Full Paper

3-Methyl-2-(4-substituted phenyl)-4,5-dihydronaphtho[1,2-*c*]-pyrazoles: Synthesis and *in-vitro* Biological Evaluation as Antitumour Agents

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The synthetic strategies and characterization of some novel derivatives of 3-methyl-2-(4-substituted phenyl)-4,5-dihydronaphtho[1,2-*c*]pyrazoles carrying different pharmacophores and heterocyclic rings that are relevant to potential antitumour and cytotoxic activities are described. The antitumour activities of the newly synthesized compounds were evaluated according to the protocol of the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay. The results revealed that six compounds, namely **6**, **8**, **11**, **15**, **17** and **18**; displayed promising *in-vitro* antitumour activity in the 60-cell lines assay. The sulfonylthioureido group emerged as the most favourable pharmacophore. Incorporating such thioureido counterpart into the 6-membered 1,3-thiazinan-5-one resulted in better antitumour activities than those displayed by the 5-membered thiazoles and the 6-membered 1,3-thiazinan-4-one ring systems. Further ring expansion led to a total loss of the antitumour activity. The analog **18**, 3-benzyl-2-[4-(3-methyl-4,5-dihydronaphtho-[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-1,3-thiazinan-5-one, proved to be the most active member identified in this series of compounds (GI_{50}, TGI, and LC_{50} MG-MID values of 34.7, 85.1 and 97.7 μM, respectively). The differential cytotoxicity of the six active compounds to cancer and normal cells was studied utilizing the standard MTT cell viability assay. Compounds **17** and **18** were totally selective for the breast cancer cell line MCF7 (IC_{50} 8.5 and 4.7 μM), without exerting any inhibitory effect on the normal breast cell line MCF-10A at the concentration level used (25 μM).

Keywords: Antitumour activity / Cytotoxicity / Dihydronaphtho[1,2-*c*]pyrazoles / Thiazinanes / Thiazoles

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Introduction

The design of new antitumour agents is one of the most active areas in contemporary medicinal chemistry. Nevertheless, the number of drugs available for the treatment of some types of disseminated cancers is still very limited. This might be attributed to the difficulties faced during the search of new anticancer agents; principally the unknown and unexpected etiology of neoplasm and the difficulty in designing selective agents against transformed cells rather than normal cells [1]. Random screening continues to be one of the main routes to discover new structure leads with antitumour activity and the National Cancer Institute (NCI), Bethesda, MD, USA, still playing a pivotal role in this field, through the selection of novel compounds with unique chemical structures [2]. Therefore, new chemical classes of agents that have not had extensive clinical evaluation are prime targets. A common feature of several types of antitumour agents is the presence of primary or secondary sulfonamide moieties in their molecules [3 – 7]. In this regard, diarylsulfo-

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Abbreviations: GI_{50} value (growth inhibitory activity); TGI value (cytostatic activity); LC_{50} value (cytotoxic activity); mean-graph midpoint values (MG-MID)

nylureas and their congeners [8–10] have been focused upon as new chemical entities with a distinct mode of action, which has not been explored [11–13]. Meanwhile, among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, pyrazole-containing compounds have attracted synthetic interest for being an essential structure in many chemotherapeutic agents with potential antimicrobial [14–16], antiparasitic [17, 18], antimalarial [19] and antiviral activities [20, 21]. As far as the anticancer activity is concerned, literature citation revealed that a wide range of pyrazole derivatives were reported to contribute to a variety of antineoplastic potentials against a wide range of cancer cell lines [22–25].

During our ongoing studies aimed at the discovery of new structures endowed with potential chemotherapeutic activities [26–31], a series of 3-(4-chlorophenyl)-indeno[1,2-*c*]pyrazol(in)es substituted with benzenesulfonamide, sulfonylurea, sulfonylthiourea pharmacophores and some derived thiazole-ring systems, was identified as promising antitumour agents [32]. Some compounds showed an interesting antitumour activity often associated with high or moderate specificity for certain human tumour cell lines. In particular, it was found that the oxidized pyrazole derivatives showed better antitumour activity than their parent pyrazoline analogs, whereas the benzenesulfonamides and sulfonylureas showed significant better antitumour spectrum than that of the sulfonylthioureido and derived thiazole analogs. These findings prompted the design and synthesis of a new series of analogs in which the 4-chlorophenyl-indeno[1,2-*c*]pyrazol(in)e moiety was replaced by a 3-methyl-dihydronaphtho[1,2-*c*]pyrazole counterpart in order to evaluate the impact of ring size and lipophilicity alteration on the antitumour activity. All the newly synthesized compounds comprise the pyrazole ring that proved to contribute to a better antitumour activity [32]. In an attempt to add some synergism to the target compounds, it was considered of interest to incorporate some of the sulfonylthioureido derivatives into 6-membered thiazinone, thiazolo[4,3-*c*][1,2,4]triazole and pyrazolo[3,4-*d*]thiazole, in addition to the 5-membered thiazole ring systems, in order to explore the influence of such structure rigidization on the anticipated biological activity. The antitumour activities of the newly synthesized compounds were evaluated according to the protocol of the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay [33–35]. It has been well established that many adverse effects on normal cells and organs are common for most chemotherapeutic drugs, basically due to lack of specificity. These side effects include nephrotoxicity, hematotoxicity, neurotoxicity and impairment of other functions [36]. Consequently, as a complementary part to the present research, it was thought worthwhile to investigate the differential cytotoxic effect of the NCI-active antitumour analogs on both cancerous and normal cells. Their *in-vitro* cytotoxic effect was determined via a modification of the MTT cell viability technique [37], against a panel of two cell types: breast cancer cell line MCF7 and normal breast cell line MCF-10A.

