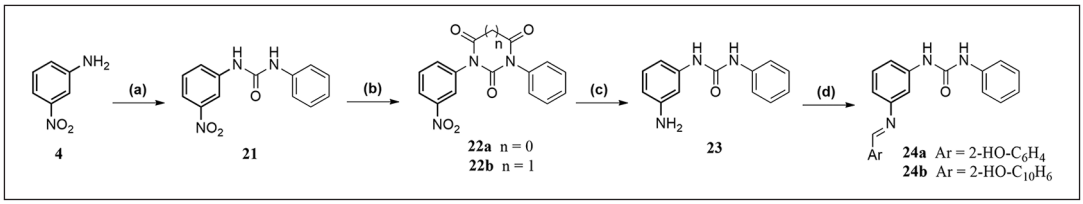
Scheme 2. Synthetic pathway to compounds **7a–d** and **12a,b**. Reagents and conditions: (a) benzoyl chloride/phenylacetyl chloride, Et_3N, CH_2Cl_2, reflux, 5 h, yield 69%–86%; (b) $SnCl_2{\cdot}2H_2O$, conc. HCl, absolute EtOH, RT, 48 h, yield 45%–80%; (c) appropriate aldehyde, glacial CH_3COOH, absolute EtOH, 70°C, 6 h, yield 65%–86%; (d) $ClCH_2COCl$, CH_2Cl_2, reflux, 5 h, yield 88%; and (e) anhydrous K_2CO_3, absolute EtOH, reflux, 6 h, yield 74%.



Scheme 3. Synthetic pathway to compounds **16a,b**. Reagents and conditions: (a) benzaldehyde, NaOH, EtOH, RT, 2 h, yield 92%; (b) $SnCl_2{\cdot}2H_2O$, conc. HCl, absolute EtOH, reflux, 4 h, yield 56%; and (c) appropriate aldehyde, absolute EtOH, glacial CH_3COOH, 70°C, 12–15 h, yield 74%–79%.



Scheme 4. Synthetic pathway to compounds **20a,b**. Reagents and conditions: (a) benzylamine, triethylamine, CH_2Cl_2, RT, 2 h, yield 77%; (b) $SnCl_2{\cdot}2H_2O$, absolute EtOH, RT, overnight, yield 58%; and (c) appropriate aldehyde, glacial CH_3COOH, absolute EtOH, 70°C, 5 h, yield 81%–87%.



Scheme 5. Synthetic pathway to compounds **24a,b**. Reagents and conditions: (a) PhNCO, CH_2Cl_2, Et_3N, RT, 4 h, yield 92%; (b) $(COCl)_2/CH_2(COCl)_2$, dioxane, RT, 48 h, yield 79%–83%; (c) $SnCl_2{\cdot}2H_2O$, HCl, EtOH, reflux, 2 h, yield 20%–25%; and (d) appropriate aldehyde, absolute EtOH, glacial CH_3COOH, 70°C, 4 h, yield 58%–65%.

compounds **20a,b** were prepared by reacting compound **19** with the corresponding aldehydes in refluxing ethanol (Scheme 4).

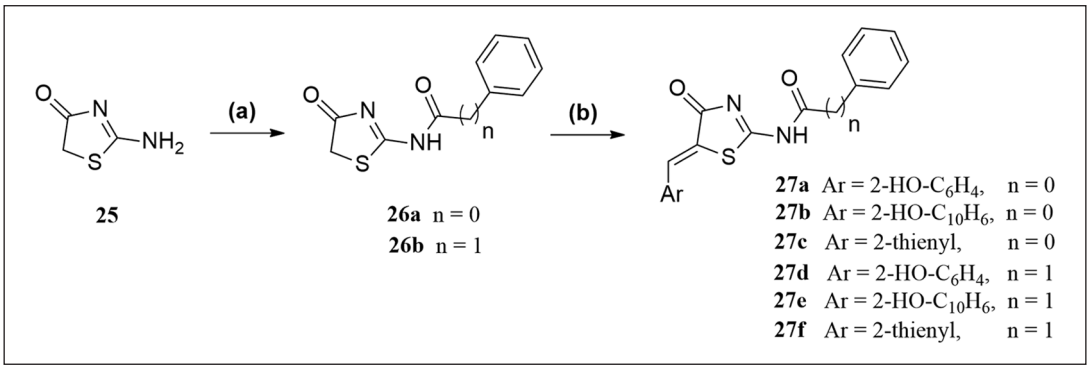
In attempts to prepare new compounds with a rigidified linker between the phenyl core and the side chain, the intermediate imidazolidine-trione derivative **22a** and pyrimidine-trione derivative **22b** were prepared through the reaction of *N,N′*-disubstituted urea derivative **21** with oxalyl chloride or malonyl chloride in dioxane (Scheme 5). Upon reduction of the nitro group in intermediates **22a,b** with $SnCl_2$/HCl, reductive cleavage of the heterocyclic rings occurs affording the urea derivative **23**. The ^{1}H NMR spectrum of **23** revealed the presence of three exchangeable protons at 5.11, 8.36 and 8.54 ppm correlating with the protons of the primary aromatic amine and protons of the secondary amino groups of urea, respectively, confirming reductive cleavage of the heterocyclic ring of the intermediates **22a,b**. Moreover, reduction of the nitro derivatives **22a,b** with Fe/NH_4Cl or Fe/acetic acid did not afford the target amino derivatives. The final compounds **24a,b** were obtained by the reaction of the amino derivative **23** with the appropriate aldehyde. The ^{1}H NMR spectra of the analogues **24a,b** showed the presence of two singlets at 8.94, 9.66 and 13.08, 15.66 ppm corresponding to the azomethine protons and OH protons, respectively. Moreover, the ^{13}C NMR spectra of compounds **24a,b** showed signals at 159.2–160.7 and 163.8–171.1 corresponding to the C=N and C=O carbons, respectively, in addition to aromatic signals confirming their carbon skeletons.

The target compounds bearing a thiazolidinone core **27a–f** were synthesized according to the pathway depicted in Scheme 6. According to the reported procedure, 2-aminothiazolidin-4-one (**25**) was prepared by reacting thiourea with ethyl chloroacetate.[18] Reaction of the precursor **25** with benzoyl chloride or phenylacetyl chloride afforded the intermediates **26a,b**. Condensation of compounds **26a,b** with different aldehydes gave the target analogues **27a–f**. The structures of the target compounds were confirmed by elemental analysis and spectral data. ^{1}H NMR and ^{13}C NMR spectra of some of the final compounds are given as supplementary materials.

Biological evaluation

In vitro cytotoxic activity against SNB-75 and HOP-92 cell lines

The prepared compounds **3a,b**, **7a–d**, **12a,b**, **16a,b**, **20a,b**, **24a,b** and **27a–f** were assayed in vitro for their cytotoxicity utilizing the MTT assay against the brain cancer cell line SNB-75 and the non-small lung cancer cell line HOP-92 using



Scheme 6. Synthetic pathway to compounds **27a–f**. Reagents and conditions: (a) appropriate acid chloride, pyridine, reflux, 4 h, yield 60%–78%; (b) appropriate aldehyde, absolute EtOH, CH_3COONa, reflux, 4 h, yield 62%–85%.

sorafenib as a positive control. The synthesized compounds can be classified into derivatives with a phenyl core **3a,b**, **7a–d**, **12a,b**, **16a,b**, **20a,b** and **24a,b** and derivatives with a thiazolidinone core **27a–f**. As shown in Table 1, almost all the synthesized compounds exhibited mild to moderate cytotoxic activities against both cell lines. Moreover, compound **12b** displayed submicromolar activity against the brain cancer cell line SNB-92. On the other hand, derivatives **7b**, **16a** and **27d** displayed submicromolar activity against the non-small lung cancer cell line HOP-92.

