Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 6475–6485
Bioorganic & Medicinal Chemistry

Synthesis and in vitro antitumor evaluation of some indeno[1,2-*c*]-pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems

Sherif A. F. Rostom*

Department of Medicinal Chemistry, Faculty of Medicine, King Abdulaziz University, PO Box 80205, Jeddah 21589, Saudi Arabia

Received 28 March 2006; revised 1 June 2006; accepted 8 June 2006
Available online 27 June 2006

Abstract—The synthesis of a series of 3-(4-chlorophenyl)-[1,2-*c*]pyrazol(in)es substituted with benzenesulfonamide, N^1,N^3-disubstituted sulfonylurea, sulfonylthiourea pharmacophores, and some derived thiazolidinone and thiazoline ring systems is described. All the newly synthesized target compounds were subjected to the NCI-in vitro disease-oriented antitumor screening to be evaluated for their antitumor activity. Eight compounds namely; **2**–**4**, **7**, **8**, **10**, **13**, and **16**; showed promising broad spectrum antitumor activity against most of the tested subpanel tumor cell lines ($GI_{50} < 100$ μM). Compound **3**, 4-(3-(4-chlorophenyl)-4*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonamide; although it did not show the highest growth inhibitory value (GI_{50} (MG-MID) 13.2 μM), it proved to be the most active analog in this study with the highest cytostatic and cytotoxic potentials (TGI and LC_{50} (MG-MID) concentrations of 33.1 and 66.1 μM, respectively). In general, the oxidized pyrazoles displayed better antitumor activity than their parent pyrazoline analogs, whereas the benzenesulfonamides and the N^1, N^3-disubstituted sulfonylureas showed significant better antitumor spectrum than the sulfonylthioureido and the derived thiazole analogs.

1. Introduction

Cancer is one of the most formidable afflictions in the world. Despite immense advances in the field of basic and clinical research, which have resulted in higher cure rates for a number of malignancies, cancer remains the second leading cause of death after heart disorders in developing as well as advanced countries.[1,2] Although major advances have been made in the chemotherapeutic management of some patients, the continued commitment to the arduous task of discovering new anticancer agents remains critically important. Among the wide range of compounds tested as potential anticancer agents, derivatives comprising the sulfonamide, N^1,N^3-diarylsulfonylurea and -thiourea functionalities have attracted great attention,[3–8] especially after the discovery of sulofenur (LY 186641) **A** and its structure analogs[8] (LY 295501) **B**, **C**, and **D** (Fig. 1). Sulofenur is an antineoplastic sulfonylurea that has been clinically evaluated in lung, breast, colon, ovarian, pancreatic, and gastric cancer.[9] It is generally assumed that the strong cytotoxicity and, as a consequence, the antitumor properties of the diarylsulfonylurea is due to the uncoupling of mitochondria[4,5] but other mechanisms, such as inhibition of the mitochondrial isozyme V of carbonic anhydrase (CA V), have also been hypothesized, since hydrolysis of the cytotoxic agent, leading to the formation of unsubstituted sulfonamides as the principal products, has been reported both in vivo and in vitro.[10] It is well known that aromatic/heterocyclic sulfonamides (formed after such a hydrolytic process) act as very potent inhibitors of CAs,[11,12] and these enzymes are involved in a multitude of crucial physiologic processes.[13] However, clinical trials of sulofenur have yielded unsatisfactory results because of its high protein binding and dosing being limited by the appearance of anemia due to methemoglobinemia, a side effect likely associated with its aniline-related metabolites.[14] In contrast LY 295501 (**B**) known now as ILX-295501; is principally metabolized by hydroxylation with negligible formation of aniline metabolites at relevant doses in experimental animals and demonstrated impressive activity against a broad spectrum of human tumor xenografts.[15]

On the other hand, over the past few years, much interest has been given to the chemotherapeutic activity of

Keywords: Antitumor activity; Sulofenur; Sulfonamides; Diarylsulfonylureas; Pyrazoles; Thiazoles.
* Tel.: +966 507654566; e-mail: sherifrostom@yahoo.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.06.020