Results and discussion

Chemistry

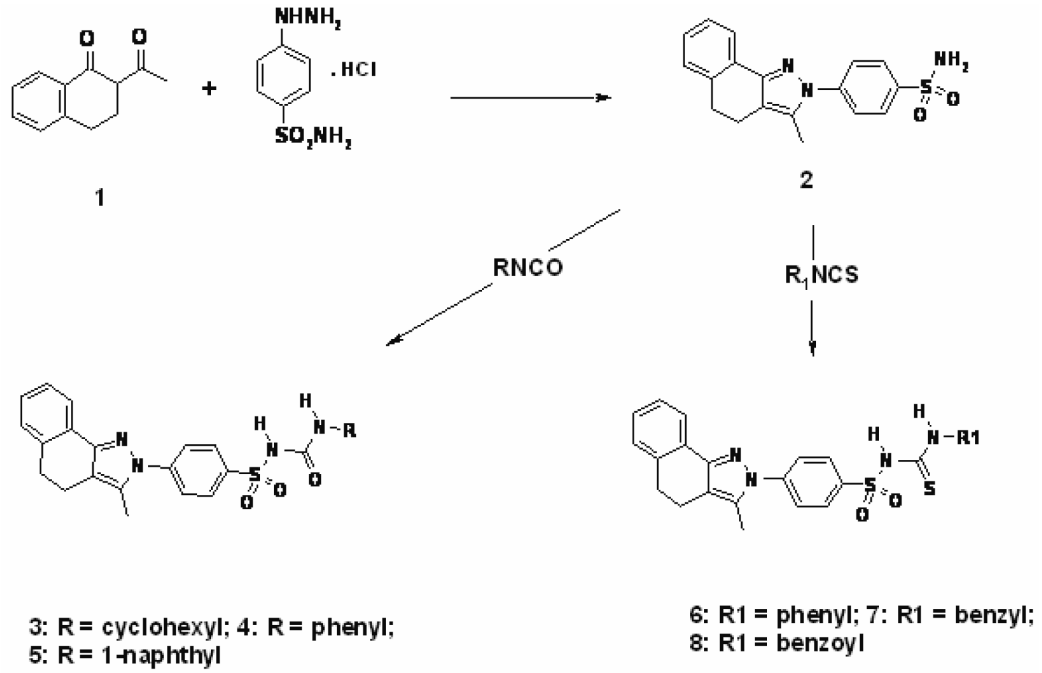
The suggested synthetic plans to obtain the target compounds are shown in Schemes 1–3. The key intermediate in this study is 3-methyl-2-(4-sulfamylphenyl)-4,5-dihydronaphtho[1,2-*c*]pyrazole **2**, which was synthesized by a simple reproducible procedure involving the 1,3-cyclocondensation of 2-acetyl-1-tetralone **1** with 4-hydrazinobenzenesulfonamide hydrochloride. Condensation of **2** with different isocyanates and isothiocyanates yielded the corresponding substituted benzene-sulfonylureas **3–5** and sulfonylthioureas **6–8**, respectively (Scheme 1). Cyclization of the sulfonylthioureido derivatives **6–8** to the corresponding 4-oxothiazolidines **9–11** and thiazolines **12–14** was effected by reacting the former with ethyl bromoacetate and phenacyl bromide in the presence of sodium acetate, respectively. Analogously, it is considered of interest to cyclize the sulfonylthioureido derivatives **6, 7** to the respective 1,3-thiazinan-4-ones **15, 16** and 1,3-thiazinan-5-ones **17, 18** target compounds using ethyl 3-bromopropionate and 1,3-dichloroacetone, respectively, as interesting structure variants (Scheme 2).

At this stage, it was attempted to prepare some condensed ring systems starting from the 4-oxothiazolidine analogs. In this view, when **11** (R = C_6H_5CO) was reacted with hydrazine hydrate in refluxing ethanol, the corresponding thiazolo[4,3-*c*][1,2,4]triazole derivative **19** was obtained. Moreover, condensing the 4-oxothiazolidine analog **9** (R = C_6H_5) with benzaldehyde in the presence of sodium hydroxide resulted in the formation of the arylidine compound **20**. The latter chalcone **20** in its turn, was condensed with methylhydrazine to afford the targeted pyrazolo[3,4-*d*]thiazole **21** (Scheme 3).

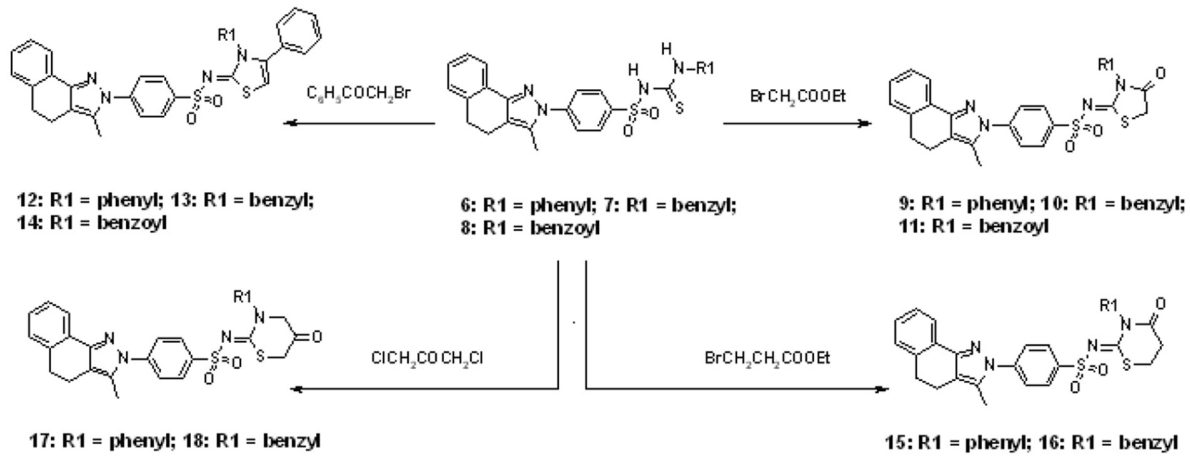
In-vitro antitumour activity

Primary in-vitro one-dose 3-cell line assay

Out of the newly synthesized analogs, twelve compounds, namely **2**, **4**, **6–9**, **11**, **15**, **17–19** and **21** were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be



Scheme 1. Synthesis route of compounds **1 – 8**.