Regarding the brain cancer cell line SNB-75, derivatives with a phenyl core, **3a,b**, **7a–d**, **12a,b**, **16a,b**, **20a,b** and **24a,b**, displayed a wide range of anticancer activity with IC_{50} values ranging from 0.14 to >20 μM. Compounds **3a**, **7a,b**, **12b**, **20a**, **24b**, **27a,d** and **27e** (IC_{50} range = 0.14–3.25 μM) presented higher antiproliferative activity than the positive control sorafenib (IC_{50} = 14.86 μM). In particular, compound **12b** showed superior potency with an IC_{50} value of 0.14 μM. With respect to the effect of the substitution pattern on the derivatives with a phenyl core, compounds **3a,b**, **7a–d**, **12a,b**, **16a,b**, **20a,b** and **24a,b**, and the 2-hydroxyphenyl derivatives **3a**, **7a**, **7c**, **16a** and **20a** exhibited higher anticancer activity than the corresponding 2-hydroxynaphthyl derivatives **3b**, **7b**, **7d**, **16b** and **20b**, except for derivatives bearing ureido linker **24a,b** and compounds featuring a mercaptodimethylpyrimidine side chain **12a,b**.

Dealing with the linker length, compounds **7a,b** with an amide linker showed good anticancer activity with IC_{50} values of 1.56 and 2.27 μM, respectively. Increasing the spacer length by attaching a benzyl group to the amide linkage as in **7c,d** decreased the activity more than four times. Meanwhile, replacing the amide linkage in the derivatives **7c,d** with a reversed amide linkage **3a,b** or sulfamoyl linkage **20a,b** resulted in compounds with comparable antiproliferative activity. Replacing the acetamide linkage in the derivatives **7c,d** with a ureido linkage as in **24a,b** increased the anticancer effect of the 2-hydroxynaphthyl derivative. Conversely, an α,β-unsaturated ketone linkage as in **16a,b** resulted in a sharp decrease in the antiproliferative activity of the 2-hydroxynaphthyl derivative **16b**.

Furthermore, the mercaptodimethylpyrimidine derivative **12b** with the longest linker displayed superior anticancer activity against the SNB-92 cancer cell line with IC_{50} = 0.14 μM. On the other hand, replacing the 2-hydroxynaphthyl moiety in **12b** with a 2-hydroxyphenyl moiety as in the congener **12a** (IC_{50} = 25.46 μM) was detrimental to the antiproliferative activity.

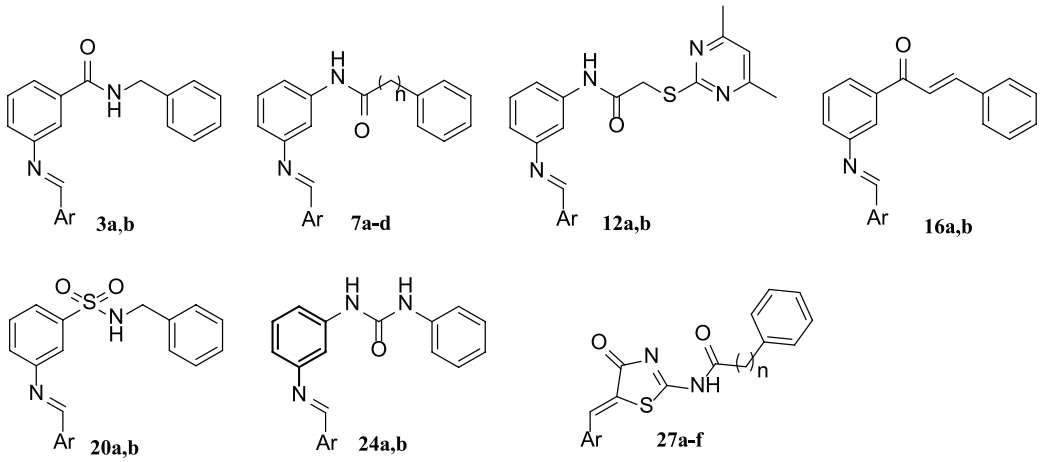
Concerning the thiazolidinone derivatives **27a–f**, compounds with an acetamide linker between the thiazolidinone ring and the pendent phenyl ring, that is, **27d–f**, displayed comparable cytotoxic activity to the corresponding analogues with an amide linker (**27a–c**). The 2-hydroxyphenyl derivatives **27a,d** displayed the highest anticancer activity with IC_{50} values of 1.59 and 1.39 μM. Replacing the 2-hydroxyphenyl moiety in **27a,d** with a 2-thienyl moiety as in compounds **27c,f** decreased the antiproliferative activity, which highlights the impact of the 2-hydroxyphenyl moiety on the anticancer activity.

With regard to the non-small lung cancer cell line HOP-92, compounds with a phenyl core, **3a,b**, **7a–d**, **12a,b**, **16a,b**, **20a,b** and **24a,b**, displayed a wide range of anticancer activity with IC_{50} values ranging from 0.73 to 16.49 μM. Compounds **3a**, **7a,b**, **16a** and **27d** (IC_{50} = 0.74–1.62 μM) presented higher anticancer activity than the positive control sorafenib (IC_{50} = 6.87 μM). In contrast with the SNB-75 cancer cell line, the 2-hydroxynaphthyl derivatives **7b**, **7d**, **12b**, **20b** and **24b** showed higher antiproliferative activity than the corresponding 2-hydroxyphenyl derivatives **7a**, **7c**, **12a**, **20a** and **24a**, with the exception of compounds bearing the reversed amide linkage (**3a,b**) and an α,β-unsaturated ketone linkage (**16a,b**).

Studying the effect of the spacer, derivatives **7a,b** with an amide linker between the phenyl core and the pendent phenyl showed good anticancer activity with IC_{50} values of 1.08 and 0.73 μM, respectively. Increasing the spacer length by attaching a benzyl group to the amide linkage (**7c,d**) decreased the antiproliferative activity by six- and fourfold, respectively. Meanwhile, replacing the acetamide linkage in the analogues **7c,d** with a reversed acetamide linkage as in **3a,b** or an α,β-unsaturated ketone linkage as in **16a,b** resulted in an increase in the antiproliferative activity of the 2-hydroxyphenyl derivatives; whereas, replacing the acetamide linkage in the derivatives **7c,d** with a urea linkage (**24a,b**), a sulfamoyl linkage (**20a,b**) or increasing the linker length as in the mercaptodimethylpyrimidine derivatives (**12a,b**) did not have a marked effect on the anticancer activity of the corresponding derivatives.

Concerning the compounds with a thiazolidinone core **27a–f**, they displayed a wide range of anticancer activity

Table 1. Cytotoxic activity of the synthesized compounds (IC_{50}, μM) against the brain cancer cell line SNB-75 and the non-small lung cancer cell line HOP-92.