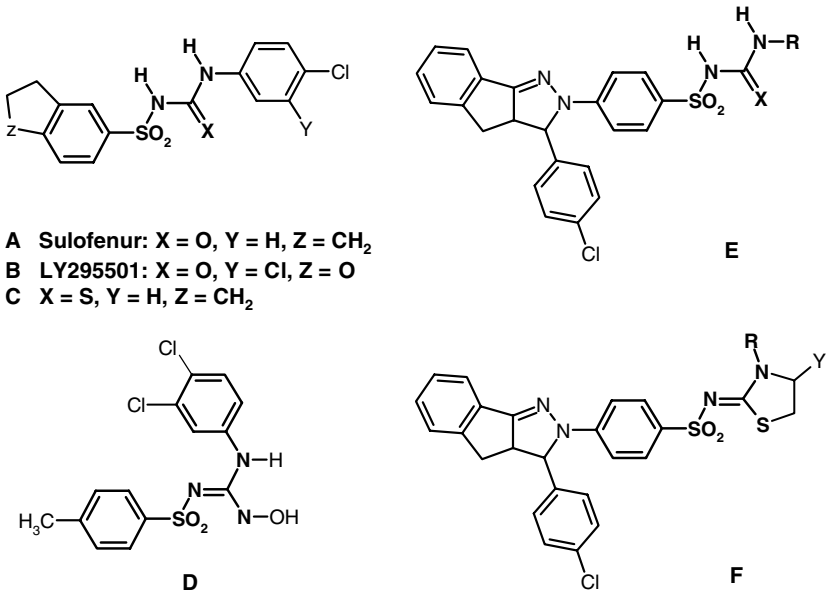


Figure 1. Structures of sulofenur, its analogs **A**–**D**, and the novel series of compounds **E** and **F**.

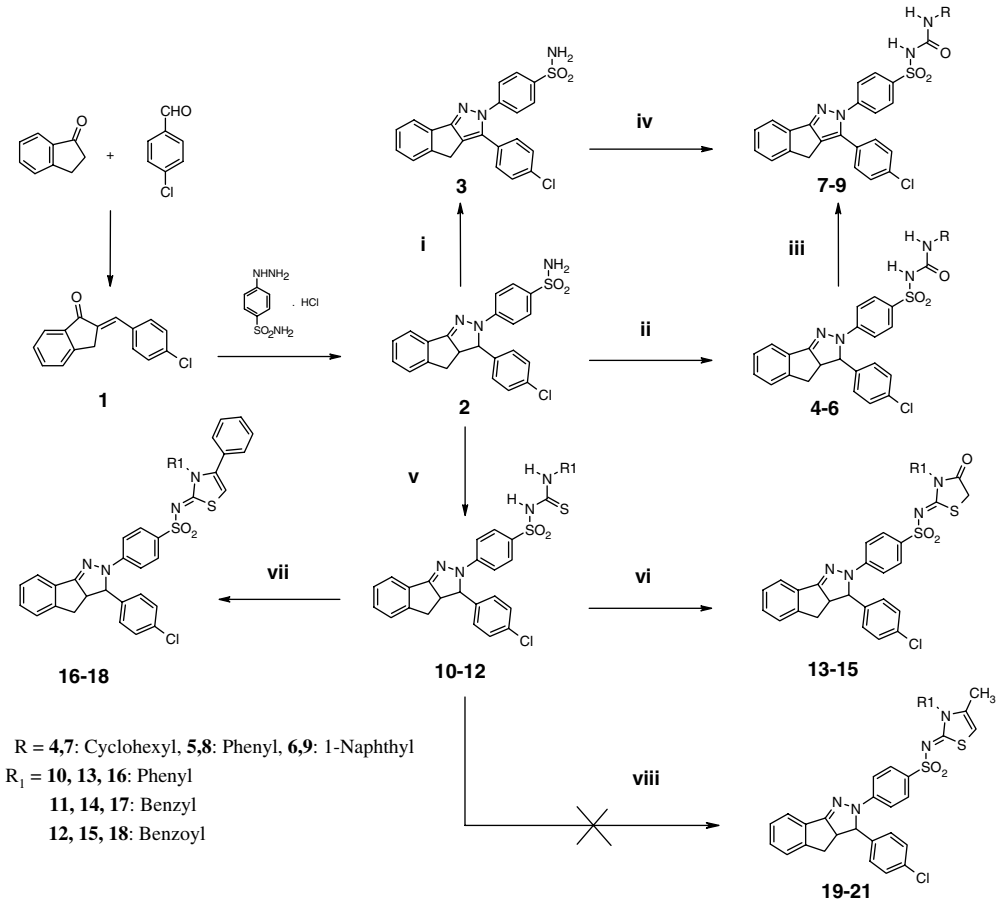
pyrazoles as antimicrobial,[16–18] antiviral,[19–21] and potential anticancer agents.[22–24] It has been also reported that some pyrazoles have remarkable antiproliferative effect on certain cell lines in addition to their pronounced inhibitory effect on the cyclin-dependent kinase (CDK) enzyme which plays a key role during cell division.[25] In addition, some fused pyrazoles proved to have potent glycogen synthase-3 inhibitory activity.[26,27]