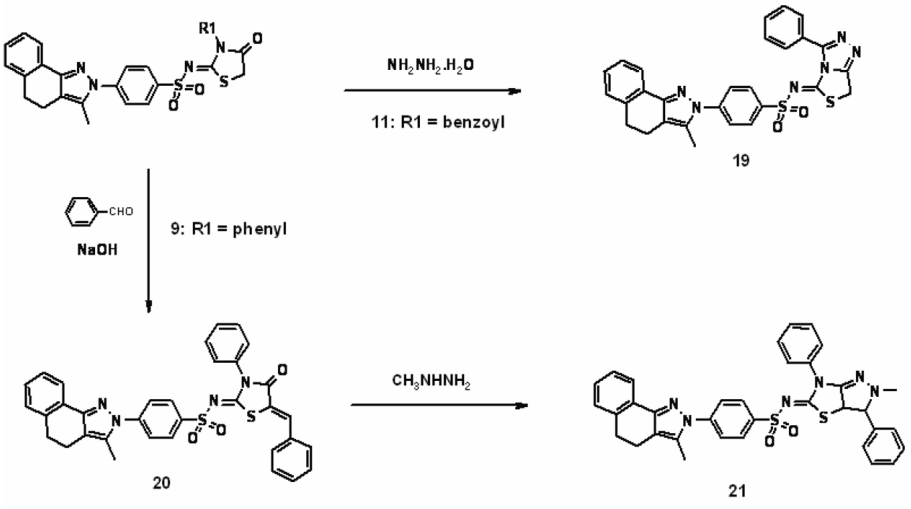


Scheme 2. Synthesis route of compounds **9 – 18**.

evaluated for their *in-vitro* antitumour activity. Primary *in-vitro* one-dose anticancer assay was performed using the 3-cell line panel consisting of NCI-H460 (lung), MCF7 (breast) and SF-268 (CNS) in accordance with the protocol of the Drug Evaluation Branch, NCI, Bethesda, MD, USA [33 – 35]. The compounds were added at a single concentration (10^{-4} M) and the culture was incubated for 48 h. End point determinations were made with a protein-binding dye, sulphorhodamine B (SRB). Results for each compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. All the compounds which reduced the growth of any one of the cell lines to 32% or less were passed on for evaluation in the full panel of 60 human tumour cell lines [33 – 35]. The results revealed that six compounds, namely **6**, **8**, **11**, **15**, **17** and **18** passed this primary anticancer assay and consequently were carried over to be tested against a panel of 60 different tumour cell lines.

In-vitro full-panel 60-cell line assay

About 60 cell lines of nine tumour subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma,



Scheme 3. Synthesis of compounds **19–21**.

ovarian, renal, prostate and breast cancer cell lines, were incubated with five concentrations (0.01–100 μM) for each compound and were used to create: log concentration - %growth inhibition curves. Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compounds causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compounds resulting in total growth inhibition and the LC_{50} value (cytotoxic activity) is the concentration of the compounds causing net 50% loss of initial cells at the end of the incubation period (48 h). Subpanel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI, or LC_{50} values of all cell lines in the subpanel or the full panel, respectively. The NCI antitumour drug discovery was designed to distinguish between broad spectrum antitumour compounds and tumours or subpanel-selective agents.

In the present study, the active compounds exhibited remarkable antitumour activities against most of the tested subpanel tumour cell lines (GI_{50}, TGI and LC_{50} values <100 μM). These compounds showed a distinctive pattern of sensitivity against some individual cell lines (Table 1), as well as a broad spectrum of antitumour activity (Tables 2 and 3).

With regard to the sensitivity against some individual cell lines, compound **6** showed high sensitivity against melanoma MALME-3M and renal CAKI-1 cell lines with GI_{50} values of 1.15 and 1.85 μM, respectively. Whereas, the analog **8** exhibited remarkable sensitivity against three leukemia cell lines, namely HL-60 (TB), K-562 and SR with GI_{50} values of 3.85, 6.09 and 0.76 μM, respectively. Moreover, compound **17** proved to be sensitive to the leukemia subpanel with GI_{50} range of 1.88–28.10 μM. Furthermore, compound **18** proved to be sensitive towards some of the tested subpanel tumour cell lines including the leukemia subpanel with GI_{50} range of 6.45–31.1 μM. It showed noticeable sensitivity against melanoma LOX IMVI, SK-MEL-5 and breast MCF7 cell lines with GI_{50} values of 10.7, 9.09 and 3.74 μM, respectively (Table 1).

With regard to the broad spectrum antitumour activity (Tables 2 and 3), the results revealed that all of the active compounds showed effective growth inhibition GI_{50} (MG-MID) beside a cytostatic activity TGI (MG-MID). In addition, only one compound, namely **18**, exhibited weak cytotoxic activity LC_{50} (MG-MID). Further interpretation of the results revealed that, compound **18** (GI_{50}, TGI and LC_{50} MG-MID values of 34.7, 85.1 and 97.7 μM, respectively) proved to be the most active member in this series with a broad spectrum of activity against all the tested subpanel tumour cell lines and particular effectiveness on the leukemia subpanel at the GI_{50} level (20.0 μM). On the other hand, compounds **8**, **11** and **17** displayed moderate antitumour activity lying almost in the same range (GI_{50} MG-MID values 42.6–44.8 μM), while their cytostatic values were significantly lower (TGI MG-MID values 79.4–91.4 μM). Finally, compound **15** proved to be the least active member within this series as evidenced by its GI_{50} MG-MID value (89.1 μM). Meanwhile, it was totally deprived of any cytostatic and cytotoxic activities.