Compound	Ar	n	IC_{50} (μM)	
			SNB-75	HOP-92
3a	2-HO-C_6H_4	0	2.86 ± 0.12	1.62 ± 0.03
3b	2-HO-$C_{10}H_6$	0	8.90 ± 0.38	5.11 ± 0.17
7a	2-HO-C_6H_4	0	1.56 ± 0.07	1.08 ± 0.09
7b	2-HO-$C_{10}H_6$	0	2.27 ± 0.13	0.73 ± 0.04
7c	2-HO-C_6H_4	1	7.09 ± 0.33	6.66 ± 0.33
7d	2-HO-$C_{10}H_6$	1	13.71 ± 0.82	2.91 ± 0.12
12a	2-HO-C_6H_4	0	25.46 ± 1.71	8.54 ± 0.39
12b	2-HO-$C_{10}H_6$	0	0.14 ± 0.02	5.69 ± 0.21
16a	2-HO-C_6H_4	0	4.89 ± 0.25	0.74 ± 0.04
16b	2-HO-$C_{10}H_6$	0	47.64 ± 2.81	1.97 ± 0.07
20a	2-HO-C_6H_4	0	3.25 ± 0.22	16.49 ± 0.95
20b	2-HO-$C_{10}H_6$	0	8.55 ± 0.61	3.45 ± 0.08
24a	2-HO-C_6H_4	0	4.73 ± 0.25	11.35 ± 0.05
24b	2-HO-$C_{10}H_6$	0	3.15 ± 0.15	3.98 ± 0.12
27a	2-HO-C_6H_4	0	1.59 ± 0.07	3.26 ± 0.19
27b	2-HO-$C_{10}H_6$	0	5.30 ± 0.19	6.38 ± 0.36
27c	2-Thienyl	0	8.02 ± 0.36	9.36 ± 0.47
27d	2-HO-C_6H_4	1	1.39 ± 0.07	0.81 ± 0.07
27e	2-HO-$C_{10}H_6$	1	2.32 ± 0.09	6.38 ± 0.25
27f	2-Thienyl	1	8.98 ± 0.25	14.31 ± 0.81
Sorafenib			14.86 ± 0.43	6.87 ± 0.41

with IC_{50} values ranging from 0.81 to 14.31 μM. The 2-hydroxyphenyl derivatives **27a,d** displayed the highest anticancer activity with IC_{50} = 3.26 and 0.81 μM, whereas the thienyl analogues **27c,f** exhibited the lowest antiproliferative activity (IC_{50} = 9.36 and 14.31 μM). As for the influence of the linker length, replacing the amide linker in compounds **27a–c** by an acetamide linker as in **27d–f** afforded congeners with comparable activity except for the 2-hydroxyphenyl derivative **27d** which showed enhanced cytotoxicity (IC_{50} = 0.81 μM).

In vitro cytotoxic activity towards non-tumorigenic human WI-38 cells

The cytotoxic effects of the most active compounds **7b**, **12b**, **16a** and **27d** were examined against the non-tumorigenic human lung fibroblast cell line WI-38 to investigate their potential safety towards normal cells. The results in Table 2 showed that the tested compounds **7b**, **12b**, **16a** and **27d** exhibited non-significant cytotoxic impact towards the normal cell line WI-38. In addition, the derivatives **7b**, **16a** and **27d** displayed good selectivity indices, thereby providing good safety as anticancer agents.

Conclusion

In the present work, new series of 2-hydroxy-1-phenyl/2-hydroxy-1-naphthyl azomethine compounds **3a,b**, **7a–d**, **12a,b**, **16a,b**, **20a,b** and **24a,b** in addition to 5-arylidene thiazolidinones **27a–f** were synthesized and screened for their anticancer activity against the brain cancer cell line SNB-75 and the non-small lung cancer cell line HOP-92. The target compounds **7b**, **12b**, **16a** and **27d** displayed submicromolar activity against both cell lines. Moreover, studying the

Table 2. Cytotoxic activity of the most active compounds against normal WI-38 cells and their selectivity indices.

Compound	IC_{50} (μM)		Selectivity index
	WI-38	HOP-92	
7b	30.82 ± 1.72	0.73 ± 0.04	42
12b	21.88 ± 1.61	5.69 ± 0.21	4
16a	29.90 ± 2.04	0.74 ± 0.04	40
23d	24.55 ± 1.38	0.81 ± 0.07	30

cytotoxic effects of the most active compounds **7b**, **16a** and **27d** against normal lung cells WI-38 showed good selectivity indices ranging from 30 to 42; thus, these compounds provide high safety profiles as anticancer agents.

Materials and methods

Chemistry

Starting materials and solvents were purchased from commercial suppliers and were used without further purification. Melting points were determined using an Electrothermal Stuart SMP3 digital melting point apparatus and are uncorrected. Elemental microanalyses were performed at the Regional Centre for Mycology and Biotechnology, Al-Azhar University. The infrared (IR) spectra were recorded on a Shimadzu FTIR 8400S, Faculty of Pharmacy, Cairo University. The proton nuclear magnetic resonance ^{1}H NMR spectra were performed in $CDCl_3$ or DMSO-d_6 using a Bruker, 400 MHz NMR spectrometer, Microanalytical Unit, Faculty of Pharmacy, Cairo University. ^{13}C NMR spectra were recorded using a Bruker, 100 MHz NMR spectrometer, Microanalytical Unit, Faculty of Pharmacy, Cairo University. Mass spectra were obtained using a Finnigan SSQ 7000 GC/MS, at the Micro Analytical Centre, Faculty of Science, Cairo University. Reactions were followed by thin-layer chromatography (TLC), using silica gel TLC plates with a fluorescence indicator (F254) obtained from Merck and the spots were visualized using a Vilber Lourmat ultraviolet lamp at $\lambda\iota = 254$ nm. The purity of the newly synthesized compounds was assessed by TLC. Compounds **2a**,[19] **2b**,[14] **5a**,[20] **5b**,[21] **6a**,[16] **6b**,[21] **8**,[22] **9**,[23] **14**,[24] **15**,[16] **18**,[25] **19**,[25] **21**,[26] **25**[18] and **26a**[27] were prepared according to the reported procedures.

General procedure for the preparation of compounds 3a,b

A solution of the appropriate 3-aminobenzoic acid derivative **2a,b** (2 mmol) in dry dichloromethane containing 5%–20% volume DMF was cooled to 0°C in an ice bath. CDI (0.49 g, 3 mmol) was added, and the mixture was stirred for 1 h. Next, benzylamine (0.65 mL, 6 mmol) was added, and the mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with dichloromethane and washed with 1M HCl and brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure.

N-Benzyl-3-[(2-hydroxybenzylidene)amino]benzamide (**3a**): The product was separated as yellow crystals, m.p. 46–48°C, yield 0.34 g (52%). IR ν (cm^{-1}): 3396 (OH), 3059–3029 (CH aromatic), 2922 (CH aliphatic), 1631 (C=O), 1582 (C=N), 1496–1456 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.82 (s, 2H, CH_2), 6.88–6.94 (m, 3H, aromatic H), 7.28–7.40 (m, 9H, aromatic H and NH), 7.48 (d, $J = 7.56$ Hz, 2H, aromatic H), 8.72 (s, 1H, CH=N), 13.46 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 62.4 (CH_2), 116.9, 119.1, 119.2, 127.7, 128.3, 129.1, 132.2, 132.9, 139.1 (aromatic carbons), 161.0 (C=N), 167.0 (C=O). Anal. calcd for $C_{21}H_{18}N_2O_2$ (330.39): C, 76.34; H, 5.49; N, 8.48; found: C, 76.01; H, 5.63; N, 8.67%.