In view of these facts, and in continuation of an ongoing program aiming at finding new structure leads with potential chemotherapeutic activities,[28–33] it was rationalized to synthesize and investigate the antitumor activity of some new compounds comprising the sulfonamido and the derived N^1,N^3-disubstituted sulfonylurea and thiourea pharmacophores having the general structure **E** that are structurally related to sulofenur **A** and its congeners **B** and **C** (Fig. 1). The thrust of efforts in the area of derivatization of such type of compounds concentrated mainly on the aryl moiety of the sulfonamide portion of the diarylsulfonylureas. In the present study, such N^3-aryl moiety was substituted basically with 3-(4-chlorophenyl)-indeno[1,2-*c*]pyrazol(in)e moieties because of the reported potential anticancer activity of pyrazoles hoping to induce some biological synergism. In addition, isosteric replacement of the sulfonylurea with a sulfonylthiourea functionality was considered as an interesting structure variation in order to study the influence of such modification on the anticipated antitumor activity. The substitution pattern of the N^1 part of the sulfonylurea, thiourea pharmacophores was selected so as to confer different electronic environment that would affect the lipophilicity, and hence the activity of the target molecules. Owing to the well-documented chemotherapeutic activities associated with thiazole derivatives,[34–36] it was considered of interest to investigate the effect of increasing compounds' rigidity by cyclizing the sulfonylthioureido derivatives to the sulfonylimino thiazolidinone and thiazoline ring systems **F** that are structurally related to reported anticancer agent **D** (Fig. 1). The objective of forming these hybrids is an attempt to reach an active antitumor agent with potentiated activity and selectivity toward cancerous cells.

2. Results and discussion

2.1. Chemistry

The synthetic pathways adopted for the preparation of the intermediate and target products are illustrated in Scheme 1. The starting chalcone **1** was prepared by condensing equimolar amounts of 1-indanone with 4-chlorobenzaldehyde in an aqueous ethanolic solution of sodium hydroxide according to a previously described procedure.[37] Cyclocondensation of **1** with 4-hydrazinobenzenesulfonamide hydrochloride in boiling ethanol afforded the indeno[1,2-*c*]-pyrazoline **2** in a good yield. Its ^{1}H NMR spectrum exhibited a doublet at δ 2.25 ppm due to two aliphatic protons at C-4, a characteristic doublet at δ 5.75 ppm attributed to the C-3 proton, and multiplet at δ 2.64 ppm due to the C-3a proton. Mild oxidation of the substituted indeno[1,2-*c*]pyrazoline **2** with bromine water at room temperature furnished the corresponding indeno[1,2-*c*]pyrazole **3**. The ^{1}H NMR of **3** lacked the characteristic doublet and multiplet of C-3 and C-3a protons of the parent pyrazoline **2**. The substituted sulfonylureas **4**–**6** were successfully obtained by condensing the key intermediate sulfonamide **2** with the appropriate isocyanate in the presence of anhydrous potassium carbonate as a mild basic catalyst. At this stage, the targeted substituted indeno[1,2-*c*]pyrazole benzenesulfonylureas **7**–**9** could be synthesized via either of the following procedures. The first method contributes to the condensation of the pyrazolylbenzenesulfonamide **3** with the appropriate isocyanate, while the second involves mild oxidation of the substituted indeno[1,2-*c*]pyrazoline benzenesulfo-



Scheme 1. Reagents and conditions: (i) bromine water, r.t., 3 h; (ii) RNCO, K_2CO_3, reflux, 18 h; (iii) bromine water, r.t., 6 h; (iv) RNCO, K_2CO_3, reflux, 18 h; (v) RNCS, K_2CO_3, reflux, 10 h; (vi): $BrCH_2COOC_2H_5$, Na acetate, reflux, 2 h; (vii) $C_6H_5COCH_2Br$, Na acetate, reflux, 3 h; (viii) CH_3COCH_2Cl.