The ratio obtained by dividing the full panel MG-MID (μM) of the compounds by their individual subpanel MG-MID (μM) is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity towards

Table 1. Growth inhibitory concentration (GI_{50}, μM) values of the *in-vitro* tumour cell lines.[a)]

Cell Lines	**6**	**8**	**11**	**15**	**17**	**18**
Leukemia						
CCRF-CEM	NT[b)]	– [c)]	21.0	-	28.1	6.45
HL-60 (TB)	–	3.85	65.5	–	–	34.1
K-562	–	6.09	79.2	–	17.6	30.1
MOLT-4	–	–	–	–	19.6	9.62
PRMI-8226	15.5	36.3	NT	NT	7.85	31.1
SR	–	0.76	69.9	65.0	1.88	8.35
Non-Small Cell Lung Cancer						
A 549 / ATCC	–	54.1	–	–	–	46.6
EKVX	–	NT	40.5	94.0	–	46.7
HOP-62	NT	–	33.5	46.4	12.7	–
HOP-92	–	–	30.9	81.3	–	–
NCI – H226	–	62.2	24.0	84.4	9.75	38.5
NCI – H23	46.8	53.9	27.7	–	23.2	49.8
NCI – H322M	–	24.5	–	23.5	–	–
NCI – H460	NT	45.0	47.5	–	15.2	29.4
NCI – H522	21.5	50.4	96.2	91.4	15.4	22.6
Colon Cancer						
COLO 205	–	44.9	NT	–	–	63.4
HCC – 2998	NT	NT	98.6	–	NT	NT
HCT – 116	18.2	89.2	26.4	–	6.50	72.9
HCT – 15	38.8	39.2	22.4	–	21.2	31.7
HT29	–	–	63.5	–	34.5	51.4
KM12	25.6	34.4	51.4	–	46.5	60.1
SW – 620	–	–	–	NT	19.1	25.5
CNS Cancer						
SF-268	18.7	29.3	44.6	35.0	20.2	17.6
SF-295	–	17.4	21.6	75.7	–	25.7
SF-539	–	NT	NT	NT	22.4	29.5
SNB-19	–	96.4	NT	–	–	–
SNB-75	–	59.5	NT	NT	–	75.9
U251	39.6	43.3	23.3	–	21.1	21.8
Melanoma						
LOX IMVI	25.8	50.3	–	–	5.59	10.7
MALME-3M	1.15	NT	NT	NT	–	41.4
M14	–	–	22.9	–	13.4	46.5
SK-MEL-2	–	–	–	–	18.2	58.2
SK-MEL-28	–	41.1	52.5	–	28.5	24.6
SK-MEL-5	–	30.7	25.1	95.5	8.11	9.09
UACC-257	–	10.7	92.5	–	45.3	38.2
UACC-62	–	29.0	18.7	–	16.0	28.3
Ovarian Cancer						
IGROV1	20.6	–	–	–	8.31	24.0
OVCAR-3	36.8	13.7	15.8	–	–	25.2
OVCAR-4	–	–	77.5	–	37.6	97.6
OVCAR-5	–	–	49.9	–	–	–
OVCAR-8	–	72.4	68.8	89.6	–	41.0
SK-OV-3	–	–	NT	–	43.3	88.9
Renal Cancer						
786-0	26.4	–	28.9	81.7	13.3	89.2
ACHN	–	41.0	24.6	–	24.1	32.9
CAKI-1	1.85	16.1	26.6	–	18.1	26.6
RXF-393	–	NT	28.8	70.9	31.5	22.6
SN12C	–	34.4	19.2	87.5	17.8	26.7
TK-10	10.0	28.9	98.0	–	–	50.4
UO-31	–	–	67.4	–	28.4	64.3
Prostate Cancer						
PC-3	39.9	42.0	42.1	–	20.4	27.7
DU-145	–	23.9	38.6	–	43.9	50.5
Breast Cancer						
MCF7	11.5	35.9	NT	NT	7.79	3.74
NCI/ADR-RES	–	–	–	–	14.5	39.3
MDA-MB-231/ATCC	-	42.5	24.0	35.1	15.4	24.8
HS 578T	44.6	NT	NT	NT	15.5	NT
MDA-MB-435	14.4	27.9	26.1	–	16.8	20.6
BT-549	–	NT	38.6	–	11.1	22.5
T-47D	–	44.9	NT	–	24.7	82.9

a) The data obtained from NCI's *in-vitro* disease-oriented human tumour cell screen.
b) NT = not tested.
c) GI_{50} value >100 μM.

the corresponding cell line, while compounds not meeting either of these criteria are rated non-selective [38]. In this context, the active compounds in the present study were found to be non-selective with broad spectrum antitumour activity against the nine tumour subpanels tested, with selectivity ratios ranging between 0.5 – 1.73.

A close examination of the structures of the active compounds revealed that the benzene-sulfonamide parent-compound **2** and the sulfonylureido derivative **4** were totally inactive, whereas the sulfonylthioureido derivatives **6** and **8** showed moderate antitumour activity which varied greatly according to the nature of the substituent. Compound **6** (R_1 = phenyl) showed GI_{50} MG-MID value of 69.4 μM. Replacing the phenyl to a benzoyl moiety as in compound **8** resulted in a remarkable improvement in the growth inhibitory activity (GI_{50} MG-MID value 69.4 μM). Meanwhile, at the cytostatic level, both compounds **6** and **8** displayed almost the same level of activity (TGI MG-MID values 98.0 and 93.3 μM, respectively). Introducing a benzyl substituent as in compound **7** resulted in a complete abolishment in the antitumour activity.

On the other hand, cyclization of the sulfonylthioureido derivative **8** to the thiazolidinone **11** (R = phenyl) did not result in a significant improvement in the antitumour activity (GI_{50} MG-MID values 42.6 vs 44.7 μM, respectively). However, the cytostatic potential of **11** has been remarkably improved when compared with **8** (79.4 vs 93.3 μM, respectively). Nevertheless, a dramatic reduction in the antitumour activity was encountered with ring enlargement to the 6-membered 1,3-thiazinan-4-one **15** (R_1 = phenyl), which proved to be the least active member within this series (GI_{50} MG-MID value 89.1 μM), and without any cytostatic and cytotoxic effects. On the con-

Table 2. Median growth inhibitory concentrations (GI_{50}, μM) of *in-vitro* subpanel tumour cell lines.[a)]

Cpd. No.	Subpanel tumour cell lines[b)]									MG-MID[c)]
	I	II	III	IV	V	VI	VII	VIII	IX	
6	86.2	91.0	77.6	86.0	81.8	85.8	68.0	90.0	73.6	69.4
8	41.2	61.0	68.0	49.2	51.7	81.0	53.4	33.0	50.2	42.6
11	67.1	55.6	60.4	29.8	58.8	62.4	41.9	40.3	47.2	44.7
15	93.0	80.1	–[d)]	77.7	99.4	98.3	91.4	–	87.0	89.1
17	41.6	63.4	59.2	72.2	48.0	79.8	52.4	64.2	30.2	44.8
18	20.0	59.3	50.8	45.1	36.3	62.6	44.7	39.1	32.3	34.7
M*	20.1	38.5	42.1	17.1	31.9	43.0	34.4	34.7	39.2	27.1

a) Data obtained from NCI's *in-vitro* disease-oriented human tumour cell screen.
b) I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.
c) GI_{50} (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent.
d) Subpanel GI_{50} value >100 μM.
* Melphalan (A reference standard antineoplastic agent).