N-Benzyl-3-{[(2-hydroxynaphthalen-1-yl)methylidene] amino}benzamide (**3b**): The title compound was separated as yellow crystals and purified by preparative TLC using chloroform as the mobile phase, m.p. 73–75°C, yield 0.58 g (76%). IR ν (cm^{-1}): 3398 (OH), 3290 (NH), 3059–3034 (CH aromatic), 2922 (CH aliphatic), 1632 (C=O), 1614 (C=N), 1595–1541 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.89 (d, $J = 3.6$ Hz, 2H, CH_2), 6.77 (d, $J = 9.32$ Hz, 1H, aromatic H), 7.22 (t, $J = 7.56$ Hz, 1H, aromatic H), 7.32 (t, $J = 6.60$ Hz, 1H, aromatic H), 7.38–7.48 (m, 10H, aromatic H and NH), 7.66 (d, $J = 7.72$ Hz, 1H, aromatic H), 7.75 (d, $J = 9.32$ Hz, 1H, aromatic H), 8.14 (d, $J = 8.36$ Hz, 1H, aromatic H), 9.32 (s, 1H, CH=N), 14.42 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.4 (CH_2), 106.6, 119.1, 122.8, 125.5, 125.9, 128.2, 128.1, 128.4, 129.2, 129.4, 134.6, 137.6, 138.3 (aromatic carbons), 160.0 (C=N), 176.9 (C=O). MS (EI): *m/z* (%) = 380 (M^+, 0.01), 91 (100). Anal. calcd for $C_{25}H_{20}N_2O_2$ (380.45): C, 78.93; H, 5.30; N, 7.36; found: C, 78.70; H, 5.41; N, 7.64%.

General procedure for the preparation of the Schiff bases 7a–d, 12a,b, 16a,b, 20a,b and 24a,b

A solution of the appropriate amine **6a,b**, **11**, **15**, **19**, **23** (2.35 mmol) in absolute ethanol (20 mL) and salicylaldehyde (0.28 mL, 2.59 mmol) or 2-hydroxy-1-naphthaldehyde (0.41 g, 2.35 mmol) was heated under reflux for 4–15 h at 70°C in the presence of a catalytic amount of glacial acetic acid. The reaction mixture was left to cool, the precipitated crystals were filtered and recrystallized from an appropriate solvent(s).

N-(3-((2-Hydroxybenzylidene)amino)phenyl)benzamide (**7a**): The title compound separated as yellow crystals, recrystallized from *n*-butanol, m.p. 175–177°C, yield 0.61 g (83%). IR ν (cm^{-1}): 3445 (OH), 3308 (NH), 3055–3032 (CH aromatic), 1647 (C=O), 1620 (C=N), 1595–1558 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.98–7.01 (m, 2H, aromatic H), 7.19 (d, $J = 7.96$ Hz, 1H, aromatic H), 7.42–7.47 (m, 2H, aromatic H), 7.54–7.62 (m, 3H, aromatic H), 7.69–7.74 (m, 2H, aromatic H), 7.88 (s, 1H, aromatic H), 7.99 (d, $J = 7.36$ Hz, 2H, aromatic H), 8.98 (s, 1H, CH=N), 10.38 (br s, 1H, NH, D_2O exchangeable), 13.06 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 114.1, 116.6, 117.1, 119.3, 119.70, 119.74, 128.1, 128.9, 130.1, 132.2, 133.0, 133.9, 135.2, 140.7, 148.9, 160.8 (aromatic carbons), 163.8 (C=N), 166.2 (C=O). MS (EI): *m/z* (%) = 316 (M^+, 54.45), 105 (100).

Anal. calcd for $C_{20}H_{16}N_2O_2$ (316.36): C, 75.93; H, 5.10; N, 8.86; found: C, 76.14; H, 5.23; N, 9.15%.

N-(3-(((2-Hydroxynaphthalen-1-yl)methylene)amino) phenyl)benzamide (**7b**): The product separated as yellow crystals, recrystallized from *n*-butanol, m.p. 221–223°C, yield 0.74 g (86%). IR ν (cm^{-1}): 3460 (OH), 3323 (NH), 1653 (C=O), 1622 (C=N), 1593–1531 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.03 (d, J = 9.20 Hz, 1H, aromatic H), 7.37 (t, J = 8.16 Hz, 1H, aromatic H), 7.49–7.63 (m, 6H, aromatic H), 7.76 (br s, 1H, aromatic H), 7.81 (d, J = 7.92 Hz, 1H, aromatic H), 7.94 (d, J = 9.16 Hz, 1H, aromatic H), 7.96 (s, 1H, aromatic H), 8.01 (d, J = 7.48 Hz, 2H, aromatic H), 8.48 (d, J = 8.44 Hz, 1H, aromatic H), 9.66 (s, 1H, CH=N), 10.40 (br s, 1H, NH, D_2O exchangeable), 15.78 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 108.9, 113.3, 115.4, 118.8, 120.6, 122.8, 124.0, 127.1, 128.1, 128.7, 128.9, 129.5, 130.4, 132.2, 133.6, 135.2, 137.6, 140.8, 144.5, 155.8 (aromatic carbons), 166.2 (C=N), 171.4 (C=O). MS (EI): *m*/*z* (%) = 366 (M^+, 89.97), 59 (100). Anal. calcd for $C_{24}H_{18}N_2O_2$ (366.42): C, 78.67; H, 4.95; N, 7.65; found: C, 79.01; H, 5.12; N, 7.88%.

N-(3-((2-Hydroxybenzylidene)amino)phenyl)-2-phenylacetamide (**7c**): The title compound was obtained as yellow crystals, recrystallized from *n*-butanol, m.p. 195–197°C, yield 0.61 g (78%). IR ν (cm^{-1}): 3450 (OH), 3260 (NH), 3047–3026 (CH aromatic), 2993–2920 (CH aliphatic), 1661 (C=O), 1622 (C=N), 1589–1533 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.67 (s, 2H, CH_2), 6.97–7.00 (m, 2H, aromatic H), 7.12 (d, J = 7.84 Hz, 1H, aromatic H), 7.26 (t, J = 6.40 Hz, 1H, aromatic H), 7.32–7.44 (m, 6H, aromatic H), 7.49 (d, J = 8.20 Hz, 1H, aromatic H), 7.67–7.71 (m, 2H, aromatic H), 8.92 (s, 1H, CH=N), 10.32 (br s, 1H, NH, D_2O exchangeable), 13.02 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.8 (CH_2), 112.8, 116.2, 117.1, 118.0, 119.6, 119.7, 127.0, 128.8, 129.6, 130.2, 133.0, 133.8, 136.3, 140.7, 149.0, 160.7 (aromatic carbons), 163.8 (C=N), 169.8 (C=O). MS (EI): *m*/*z* (%) = 330 (M^+, 100). Anal. calcd for $C_{21}H_{18}N_2O_2$ (330.39): C, 76.34; H, 5.49; N, 8.48; found: C, 76.49; H, 5.67; N, 8.67%.

N-(3-{[(2-Hydroxynaphthalen-1-yl)methylidene] amino}phenyl)-2-phenylacetamide (**7d**): The product was obtained as yellow crystals, recrystallized from *n*-butanol, m.p. 194–196°C, yield 0.72 g (80%). IR ν (cm^{-1}): 3441 (OH), 3287 (NH), 3055–3028 (CH aromatic), 2943 (CH aliphatic), 1662 (C=O), 1627 (C=N), 1589–1535 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.69 (s, 2H, CH_2), 7.01 (d, J = 9.16 Hz, 1H, aromatic H), 7.26–7.42 (m, 8H, aromatic H), 7.51–7.55 (m, 2H, aromatic H), 7.78–7.81 (m, 2H, aromatic H), 7.94 (d, J = 9.24 Hz, 1H, aromatic H), 8.45 (d, J = 8.48 Hz, 1H, aromatic H), 9.60 (s, 1H, CH=N), 10.35 (br s, 1H, NH, D_2O exchangeable), 15.71 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.8 (CH_2), 108.8, 112.0, 115.0, 117.6, 120.5, 122.8, 124.0, 127.0, 127.1, 128.71, 128.74, 128.8, 129.5, 129.6, 130.5, 133.5, 136.2, 137.6, 140.8, 144.5, 155.7 (aromatic carbons), 170.0 (C=N), 171.6 (C=O). MS (EI): *m*/*z* (%) = 380 (M^+, 100). Anal. calcd for $C_{25}H_{20}N_2O_2$ (380.45): C, 78.93; H, 5.30; N, 7.36; found: C, 78.70; H, 5.49; N, 7.68%.