nylureas **4**–**6** with bromine water at room temperature to give the same products. Here, it should be mentioned that the yield of first procedure was slightly better than that of the second one. The IR spectra of compounds **4**–**9** were characterized by a urea carbonyl band at 1640–1665 cm^{-1}. On the other hand, condensing the key intermediate **2** with different isothiocyanates in the presence of anhydrous potassium carbonate gave rise to the substituted benzenesulfonylthioureas **10**–**12**.Their IR spectra revealed a characteristic C═S band at 1115–1155 cm^{-1}. The latter compounds in their turn were further utilized for the preparation of some thiazole ring systems. Thus, cyclization of the sulfonylthioureido derivatives **10**–**12** with ethyl bromoacetate in the presence of anhydrous sodium acetate afforded the corresponding thiazolidin-4-ones **13**–**15**. The IR of these compounds was characterized by a carbonyl band at 1720–1735 cm^{-1}, whereas, their ^1H NMR spectra revealed a new singlet at δ 4.15–4.28 ppm attributed to the oxothiazolidine C-5 two protons. Analogously, reacting the same derivatives **10**–**12** with phenacyl bromide under similar reaction conditions resulted in the formation of the desired 1,3-thiazolines **16**–**18**. Their ^1H NMR spectra showed a new singlet at δ 6.11–6.19 ppm due to the thiazoline C-5 proton. Here it should be pointed out that, reacting **10**–**12** with chloroacetone using various reaction conditions, basic catalysts and different solvents failed to produce the desired 1,3-thiazolines **19**–**21**.

2.2. In vitro antitumor activity

2.2.1. Primary in vitro one dose 3-cell line assay. All the synthesized compounds were selected by the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay to be evaluated for their in vitro antitumor 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.[38–40] 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, sulforhodamine 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 tumor cell lines.[38–40] The results revealed that eight compounds namely **2**, **3**, **4**, **7**, **8**, **10**, **13**, and **16**; passed this primary anticancer

assay and consequently were carried over to be tested against a panel of 60 different tumor cell lines.

2.2.2. In vitro full panel 60-cell line assay. About 60 cell lines of nine tumor subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma, 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 antitumor drug discovery program was designed to distinguish between broad spectrum antitumor compounds and tumor or subpanel-selective agents.

In the present study, the active eight compounds exhibited remarkable antitumor activities against most of the tested subpanel tumor 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 antitumor activity (Tables 2 and 3).

With regard to the sensitivity against some individual cell lines (Table 1), compound **2** showed high activity against leukemia SR cell line (GI_{50} 2.19 μM). The analog **3** also exhibited noticeable antitumor activity against non-small cell lung NCS-H23 cell line (GI_{50} 3.27 μM). Furthermore, compound **4** showed the ever highest growth inhibitory activity against the renal A498 cancer cell line with GI_{50} value of <0.01 μM. It also revealed an obvious sensitivity profile toward leukemia SR cell line with GI_{50} value of 0.41 μM, in addition to a good activity against renal UO-31 and breast MCF7 cell lines with GI_{50} values of 2.00 and 2.52 μM, respectively. Except for the SR cell line, compound **7** proved to be sensitive toward most of the tested leukemia subpanel tumor cell lines with GI_{50} values range of 1.13–4.28 μM. It also showed particular effectiveness against the melanoma LOX IMVI cell line (GI_{50} 1.96 μM). Furthermore, compound **13** exhibited a significant activity against the colon COLO 205 cell line with GI_{50} value of 1.73 μM. On the other hand, the colon SW-620 cell line proved to be sensitive toward compounds **2**, **3** and **7** with GI_{50} values of 4.71, 1.88, and 2.46 μM, respectively. Melanoma SK-MEL-5, ovarian IGROV1 and renal A498 cancer cell lines showed high sensitivity toward compounds **2**–**4**, with GI_{50} values range of 1.36–5.59 μM. Moreover, the highest sensitivity against the prostate cancer cell lines was displayed by compounds **4** and **7**, with GI_{50} values range of 4.05–5.50 and 6.28–6.39 μM, respectively.

Concerning the broad spectrum antitumor activity, the results revealed that all of the eight active compounds **2**, **3**, **4**, **7**, **8**, **10**, **13**, and **16**, showed effective growth inhibition GI_{50} (MG-MID) values of 9.12, 13.2, 9.12, 10.2, 19.5, 25.7, 30.2, and 41.7 μM, respectively, beside cytostatic activity TGI (MG-MID) values of 34.7, 33.1, 33.9, 34.7, 63.1, 61.6, 77.6, and 91.2 μM, respectively (Tables 2 and 3). In addition, except for compounds **13** and **16**, the rest of the active compounds **2**, **3**, **4**, **7**, **8**, and **10**, exhibited a variable degree of cytotoxic efficacy with LC_{50} (MG-MID) values of 83.2, 66.1, 77.6, 81.3, 97.7, and 95.5 μM, respectively (Table 3).