Table 3. Median total growth inhibitory concentrations (TGI, μM) of *in-vitro* subpanel tumour cell lines.[a)]

Cpd. No.	Subpanel tumour cell lines[b)]									MG-MID[c)]
	I	II	III	IV	V	VI	VII	VIII	IX	
6	97.0	–[d)]	–	–	–	–	95.2	–	95.6	98.0
8	–	97.7	–	89.7	92.0	90.2	88.4	–	98.3	93.3
11	89.8	87.4	84.7	73.5	76.2 (95.4)[e)]	87.7 (99.0)	76.7	-	78.1	79.4
15	–	–	–	–	–	–	–	–	–	–
17	–	94.4	88.6 (96.4)	–	86.8	86.0	–	–	91.8	91.4
18	–	95.5	–	79.1	75.0 (86.2)	94.7	91.7	–	70.9	85.1 (97.7)
M*	66.3	–	–	81.9	73.9	–	–	–	–	98.3

a) Data obtained from NCI's *in-vitro* disease-oriented human tumour cell screen.
b) For subpanel tumour cell lines, see footnote (&) of Table 2.
c) TGI (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent
d) Subpanel TGI value >100 μM.
e) LC_{50} (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines towards the test agent.
* Melphalan (A reference standard antineoplastic agent).

trary, the 1,3-thiazinan-5-one **17** (R_1 = phenyl) showed an obvious higher activity than the parent sulfonyl-thioureido derivative **6** (GI_{50} MG-MID values 44.8 vs 69.4 μM, respectively). At the cytostatic level, both compounds **6** and **17** displayed almost the same level of activity (TGI MG-MID values 98.0 and 91.4 μM, respectively). Replacing the phenyl substituent with a benzyl one gave rise to the analog **18**, which proved to be the most active member identified in this series of compounds (GI_{50}, TGI, and LC_{50} MG-MID values of 34.7, 85.1 and 97.7 μM, respectively). Finally, further ring enlargement to the fused thiazolo[4,3-*c*][1,2,4]triazole and pyrazolo[3,4-*d*]thiazole ring systems led to completely inactive derivatives **19** and **21**.

The antitumour activities of the active compounds were compared with that of the known antineoplastic agent, melphalan. Compound **18** showed an overall tumour growth inhibitory activity comparable with that displayed by melphalan (Table 2). In addition, the same compound proved to be nearly equipotent to melphalan against the leukaemia, melanoma, prostate and breast subpanel tumour cell lines (Table 2). Meanwhile, except for compound **6**, the other five active compounds were more active than melphalan as cytostatic agents as indicated from their TGI MG-MID values (Table 3).

In-vitro MTT cytotoxicity assay

Chemotherapy very often induces severe side effects, which are in part a consequence of destruction of normal cells. As a complementary part for this study, it was thought worthwhile to evaluate the differential cyto-

toxic effect of the NCI-active antitumour analogs: **6**, **8**, **11**, **15**, **17** and **18** on both cancerous and normal cells. Their *in-vitro* cytotoxic effect was determined via a modification of the MTT method [37], against a panel of two cell types: breast cancer cell line MCF7 and normal breast cell line MCF-10A.

MTT assay is a standard colorimetric assay for measuring cell growth. It is used to determine cytotoxicity of potential medicinal agents and other toxic materials. In brief, yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is reduced to purple formazane by mitochondrial dehydrogenases of living cells. A suitable solvent is added to dissolve the insoluble purple formazane product into a coloured solution. The absorbance of this coloured solution can be quantified by measuring at a certain wavelength. When the amount of purple formazane produced by cells treated with an agent is compared with that produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve. The results represent the mean of four independent experiments and are expressed as IC_{50}, i. e., the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.

Particular interest was focussed on the compounds that were able to preferentially inhibit the growth of the MCF7 human breast cancer cell line over the normal breast epithelial cell line MCF-10A. In this respect, compounds **17** and **18** were totally selective for the cancer cell line at low micromolar concentrations (IC_{50} 8.5 and 4.7 μM), without any inhibitory effect on the normal breast epithelial cell line MCF-10A (IC_{50} > 25 μM). On the other hand, in the presence of compounds **5** and **8**, growth of the human breast cancer cell line MCF7 was inhibited with obviously higher IC_{50} values ranging from 16.3 to 19.7 μM. Cell growth in the normal breast epithelial cell line MCF-10A showed almost the same level of growth inhibition at IC_{50} values of 22.4 and 24.1 μM, respectively. Finally, cell growth in both cancerous and normal breast cell lines appeared unaffected at the maximum concentration utilized (25 μM) in the presence of the test compounds **11** and **15**.

Conclusion

In conclusion, according to the protocol of the NCI *in-vitro* disease-oriented human cells screening panel assay, the obtained data suggests that the sulfonylthioureido group proved to be the most favourable pharmacophore rather than the parent benzenesulfonamide and the corresponding sulfonylureido isosteres. On the other hand, the ring size at the benzenesulfonylimino functionality together with their substitution pattern seemed be relevant to their biological activity. Incorporating the thioureido counterpart into the 6-membered 1,3-thiazinan-5-one ring system resulted in better antitumour activities than those displayed by the 5-membered thiazoles and the 6-membered 1,3-thiazinan-4-ones. Further ring expansion led to a total loss of the antitumour activity. In general, the overall antitumour activity of such type of dihydronaphtho[1,2-*c*]pyrazoles was less than their corresponding indeno[1,2-*c*]pyrazol(in)es substituted with the same pharmacophores [32]. Interestingly, two compounds, namely **17** and **18** were preferentially able to inhibit the growth of human breast cancer cell line MCF7, as compared to normal epithelial cells MCF-10A in a modified MTT assay. However, the broad spectrum antitumour effects expressed by the active compounds against most of the tested tumour cell lines increases the likelihood of their future derivatization in order to explore the scope and limitations of their activities.