2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(3-((2-hydroxybenzylidene)amino)phenyl) acetamide (**12a**): The title compound separated as yellow crystals, crystallized from methanol, m.p. 139–140°C, yield 0.60 g (65%). IR ν (cm^{-1}): 3479 (OH), 3263 (NH), 3082 (CH aromatic), 2970 (CH aliphatic), 1666 (C=O), 1605 (C=N), 1577–1543 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.34 (s, 6H, 2 CH_3), 4.07 (s, 2H, CH_2), 6.96–7.00 (m, 3H, aromatic H), 7.13 (d, J = 7.72 Hz, 1H, aromatic H), 7.38–7.47 (m, 3H, aromatic H), 7.67–7.70 (m, 2H, aromatic H), 8.92 (s, 1H, CH=N), 10.39 (br s, 1H, NH, D_2O exchangeable), 13.00 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.8 (2 CH_3), 36.0 (CH_2), 112.9, 116.2, 116.5, 117.1, 118.0, 119.6, 119.7, 130.3, 133.0, 133.8, 140.5, 149.0, 160.7, 163.8, 167.3, 167.4 (aromatic carbons), 169.7 (C=N), 192.0 (C=O). MS (EI): *m*/*z* (%) = 392 (M^+, 44.38), 181 (100). Anal. calcd for $C_{21}H_{20}N_4O_2S$ (392.48): C, 64.27; H, 5.14; N, 14.28; found: C, 64.49; H, 5.26; N, 14.49%.

2-((4,6-Dimethylpyrimidin-2-yl)thio)-N-(3-(((2-hydroxynaphthalen-1-yl)methylene) amino)phenyl)acetamide (**12b**): The obtained product separated as yellow crystals, crystalized from *n*-butanol, m.p. 204–206°C, yield 0.70 g (67%). IR ν (cm^{-1}): 3444 (OH), 3294 (NH), 3059–3032 (CH aromatic), 2997–2920 (CH aliphatic), 1666 (C=O), 1620 (C=N), 1578–1535 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.35 (s, 6H, CH_3), 4.09 (s, 2H, CH_2), 6.98 (s, 1H, aromatic H), 7.01 (d, J = 9.20 Hz, 1H, aromatic H), 7.34 (t, J = 7.20 Hz, 1H, aromatic H), 7.44–7.47 (m, 3H, aromatic H), 7.55 (t, J = 7.08 Hz, 1H, aromatic H), 7.76 (br s, 1H, aromatic H), 7.80 (d, J = 7.48 Hz, 1H, aromatic H), 7.94 (d, J = 9.20 Hz, 1H, aromatic H), 8.45 (d, J = 8.44 Hz, 1H, aromatic H), 9.61 (s, 1H, CH=N), 10.41 (br s, 1H, NH, D_2O exchangeable), 15.70 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.7 (2 CH_3), 35.9 (CH_2), 108.8, 112.0, 115.0, 116.6, 117.6, 120.5, 122.8, 124.1, 127.1, 128.8, 129.5, 130.6, 133.5, 137.7, 140.5, 144.4, 155.6, 167.5, 169.6 (aromatic carbons), 171.7 (C=N), 193.4 (C=O). MS (EI): *m*/*z* (%) = 442 (M^+, 70.74), 153 (100). Anal. calcd for $C_{25}H_{22}N_4O_2S$ (442.54): C, 67.85; H, 5.01; N, 12.66; found: C, 67.53; H, 5.24; N, 12.48%.

(2E)-1-{3-[(2-Hydroxybenzylidene)amino]phenyl}-3-phenylprop-2-en-1-one (**16a**): The title compound separated as pale yellow crystals on cooling the reaction mixture, recrystallized from 90% ethanol, m.p. 115–117°C, yield 0.61 g (79%). IR ν (cm^{-1}): 3419 (OH), 3062–3022 (CH aromatic), 1662 (C=O), 1618 (C=N), 1607–1574 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.02 (t, J = 8.12 Hz, 2H, aromatic H), 7.44–7.49 (m, 4H, aromatic H), 7.65–7.74 (m, 3H, aromatic H), 7.81 (d, J = 15.60 Hz, 1H, ethylenic H), 7.93–7.95 (m, 2H, aromatic H), 8.03 (d, J = 15.60 Hz, 1H, ethylenic H), 8.09 (d, J = 7.44 Hz, 1H, aromatic H), 8.18 (s, 1H, aromatic H), 9.08 (s, 1H, CH=N), 12.90 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 117.1, 119.7, 119.8, 121.6, 122.5, 126.7, 127.2, 129.2, 129.4, 129.5, 130.4, 131.2, 133.2, 134.0, 135.1, 139.3, 144.9, 149.3, 160.8, 165.1 (aromatic carbons), 189.3 (C=N), 192.3 (C=O). MS (EI): *m*/*z* (%) = 327 (M^+, 100). Anal. calcd for $C_{22}H_{17}NO_2$ (327.38): C, 80.71; H, 5.23; N, 4.28; found: C, 80.53; H, 5.60; N, 4.32%.

(2E)-1-(3-{[(2-Hydroxynaphthalen-1-yl)methylidene]amino}phenyl)-3-phenylprop-2-en-1-one (**16b**): The obtained product was filtered while hot and crystallized from *n*-butanol as yellow crystals, m.p. 203–205°C, yield 0.66 g (74%). IR ν (cm^{-1}): 3414 (OH), 3059–3028 (CH aromatic), 1655 (C=O), 1620 (C=N), 1605–1574 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.07 (d, J = 9.12 Hz, 1H, aromatic H), 7.38 (t, J = 8.00 Hz, 1H, aromatic H), 7.49–7.53 (m, 3H, aromatic H), 7.58 (t, J = 8.00 Hz, 1H, aromatic H), 7.70 (t, J = 8.00 Hz, 1H, aromatic H), 7.81–7.84 (m, 2H, ethylenic H and aromatic H), 7.95–7.99 (m, 4H, aromatic H), 8.04 (d, J = 15.52 Hz, 1H, ethylenic H), 8.05 (d, J = 7.92 Hz, 1H, aromatic H), 8.33 (s, 1H, aromatic H), 8.56 (d, J = 8.48 Hz, 1H, aromatic H), 9.78 (s, 1H, CH=N), 15.63 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 109.2, 121.0, 121.1, 122.3, 122.6, 124.1, 125.6, 126.8, 127.3, 128.7, 129.1, 129.4, 129.5, 130.6, 131.3, 133.6, 135.1, 137.6, 138.9, 139.5, 145.0, 145.4, 157.5, 170.4 (aromatic carbons), 189.4 (C=N), 193.3 (C=O). MS (EI): m/z (%) = 377 (M^+, 100). Anal. calcd for $C_{26}H_{19}NO_2$ (377.44): C, 82.74; H, 5.07; N, 3.71; found: C, 82.41; H, 5.39; N, 3.98%.