Further interpretation of the obtained results revealed that, compounds **2** (GI_{50}, TGI, and LC_{50} (MG-MID) values 9.12, 34.7, and 83.2 μM, respectively) and **4** (GI_{50}, TGI, and LC_{50} (MG-MID) values 9.12, 33.9, and 77.6 μM, respectively) are nearly equipotent with almost the same range of activity against most of the tested subpanel tumor cell lines (Tables 2 and 3). Regarding compound **3**, although it did not show the highest growth inhibitory value (GI_{50} (MG-MID) 13.2 μM; Table 2), it proved to be the most active member in this study with the highest cytostatic and cytotoxic potentials with TGI and LC_{50} (MG-MID) concentrations of 33.1 and 66.1 μM, respectively (Table 3). It revealed an obvious activity against most of the tested subpanel tumor cell lines and relative effectiveness on the leukemia subpanel at both the GI_{50} and TGI levels (5.93 and 19.1 μM, respectively). Moreover, compounds **2**, **3**, and **4** displayed almost the same level of growth inhibition against the leukemia subpanel (GI_{50} (MG-MID) values 5.92, 5.93, and 5.73 μM, respectively). This pattern was not maintained at the TGI level where compound **2** showed weak cytostatic activity (TGI (MG-MID) 73.7 μM) relative to **3** and **4.** When compared with the NCI-DTP data of the standard diarylsulfonylurea analog sulofenur (**A**); the growth inhibitory activity of compound **7** was almost the half against the leukemia subpanel (GI_{50} (MG-MID) values 1.27 vs 2.50 μM, respectively); whereas the cytostatic activity of the same compound was about 25% of that of sulofenur (TGI (MG-MID) 7.51 vs 1.98, respectively) (Tables 2 and 3). In addition, particular sensitivity has been shown by compound **4** toward the prostate cancer subpanel at the $GI_{50,}$ TGI, and LC_{50} levels (4.77, 20.0 and 50.7 μM, respectively). On the other hand, compound **16** was found to be the least effective antitumor agent in the present investigation with GI_{50} and TGI (MG-MID) values of 41.7 and 91.2 μM, respectively; and without any cytotoxic effect (Tables 2 and 3).

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 toward the corresponding cell line, while compounds not meeting either of these criteria are rated non-selective.[41] In this context, the active compounds in the present study were found to be non-selective with broad spectrum antitumor activity against the nine tumor subpanels tested with selectivity ratios ranging

Table 1. Growth inhibitory concentration (GI_{50}, μM) of compounds **2**–**4**, **7**, **8**, **10**, **13**, and **16**[a]