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The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus (Weiss-Gallenkamp, London, UK) and were uncorrected. The infrared (IR) spectra were recorded on Perkin-Elmer 297 infrared spectrophotometer (Perkin Elmer, Beaconsfield, UK) using the NaCl plate technique. The ^{1}H-NMR spectra were recorded on a Varian EM 360 spectrometer (Varian Inc., Palo Alto, CA, USA) using tetramethylsilane as the internal standard and DMSO-d_6 as the solvent (Chemical shifts in (d, ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet. Elemental analyses were performed at the Microanalytical Unit, Faculty of Science, King Abdul-Aziz University, Jeddah, Saudi Arabia, and the found values were within ± 0.4% of the theoretical values. Physicochemical and analytical data are recorded in Table 4. Follow up of the reactions and checking the homogeneity of the compounds were

made by TLC on silica gel-protected aluminium sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV-lamp at λ 254.

3-Methyl-2-(4-sulfanylphenyl)-4,5-dihydronaphtho[1,2-c]pyrazole ***2***

A solution of 2-acetyl-1-tetralone (3.8 g, 20 mmol) in ethanol (30 mL) was heated under reflux with 4-hydrazinobenzenesulfonamide hydrochloride (4.9 g, 22 mmol) for 3 h. On concentration, the separated solid product was filtered, washed with cold ethanol and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 4. IR (cm^{-1}): 1310 and 1175 (SO_2N), 3240 and 3345 (NH_2). ^{1}H-NMR (δ, ppm): 2.29 (s, 3H, CH_3), 2.67 (m, 2H, C_4-2H), 3.02 (m, 2H, C_5-2H), 6.84 – 8.02 (m, 10H, Ar-H + 2NH).

N^1-Substituted N^3-[4-(3-methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonyl]-ureas ***3–5***

A mixture of the pyrazole **2** (1.7 g, 5 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol) in dry acetone (20 mL) was heated with the appropriate isocyanate (6 mmol) under reflux with stirring for 18 h. The solvent was removed under reduced pressure and the remaining solid residue was dissolved in water (20 mL). After acidification of the resulting solution with 2 N hydrochloric acid, the precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 4. IR cm^{-1}: 1300 – 1335 and 1165 – 1200 (SO_2N), 1640 – 1655 (C=O). ^{1}H-NMR (δ, ppm) or **3** (R = cyclohexyl): 1.58 – 2.13 (m, 11H, cyclohexyl-H), 2.28 (s, 3H, CH_3), 2.64 (m, 2H, C_4-2H), 3.19 (m, 2H, C_5-2H), 6.39 – 7.89 (m, 10H, 2 NH + 8 Ar-H); for **4** (R = phenyl): 2.25 (s, 3H, CH_3), 2.63 (m, 2H, C_4-2H), 3.05 (m, 2H, C_5-2H), 6.70 – 8.04 (m, 15H, 2 NH + 13 Ar-H); for **5** (R = 1-naphthyl): 2.30 (s, 3H, CH_3), 2.71 (m, 2H, C_4-2H), 3.08 (m, 2H, C_5-2H), 6.88 – 8.22 (m, 17H, 2 NH + 15 Ar-H).

N^1-Substituted N^3-[4-(3-methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonyl]-thioureas ***6–8***

A solution of the appropriate isothiocyanate (6 mmol) in dry acetone (5 mL) was added to a stirred mixture of the pyrazole **2** (1.7 g, 5 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol) in dry acetone (20 mL), and the reaction mixture was heated under reflux with stirring for 10 h. The solvent was removed under reduced pressure and the remaining residue was dissolved in water (20 mL) and acidified with 2 N hydrochloric acid. The precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 4. IR cm^{-1}: 1300 – 1335 and 1165 – 1300 (SO_2N), 1155 – 1165 (C=S), 1650 (C=O, **8**: R_1 = CO-C_6H_5). ^{1}H-NMR (δ, ppm) for **6** (R_1 = phenyl): 2.32 (s, 3H, CH_3), 2.59 (m, 2H, C_4-2H), 3.11 (m, 2H, C_5-2H), 6.86 – 8.12 (m, 15H, 2 NH + 13 Ar-H); for **7** (R_1 = benzyl): 2.28 (s, 3H, CH_3), 2.61 (m, 2H, C_4-2H), 3.08 (m, 2H, C_5-2H), 4.75 (d, 2H, CH_2-C_6H_5), 6.90-8.05 (m, 15H, 2 NH + 13 Ar-H); for **8** (R_1 = benzoyl): 2.31 (s, 3H, CH_3), 2.72 (m, 2H, C_4-2H), 3.17 (m, 2H, C_5-2H), 6.86 – 7.95 (m, 15H, 2 NH + 13 Ar-H).