N-Benzyl-3-((2-hydroxybenzylidene)amino)benzenesulfonamide (**20a**): The title product was obtained as pale yellow crystals, recrystallized from methanol/chloroform mixture (5:1), m.p. 153–154°C, yield 0.70 g (81%). IR ν (cm^{-1}): 3475 (OH), 3302 (NH), 3063 (CH aromatic), 2980 (CH aliphatic), 1620 (C=N), 1610 (NH bending), 1570 (C=C), 1350, 1150 (SO_2). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.05 (d, J = 5.48 Hz, 2H, CH_2), 7.00–7.04 (m, 2H, aromatic H), 7.20–7.30 (m, 5H, aromatic H), 7.45 (t, J = 7.76 Hz, 1H, aromatic H), 7.65–7.66 (m, 2H, aromatic H), 7.72–7.74 (m, 3H, aromatic H), 8.23 (t, J = 5.72 Hz, 1H, NH, D_2O exchangeable), 8.97 (s, 1H, CH=N), 12.62 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 46.6 (CH_2), 111.6, 117.2, 117.7, 119.7, 125.6, 127.9, 128.1, 128.6, 128.7, 129.9, 130.9, 133.0, 136.9, 137.9, 142.5, 149.4, 160.6, 165.2 (aromatic carbons), 192.5 (C=N). MS (EI): m/z (%) = 366 (M^+, 100). Anal. calcd for $C_{20}H_{18}N_2O_3S$ (366.44): C, 65.56; H, 4.95; N, 7.65; found: C, 65.80; H, 5.12; N, 7.83%.

N-Benzyl-3-(((2-hydroxynaphthalen-1-yl)methylene)amino)benzenesulfonamide (**20b**): The product separated as yellow crystals, recrystallized from *n*-butanol, m.p. 182–184°C, yield 0.85 g (87%). IR ν (cm^{-1}): 3417 (OH), 3244 (NH), 3059–3028 (CH aromatic), 2935 (CH aliphatic), 1620 (C=N), 1574 (C=C), 1350, 1150 (SO_2). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.07 (d, J = 6.32 Hz, 2H, CH_2), 7.09 (d, J = 9.16 Hz, 1H, aromatic H), 7.19–7.27 (m, 5H, aromatic H), 7.40 (t, J = 7.20 Hz, 1H, aromatic H), 7.60 (t, J = 8.32 Hz, 1H, aromatic H), 7.66–7.73 (m, 2H, aromatic H), 7.83–7.90 (m, 3H, aromatic H), 8.00 (d, J = 9.16 Hz, 1H, aromatic H), 8.23 (t, J = 6.32 Hz, 1H, NH, D_2O exchangeable), 8.53 (d, J = 8.48 Hz, 1H, aromatic H), 9.69 (s, 1H, CH=N), 15.39 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 47.4 (CH_2), 109.1, 118.4, 119.3, 121.1, 123.9, 124.6, 125.5, 127.9, 128.0, 128.4, 128.7, 129.4, 130.4, 132.9, 136.0, 137.0, 141.7, 147.3, 157.2, 168.1 (aromatic carbons), 193.3 (C=N). MS (EI): m/z (%) = 416 (M^+, 100). Anal. calcd for $C_{24}H_{20}N_2O_3S$ (416.50): C, 69.21; H, 4.84; N, 6.73; found: C, 69.03; H, 4.97; N, 6.94%.

1-{3-[(2-Hydroxybenzylidene)amino]phenyl}-3-phenylurea (**24a**): The title compound separated as yellow crystals, recrystallized from ethanol, m.p. 227–229°C, yield 0.51 g (65%). IR ν (cm^{-1}): 3290 (OH), 3200, 3125 (2 NH), 3059–3035 (CH aromatic), 1732 (C=O), 1647 (NH bending), 1620 (C=N), 1593–1558 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.98–7.05 (m, 4H, aromatic H), 7.27–7.31 (m, 3H, aromatic H), 7.38 (t, J = 7.88 Hz, 1H, aromatic H), 7.41–7.48 (m, 3H, aromatic H), 7.59 (s, 1H, aromatic H), 7.69 (d, J = 7.60 Hz, 1H, aromatic H), 8.73 (br s, 1H, NH, D_2O exchangeable), 8.81 (br s, 1H, NH, D_2O exchangeable), 8.94 (s, 1H, CH=N), 13.08 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 111.8, 114.8, 117.1, 118.8, 119.7, 122.5, 127.1, 129.3, 129.8, 130.3, 133.0, 133.8, 136.9, 141.2, 149.1, 153.0 (aromatic carbons), 160.7 (C=N), 163.8 (C=O). MS (EI): m/z (%) = 331 (M^+, 10.43), 93 (100). Anal. calcd for $C_{20}H_{17}N_3O_2$ (331.38): C, 72.49; H, 5.17; N, 12.68; found: C, 72.68; H, 5.40; N, 12.50%.

1-(3-{[(2-Hydroxynaphthalen-1-yl)methylidene]amino}phenyl)-3-phenylurea (**24b**): The title compound separated as yellow crystals, recrystallized from ethanol, m.p. 262–265°C, yield 0.56 g (58%). IR ν (cm^{-1}): 3541 (2 NH), 3398 (OH), 3067 (CH aromatic), 1732 (C=O), 1628 (C=N), 1593–1535 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.04 (d, J = 9.16 Hz, 1H, aromatic H), 7.18 (t, J = 9.16 Hz, 1H, aromatic H), 7.39–7.43 (m, 3H, aromatic H), 7.51–7.57 (m, 3H, aromatic H), 7.81–7.90 (m, 4H, aromatic H), 7.96 (d, J = 9.20 Hz, 1H, aromatic H), 8.07 (s, 1H, aromatic H), 8.46 (d, J = 8.44 Hz, 1H, aromatic H), 9.66 (s, 1H, CH=N), 10.90 (br s, 1H, NH, D_2O exchangeable), 10.97 (br s, 1H, NH, D_2O exchangeable), 15.66 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 108.9, 113.2, 116.8, 118.8, 120.6, 121.0, 122.6, 124.1, 125.2, 127.2, 128.7, 129.3, 129.6, 130.5, 133.6, 137.6, 138.0, 139.2, 144.8, 156.1, 158.9 (aromatic carbons), 159.2 (C=N), 171.1 (C=O). MS (EI): m/z (%) = 410 ($M\pm^1$, 9.74), 92 (100). Anal. calcd for $C_{24}H_{19}N_4O_3$ (411.44): C, 75.57; H, 5.02; N, 11.02; found: C, 75.79; H, 5.13; N, 11.34%.

2-[(4,6-Dimethylpyrimidin-2-yl)sulfanyl]-N-(3-nitrophenyl)acetamide (**10**): A suspension of 2-mercapto-4,6-dimethylpyrimidine hydrochloride (**9**) (1.76 g, 10 mmol) and potassium carbonate (2.76 g, 20 mmol) in absolute ethanol (20 mL) was heated under reflux for 30 min. Then, 2-Chloro-*N*-(3-nitrophenyl)acetamide (**8**) (2.15 g, 10 mmol) was added, and the mixture was heated under reflux for another 3 h. The obtained residue was filtered and washed several times with water (3 × 10 mL) and recrystallized from ethanol. The product separated as yellowish-white crystals, m.p. 138–139°C, yield 2.36 g (74%). IR ν (cm^{-1}): 3259 (NH), 3094–3024 (CH aromatic), 2978–2924 (CH aliphatic), 1678 (C=O), 1585–1535 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.32 (s, 6H, 2 CH_3), 4.09 (s, 2H, CH_2), 6.95 (s, 1H, aromatic H), 7.61 (t, J = 8.20 Hz, 1H, aromatic H), 7.90–7.93 (m, 2H, aromatic H), 8.62 (s, 1H, aromatic H), 10.73 (br s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.8 (2 CH_3), 36.0 (CH_2), 113.6, 116.6, 118.2, 125.5,

130.7, 140.6, 148.4, 167.4, 167.9 (aromatic carbons), 169.6 (C=O). MS (EI): *m/z* (%) = 318 (M^+, 5.77), 181 (100). Anal. calcd for $C_{14}H_{14}N_4O_3S$ (318.35): C, 52.82; H, 4.43; N, 17.60; found: C, 52.69; H, 4.65; N, 17.87%.