2-[4-(3-Methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonylimino]-3-substituted-thiazolidin-4-one ***9–11***

To a solution of the appropriate thioureido derivative **6 – 8** (5 mmol) in absolute ethanol (20 mL) was added ethyl bromoacetate (1 g, 6 mmol) and anhydrous sodium acetate (0.82 g, 10 mmol), and the reaction mixture was heated under reflux for 2 h. The reaction mixture was left to attain room temperature then poured into ice-cold water (30 mL). The solid product thus formed was filtered, washed with water, dried and recrystallized from ethanol-benzene mixture (1 : 1). Physicochemical and analytical data are recorded in Table 4. IR cm^{-1}: 1720 – 1735 (thiazol-C=O), 1660 (C=O, **11**: R_1 = CO-C_6H_5). ^{1}H-NMR (δ, ppm) for **9** (R_1 = phenyl): 2.29 (s, 3H, CH_3), 2.66 (m, 2H, C_4-2H), 3.01 (m, 2H, C_5-2H), 4.24 (s, 2H, thiazol-CH_2), 6.88 – 7.93 (m, 13H, Ar-H); for **10** (R_1 = benzyl): 2.27 (s, 3H, CH_3), 2.68 (m, 2H, C_4-2H), 3.05 (m, 2H, C_5-2H), 4.22 (s, 2H, thiazol-CH_2), 4.80 (s, 2H, CH_2-C_6H_5), 6.87 – 7.98 (m, 13H, Ar-H); for **11** (R_1 = benzoyl): 2.31 (s, 3H, CH_3), 2.65 (m, 2H, C_4-2H), 3.02 (m, 2H, C_5-2H), 4.28 (s, 2H, thiazol-CH_2), 6.79 – 8.02 (m, 13H, Ar-H).

Table 4. Physicochemical and analytical data of compounds **2 – 21**.

Cpd.	R or R_1	Mp. (°C)	Yield (%)	Mol. formula (Mol. weight)
2	-	270-2	92	$C_{18}H_{17}N_3O_2S$ (339.41)
3	Cyclohexyl	>310	82	$C_{25}H_{28}N_4O_3S$ (464.58)
4	Phenyl	144-6	84	$C_{25}H_{22}N_4O_3S$ (458.53)
5	1-Naphthyl	212-3	86	$C_{29}H_{24}N_4O_3S$ (508.59)
6	Phenyl	184-5	83	$C_{25}H_{22}N_4O_2S_2$ (474.60)
7	Benzyl	162-4	77	$C_{26}H_{24}N_4O_2S_2$ (488.63)
8	Benzoyl	136-8	78	$C_{26}H_{22}N_4O_3S_2$ (502.61)
9	Phenyl	222-3	74	$C_{27}H_{22}N_4O_3S_2$ (514.62)
10	Benzyl	266-7	72	$C_{28}H_{24}N_4O_3S_2$ (528.65)
11	Benzoyl	216-8	76	$C_{28}H_{22}N_4O_4S_2$ (542.63)
12	Phenyl	154-6	70	$C_{33}H_{26}N_4O_2S_2$ (574.72)
13	Benzyl	260-2	72	$C_{34}H_{28}N_4O_2S_2$ (588.74)
14	Benzoyl	214-5	74	$C_{34}H_{26}N_4O_3S_2$ (602.73)
15	Phenyl	266-7	71	$C_{28}H_{24}N_4O_3S_2$ (528.65)
16	Benzyl	272-4	74	$C_{29}H_{26}N_4O_3S_2$ (542.67)
17	Phenyl	182-4	75	$C_{28}H_{24}N_4O_3S_2$ (528.65)
18	Benzyl	209-1	76	$C_{29}H_{26}N_4O_3S_2$ (542.67)
19	–	279-1	72	$C_{28}H_{22}N_6O_2S_2$ (538.65)
20	–	110-2	70	$C_{34}H_{26}N_4O_3S_2$ (602.73)
21	–	278-9	65	$C_{35}H_{30}N_6O_2S_2$ (630.78)

2-[4-(3-Methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonylimino]-4-phenyl-3-substituted-1,3-thiazolines ***12 – 14***

A solution of the appropriate thioureido derivative **6 – 8** (5 mmol) in absolute ethanol (20 mL) was refluxed with phenacyl bromide (1.2 g, 6 mmol) and anhydrous sodium acetate (0.82 g, 10 mmol), for 3 h during which a solid product was partially crystallized out. The mixture was left to attain room temperature then filtered, washed with cold ethanol, dried and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 4. ^{1}H-NMR (d, ppm) for **12** (R_1 = phenyl): 2.33 (s, 3H, CH_3), 2.68 (m, 2H, C_4-2H), 3.09 (m, 2H, C_5-2H), 5.80 (s, 1H, thiazol-H), 6.91 – 8.18 (m, 18H, Ar-H); for **13** (R_1 = benzyl): 2.30 (s, 3H, CH_3), 2.71 (m, 2H, C_4-2H), 3.11 (m, 2H, C_5-2H), 4.77 (s, 2H, CH_2-C_6H_5), 5.83 (s, 1H, thiazol-CH), 6.85 – 8.10 (m, 18H, Ar-H); for **14** (R_1 = benzoyl): 2.33 (s, 3H, CH_3), 2.68 (m, 2H, C_4-2H), 3.05 (m, 2H, C_5-2H), 5.75 (s, 1H, thiazol-CH), 6.80-8.05 (m, 18H, Ar-H).

2-[4-(3-Methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonylimino]-3-substituted-1,3-thiazinan-4-ones ***15, 16***

To a solution of the appropriate thioureido derivative **6** or **7** (5 mmol) in absolute ethanol (20 mL) was added ethyl 3-bromopropionate (1.1 g, 6 mmol) and sodium acetate (0.82 g, 10 mmol) and the reaction mixture was heated under reflux for 4 h. The reaction mixture was worked up as described under compounds **9 – 11**. Physicochemical and analytical data are recorded in Table 4. IR cm^{-1}: 1720 – 1730 (C=O). ^{1}H-NMR (δ, ppm) for **15** (R_1 = phenyl): 2.29 (s, 3H, CH_3), 2.64 (m, 2H, C_4-H), 2.93 (m, 4H, C_5-2H + thiazinan-CH_2), 3.20 (t, 2H, thiazinan-CH_2), 6.82 – 8.00 (m, 13H, Ar-H); for **16** (R_1 = benzyl): 2.30 (s, 3H, CH_3), 2.66 (m, 2H, C_4-2H), 3.01 (m, 4H, C_5-2H + thiazinan-CH_2), 3.23 (t, 2H, thiazinan-CH_2), 4.75 (s, 2H, CH_2-C_6H_5), 6.85 – 7.99 (m, 13H, Ar-H).

2-[4-(3-Methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonylimino]-3-substituted-1,3-thiazinan-5-ones ***17, 18***

To a solution of the appropriate thioureido derivative **6** or **7** (5 mmol) in absolute ethanol (20 mL) was added 1,3-dichloroacetone (0.76 g, 6 mmol) and sodium acetate (0.82 g, 10 mmol) and the reaction mixture was heated under reflux for 5 h. The reaction mixture was worked up as described under compounds **9 – 11**. Physicochemical and analytical data are recorded in Table 4. IR cm^{-1}: 1720 – 1730 (C=O). ^{1}H-NMR (d, ppm) for **17** (R_1 = phenyl): 2.33 (s, 3H, CH_3), 2.68 (m, 2H, C_4-2H), 3.03 (m, 2H, C_5-2H), 4.39 (s, 2H, thiazinan-CH_2), 5.38 (s, 2H, thiazinan-CH_2), 6.79 – 8.06 (m, 13H, Ar-H); for **18** (R_1 = benzyl): 2.35 (s, 3H, CH_3), 2.69 (m, 2H, C_4-2H), 3.05 (m, 3H, C_5-2H), 4.39 (s, 2H, thiazinan-CH_2), 4.78 (s, 2H, CH_2-C_6H_5), 5.36 (s, 2H, thiazinan-CH_2), 6.88 – 8.03 (m, 13H, Ar-H).

5-[4-(3-Methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonylimino]-3-phenyl-7H-thiazolo[4,3-c][1,2,4]triazole ***19***

A mixture of the 3-benzoylthiazolidin-4-one **11** (2.7 g, 5 mmol) and hydrazine hydrate (0.35 g, 7 mmol) in ethanol (15 mL) was heated under reflux for 30 min. The solid product obtained on cooling was filtered, washed with ethanol and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 4. ^{1}H-NMR (δ, ppm): 2.35 (s, 3H, CH_3), 2.70 (m, 2H, C_4-2H), 2.99 (m, 2H, C_5-2H), 4.22 (s, 2H, thiazol-CH_2), 7.11 – 8.06 (m, 13H, Ar-H).

5-Benzylidene-2-[4-(3-methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonylimino]-3-phenylthiazolidin-4-one ***20***

To a solution of the 3-phenylthiazolidin-4-one **9** (5 mmol) and piperidine (3 drops) in absolute ethanol (15 mL) was added benzaldehyde (0.53 g, 5 mmol). The mixture was heated under reflux for 8 h, when a solid product partially crystallized out. The reaction mixture was left to attain room temperature, and the separated solid product was filtered, dried and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 4. ^{1}H-NMR (δ, ppm): 2.31 (s, 3H, CH_3), 2.72 (m, 2H, C_4-2H), 3.11 (m, 2H, C_5-2H), 5.80 (s, 1H, thiazol-H), 7.01 – 8.09 (m, 19H, benzylidin-CH + Ar-H).

3,6-Diphenyl-5-[4-(3-methyl-4,5-dihydronaphtho[1,2-c]pyrazol-2-yl)-benzenesulfonylimino]-2-methyl-3,3a,5,6-tetrahydro-2H-pyrazolo[3,4-d]thiazole ***21***

A mixture of **20** (3 g, 5 mmol) and methylhydrazine (0.23 g, 5 mmol) in ethanol (15 mL) was heated under reflux for 30 min. The solid product obtained on cooling was filtered, washed with ethanol and recrystallized from ethanol. Physicochemical and analytical data are recorded in Table 4. ^{1}H-NMR (δ, ppm): 2.28 (s, 3H, CH_3), 2.51 (s, 3H, N-CH_3), 2.75 (m, 2H, C_4-2H), 3.06 (m, 2H, C_5-2H), 3.47 (d, J = 9 Hz, 1H, C_{3a}-H), 4.65 (d, J = 9 Hz, 1H, C_3-H), 6.65 – 7.86 (m, 18H, Ar-H).

In-vitro antitumour activity

Out of the newly synthesized compounds, twelve compounds, namely **2**, **4**, **6 – 9**, **11**, **15**, **17 – 19** and **21** were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* antitumour activity. Primary *in-vitro* one dose anticancer assay was performed using the 3-cell line panel consisting of NCI-H460 (lung), MCF7 (breast), and SF-268 (CNS) in accordance with the protocol of the Drug Evaluation Branch, NCI, Bethesda [33 – 35]. All the compounds that reduced the growth of any one of the cell lines to 32% or less, namely **6**, **8**, **11**, **15**, **17** and **18** were passed on for evaluation in the full panel of 60 human tumour cell lines of nine tumour subpanels. These cell lines were incubated with five concentrations (0.01 – 100 μM) for each compound. A 48 h continuous drug exposure protocol was used, and a SRB protein assay was employed to estimate cell viability or growth [33 – 35].

In-vitro MTT cytotoxicity assay

A modified MTT colorimetric assay [37] was employed to determine growth inhibition. Cell lines were obtained from the American Type Culture Collection (ATCC). The human breast cancer cell line MCF7 was maintained in DMEM supplemented with 5% fetal bovine serum (FBS), 2 mM glutamine and 100 units/mL penicillin/streptomycin. The normal human breast epithelial cell line MCF-10A was maintained in 5% and 10% horse serum, respectively, supplemented with 2 mM glutamine, 100 units/mL penicillin/streptomycin, 0.02 μg/mL EGF, 0.01 mg/mL insulin and 0.1 μg/mL cholera toxin. Cells were incubated at 37°C in a 5% CO_2 atmosphere.

Cells were plated in 96-well plates and allowed to attach for 24 h. Test compounds were dissolved in DMSO at 10 mM. Cells in quadruplicate wells were exposed to the individual test compounds at 0 – 25 μM for 72 h. Thereafter, the medium was replaced with 100 μL of 1 mg/mL MTT solution diluted in serum-free DMEM in each well. After 4 h of incubation, the MTT solution was removed, and 200 μL of DMSO was added to each well to dissolve the formed formazane crystals. The absorbance was measured at 495 nm. Results were compared with those of DMSO-treated cells as control. The results represent the mean of four independent experiments and are expressed as IC_{50}, i. e., the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.

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