N-(3-Aminophenyl)-2-[(4,6-dimethyl pyrimidin-2-yl)sulfanyl]acetamide (**11**): A suspension of **10** (1.02 g, 3.23 mmol) in ethanol (9 mL) was slowly added to a solution of stannous chloride dihydrate ($SnCl_2{\cdot}2H_2O$) (2.55 g, 11.31 mmol) and 37% hydrochloric acid (3 mL) while cooling in an ice bath. After 30 min, the reaction mixture allowed to reach room temperature and stirred for a further 48 h. After completion of the reaction, the solution was diluted with water (100 mL) and neutralized with ammonia. The resulting solution was extracted with chloroform, washed with water and dried over anhydrous sodium sulfate. The obtained aniline derivative was crystallized from ethanol.

General procedure for the preparation of compounds **22a,b**

To a suspension of the diphenylurea derivative **21** (0.5 g, 1.94 mmol) in dioxane (10 mL), the appropriate acid chloride (1.94 mmol) was added dropwise while cooling in an ice bath. The reaction mixture was stirred at room temperature for 48 h. The obtained solution was poured onto ice/water and stirred for 30 min. The residue was filtered, washed with water, dried.

1-(3-Nitrophenyl)-3-phenylimidazolidine-2,4,5-trione (**22a**): The title product was separated while hot when the residue was boiled with ethanol as white crystals, m.p. 350–353°C, yield 0.50 g (83%). IR ν (cm^{-1}): 3024 (CH aromatic), 1732 (C=O), 1593–1535 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.47–7.54 (m, 3H, aromatic H), 7.59–7.63 (m, 2H, aromatic H), 7.90–7.97 (m, 2H, aromatic H), 8.36–8.39 (m, 2H, aromatic H). Anal. calcd for $C_{15}H_9N_3O_5$ (311.25): C, 57.88; H, 2.91; N, 13.50; found: C, 58.12; H, 3.17; N, 13.79%.

1-(3-Nitrophenyl)-3-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (**22b**): The title product was separated as buff crystals, recrystallized from ethanol, m.p. 163–165°C, yield 0.50 g (79%). IR ν (cm^{-1}): 3093 (CH aromatic), 2970 (CH aliphatic), 1763 (2 C=O), 1686 (C=O), 1570–1531 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.09 (s, 2H, CH_2), 7.30–7.38 (m, 2H, aromatic H), 7.42–7.52 (m, 3H, aromatic H), 7.80–7.86 (m, 2H, aromatic H), 8.28–8.36 (m, 2H, aromatic H). Anal. calcd for $C_{16}H_{11}N_3O_5$ (325.28): C, 59.08; H, 3.41; N, 12.92; found: C, 59.26; H, 3.56; N, 13.21%.

1-(3-Aminophenyl)-3-phenylurea (**23**): A suspension of **22a,b** (3.23 mmol) in ethanol (9 mL) was slowly added to a solution of stannous chloride dihydrate ($SnCl_2{\cdot}2H_2O$) (2.55 g, 11.31 mmol) and 37% hydrochloric acid (3 mL) while cooling in an ice bath. After 30 min, the reaction mixture had reached room temperature and was then refluxed for 2 h. After completion of the reaction, the solution was diluted with water (100 mL) and neutralized with ammonia solution. The resulting solution was extracted with chloroform, washed with water and dried over anhydrous sodium sulfate. The obtained aniline derivative was used without purification. The title product separated as a yellow oil, yield 0.15–0.18 g (20%–25%). IR ν (cm^{-1}): 3441 and 3329 (NH_2), 3136 (NH), 3059 (CH aromatic), 1720 (C=O), 1630 (NH bending), 1597–1539 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.11 (s, 2H, NH_2), 6.19 (dd, *J* = 7.92, 1.16 Hz, 1H, aromatic H), 6.56 (dd, *J* = 8.84, 0.96 Hz, 1H, aromatic H), 6.78 (s, 1H, aromatic H), 6.90 (t, *J* = 7.96 Hz, 1H, aromatic H), 6.96 (t, *J* = 7.32 Hz, 1H, aromatic H), 7.27 (t, *J* = 8.16 Hz, 2H, aromatic H), 7.44 (d, *J* = 7.68 Hz, 2H, aromatic H), 8.36 (s, 1H, NH), 8.54 (s, 1H, NH). Anal. calcd for $C_{13}H_{13}N_3O$ (227.27): C, 68.70; H, 5.77; N, 18.49; found: C, 68.94; H, 5.83; N, 18.71%.

N-(4-Oxo-4,5-dihydro-1,3-thiazol-2-yl)-2-phenylacetamide (**26b**): To a continuously stirred and cold suspension of pseudothiohydantoin **21** (5.8 g, 50 mmol) in anhydrous pyridine (20 mL), phenylacetyl chloride (5.83 mL, 50 mmol) was added dropwise. The mixture was heated for 4 h under reflux. The solution turned yellow and changed to a clear orange solution, which after being cooled to room temperature was poured onto finely crushed ice. After 12 h, the dark-brown crystals which had separated out were filtered, dried and recrystallized from ethanol/charcoal. The product was obtained as reddish brown crystals, m.p. 205–206°C, yield 7.02 g (60%). IR ν (cm^{-1}): 3410 (NH), 3028 (CH aromatic), 2932 (CH aliphatic), 1713 (C=O), 1666 (C=O), 1624 (NH bending), 1549 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.83 (s, 2H, CH_2), 3.86 (s, 2H, CH_2), 7.27–7.36 (m, 5H, aromatic H), 12.82 (br s, 1H, NH). Anal. calcd for $C_{11}H_{10}N_2O_2S$ (234.27): C, 56.40; H, 4.30; N, 11.96; found: C, 56.71; H, 4.53; N, 12.19%.

General procedure for the preparation of compounds **27a–f**

To a mixture of the appropriate aldehyde (2.27 mmol) and thiazolidinone derivative **22a,b** (2.27 mmol) in absolute ethanol (15 mL), fused sodium acetate (0.18 g, 2.27 mmol) was added. The reaction mixture was heated under reflux for 4 h, then transferred into a beaker and left to cool. The obtained precipitate was filtered, washed, dried and recrystallized from ethanol.

N-[5-(2-Hydroxybenzylidene)-4-oxo-4,5-dihydro-1,3-thiazol-2-yl]benzamide (**27a**): The title compound was obtained as pale brown crystals, m.p. 243–244°C, yield 0.46 g (62%). IR ν (cm^{-1}): 3387 (OH), 3155 (NH), 3062 (CH aromatic), 1701 (2 C=O), 1628 (NH bending), 1597–1539 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.99–7.04 (m, 2H, aromatic H), 7.35 (t, *J* = 7.24 Hz, 1H, aromatic H), 7.51–7.59 (m, 3H, aromatic H), 7.67 (t, *J* = 7.32 Hz, 1H, aromatic H), 8.07 (s, 1H, CH=C), 8.18 (d, *J* = 7.44 Hz, 2H, aromatic H), 10.59 (br s, 1H, OH, D_2O exchangeable), 13.08 (br s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 116.7, 120.2, 120.7, 122.9, 129.1, 129.6, 129.8, 130.0, 133.1, 133.8, 158.0 (aromatic carbons), 169.0, 176.0 (2 C=O). MS (EI): *m/z* (%) = 324 (M^+, 1.38), 77 (100). Anal. calcd for $C_{17}H_{12}N_2O_3S$ (324.35): C, 62.95; H, 3.73; N, 8.64; found: C, 63.11; H, 3.86; N, 8.88%.

N-{5-[(2-Hydroxynaphthalen-1-yl)methylidene]-4-oxo-4,5-dihydro-1,3-thiazol-2-yl}benzamide (**27b**): The product was obtained as dark-brown crystals, m.p. 180–182°C, yield 0.62 g (73%). IR ν (cm^{-1}): 3483 (OH), 3314 (NH),

3067 (CH aromatic), 1697 (2 C=O), 1628 (NH bending), 1578–1531 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.28 (d, J = 9.08 Hz, 1H, aromatic H), 7.41 (t, J = 8.00 Hz, 1H, aromatic H), 7.55–7.67 (m, 2H, aromatic H), 7.72 (t, J = 7.72 Hz, 1H, aromatic H), 7.89–7.97 (m, 2H, aromatic H), 8.05 (d, J = 7.92 Hz, 1H, aromatic H), 8.14 (d, J = 7.20 Hz, 1H, aromatic H), 8.20–8.22 (m, 2H, aromatic H), 9.27 (s, 1H, CH=C), 10.94 (br s, 1H, NH), 12.89 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 113.2, 118.6, 123.7, 123.8, 127.8, 128.3, 128.6, 128.9, 128.9, 129.0, 129.1, 129.6, 129.7, 129.8, 132.1, 132.5, 133.4, 135.7, 135.9 (aromatic carbons), 154.5, 172.5 (2 C=O). Anal. calcd for $C_{21}H_{14}N_2O_3S$ (374.41): C, 67.37; H, 3.77; N, 7.48; found: C, 67.12; H, 3.89; N, 7.63%.

N-[4-Oxo-5-(thiophen-2-ylmethylidene)-4,5-dihydro-1,3-thiazol-2-yl]benzamide (**27c**): The title compound was obtained as yellowish-green crystals, m.p. 224–226°C, yield 0.61 g (85%). IR ν (cm^{-1}): 3391 (NH), 3028 (CH aromatic), 1724 (C=O), 1697 (C=O), 1647 (NH bending), 1589–1551 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.33 (t, J = 4.32 Hz, 1H, thiophene H), 7.58 (t, J = 7.44 Hz, 2H, aromatic H), 7.68 (t, J = 7.32 Hz, 1H, aromatic H), 7.75 (d, J = 3.36 Hz, 1H, thiophene H), 8.07–8.09 (m, 2H, CH=C and thiophene H), 8.20 (d, J = 7.32 Hz, 2H, aromatic H), 13.13 (br s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 122.2, 127.6, 129.2, 129.5, 129.8, 133.9, 134.3, 135.0, 135.9, 137.9 (aromatic carbons and thiophene carbons), 168.7, 176.0 (2 C=O). Anal. calcd for $C_{15}H_{10}N_2O_2S_2$ (314.38): C, 57.31; H, 3.21; N, 8.91; found: C, 57.60; H, 3.49; N, 9.12%.

N-[5-(2-Hydroxybenzylidene)-4-oxo-4,5-dihydro-1,3-thiazol-2-yl]-2-phenylacetamide (**27d**): The product was obtained as light brown crystals, m.p. 267–268°C, yield 0.53 g (69%). IR ν (cm^{-1}): 3263 (OH), 3128 (NH), 3032 (CH aromatic), 2927 (CH aliphatic), 1724 (C=O), 1663 (C=O), 1582–1555 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.89 (s, 2H, CH_2), 6.94–6.98 (m, 2H, aromatic H), 7.27–7.37 (m, 6H, aromatic H), 7.45 (d, J = 7.68 Hz, 1H, aromatic H), 8.08 (s, 1H, CH=C), 10.54 (br s, 1H, NH, D_2O exchangeable), 13.02 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.5 (CH_2), 116.7, 120.2, 120.9, 124.9, 127.5, 128.9, 129.3, 129.9, 130.0, 130.8, 133.0, 134.4 (aromatic carbons), 158.0, 174.0 (2 C=O). Anal. calcd for $C_{18}H_{14}N_2O_3S$ (338.38): C, 63.89; H, 4.17; N, 8.28; found: C, 63.71; H, 4.38; N, 8.05%.

N-{5-[(2-hydroxynaphthalen-1-yl)methylidene]-4-oxo-4,5-dihydro-1,3-thiazol-2-yl}-2-phenylacetamide (**27e**): The title compound was obtained as dark-brown crystals, m.p. 200–201°C (decomposition), yield 0.55 g (63%). IR ν (cm^{-1}): 3395 (OH), 3186 (NH), 3062–3032 (CH aromatic), 2981 (CH aliphatic), 1717 (2 C=O), 1631 (NH bending), 1593–1535 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.91 (s, 2H, CH_2), 7.22–7.37 (m, 3H, aromatic H), 7.46–7.53 (m, 2H, aromatic H), 7.62–7.74 (m, 2H, aromatic H), 7.87–7.90 (m, 2H, aromatic H), 8.11–8.19 (m, 2H, aromatic H), 9.02 (s, 1H, CH=C), 10.15 (br s, 1H, NH, D_2O exchangeable), 13.15 (br s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.5 (CH_2), 112.0, 112.6, 114.0, 125.1, 127.1, 127.6, 127.7, 128.3, 128.7, 128.8, 128.9, 129.1, 129.7, 129.9, 130.0, 130.3, 131.1, 131.8, 133.3, 135.2 (aromatic carbons), 150.2, 178.9 (2 C=O). Anal. calcd for $C_{22}H_{16}N_2O_3S$ (388.44): C, 68.03; H, 4.15; N, 7.21; found: C, 68.21; H, 4.33; N, 7.49%.

N-[4-Oxo-5-(thiophen-2-ylmethylidene)-4,5-dihydro-1,3-thiazol-2-yl]-2-phenylacetamide (**27f**): The product was obtained as brown crystals, m.p. 280–281°C, yield 0.55 g (74%). IR ν (cm^{-1}): 3113 (NH), 3028 (CH aromatic), 2908 (CH aliphatic), 1720 (C=O), 1686 (C=O), 1589–1558 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.90 (s, 2H, CH_2), 7.28–7.36 (m, 6H, aromatic H and thiophene H), 7.71 (d, J = 3.40 Hz, 1H, thiophene H), 8.01 (d, J = 4.92 Hz, 1H, thiophene H), 8.06 (s, 1H, CH=C), 13.11 (br s, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.5 (CH_2), 124.1, 127.5, 128.4, 128.9, 129.6, 129.9, 130.0, 133.8, 134.3, 135.8, 138.5 (aromatic carbons and thiophene carbons), 172.9, 180.4 (2 C=O). Anal. calcd for $C_{16}H_{12}N_2O_2S_2$ (328.40): C, 58.52; H, 3.68; N, 8.53; found: C, 58.74; H, 3.85; N, 8.79%.

Biological evaluation

In vitro MTT cytotoxic activity screening

All target compounds were screened for their cytotoxic activity, at the Confirmatory Diagnostic Unit in VACSERA-Egypt, against the brain SNB-75 and the lung HOP-92 cancer cell lines as well as normal WI-38 fibroblasts using the MTT assay according to the reported procedure.[28] Sorafenib was used as a positive control.

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Supplemental material

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