Cell lines	**2**	**3**	**4**	**7**	**8**	**10**	**13**	**16**
Leukemia								
CCRF-CEM	6.48	2.96	3.85	1.13	3.21	19.7	13.7	18.6
HL-60 (TB)	NT[b]	NT	15.8	4.28	13.4	21.0	23.1	29.6
K-562	10.6	4.98	6.71	2.15	4.66	16.4	18.7	20.0
MOLT-4	7.58	5.51	4.04	2.77	7.05	15.9	17.2	18.5
RPMI-8226	2.73	10.4	3.57	2.11	16.5	19.6	26.4	21.5
SR	2.19	5.82	0.41	NT	2.86	14.7	11.4	17.3
Non-small cell lung cancer								
A549/ATCC	10.8	37.8	17.9	16.6	24.5	20.9	24.2	31.4
EKVX	14.1	17.1	5.38	14.2	15.8	27.4	43.2	61.1
HOP-62	21.1	30.4	15.0	35.8	72.0	41.3	56.8	93.9
HOP-92	10.3	11.6	10.8	6.45	17.1	18.1	27.0	38.6
NCI-H226	10.9	16.8	19.8	6.15	15.5	41.4	45.1	94.6
NCI-H23	11.4	3.27	12.2	NT	NT	NT	NT	21.0
NCI-H322	5.80	27.9	4.26	14.2	25.3	16.3	19.3	25.2
NCI-H460	19.7	32.1	4.45	19.8	22.3	22.2	19.7	NA[c]
NCI-H522	8.44	5.63	9.43	18.2	19.0	18.1	36.7	22.7
Colon cancer								
COLO 205	4.99	22.8	11.7	36.2	23.5	21.6	1.73	54.9
HCC-2998	10.3	18.3	3.56	NT	NT	NT	NT	NT
HCT-116	6.04	4.06	5.15	4.18	22.5	21.6	38.7	47.5
HCT-15	18.2	19.7	14.5	7.79	18.5	22.4	34.8	47.7
HT29	15.7	15.4	32.5	34.8	29.8	41.6	NT	44.0
KM12	5.55	19.9	7.90	10.6	16.0	19.4	22.0	19.9
SW-620	4.71	1.88	10.3	2.46	23.0	29.7	37.4	35.0
CNS cancer								
SF-268	22.1	22.4	6.78	20.1	14.8	20.7	22.7	NA
SF-295	16.3	29.8	15.9	11.3	18.2	35.5	35.8	40.7
SF-539	9.07	17.5	12.9	11.2	18.8	24.3	60.2	62.6
SNB-19	17.3	31.7	21.6	43.7	61.5	21.8	23.3	78.8
SNB-75	2.97	14.6	13.2	27.6	48.6	39.7	NT	NA
U251	20.2	19.5	10.1	11.7	22.3	19.7	27.8	26.1
Melanoma								
LOX IMVI	18.0	19.0	10.3	1.96	15.3	19.5	23.4	21.5
M14	10.1	13.1	10.8	13.7	34.5	36.4	59.4	NA
SK-MEL-2	8.23	13.6	24.4	19.4	27.4	23.4	NA	36.0
SK-MEL-28	10.0	18.3	29.7	21.9	33.7	29.4	NT	67.6
SK-MEL-5	2.30	1.36	3.69	12.1	22.0	20.4	32.2	25.2
UACC-257	11.4	20.6	19.7	18.4	37.1	29.5	92.9	83.5
UACC-62	5.97	13.7	14.9	10.9	12.2	18.3	30.1	32.7
Ovarian cancer								
IGROV1	3.48	2.58	5.98	18.6	28.5	20.2	37.8	25.7
OVCAR-3	17.3	17.0	18.4	13.7	18.3	46.3	48.5	44.3
OVCAR-4	9.76	15.0	38.4	28.8	45.2	42.2	NT	63.8
OVCAR-5	15.9	24.2	25.5	NT	NT	NT	NT	38.1
OVCAR-8	11.8	21.6	19.6	11.9	24.7	26.8	34.6	36.1
SK-OV-3	12.4	77.1	24.5	31.6	53.9	40.7	31.4	76.2
Renal cancer								
786-0	6.28	14.6	13.3	5.07	27.5	32.4	46.6	32.7
A498	2.55	4.70	0.01	18.5	21.8	NT	25.6	79.8
ACHN	17.5	19.8	13.4	7.93	12.9	25.8	27.7	40.1
CAKI-1	18.2	17.5	12.8	4.52	4.37	26.1	35.8	46.4
RXF 393	5.07	17.0	23.2	20.0	22.2	43.1	29.4	42.0
SN12C	6.74	5.49	16.8	7.54	23.2	24.5	37.3	50.8
TK-10	8.32	19.5	4.50	13.3	18.5	30.2	29.7	29.6
UO-31	7.88	9.57	2.00	3.64	24.5	19.0	29.1	45.4
Prostate cancer								
PC-3	7.17	14.0	4.05	6.28	16.7	13.8	22.6	19.1
DU-145	14.2	15.1	5.50	6.39	19.4	24.9	33.9	54.3
Breast cancer								
MCF7	25.0	22.9	2.52	15.3	11.2	23.5	18.7	NA
NCI/ADR-RES	10.3	19.6	20.6	NT	NT	NT	NT	51.5

(*continued on next page*)

Table 1 (*continued*)

Cell lines	**2**	**3**	**4**	**7**	**8**	**10**	**13**	**16**
MDA-MB-231/ATCC	2.89	14.8	12.8	6.43	14.7	87.1	86.0	70.1
HS 578T	4.61	12.1	4.61	5.03	19.6	30.0	29.4	33.0
MDA-MB-435	16.0	17.8	18.5	8.27	16.8	44.8	30.6	24.2
BT-549	NT	NT	7.01	3.52	21.0	33.2	35.6	41.4
T-47D	32.9	91.0	22.6	4.98	49.9	36.3	41.7	NA

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell screen.
[b] NT, Not Tested.
[c] NA, not active; GI_{50} value >100 μM.

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

Compound	Subpanel tumor cell lines[b]									MG-MID[c]
	I	II	III	IV	V	VI	VII	VIII	IX	
2	5.92	12.5	9.35	14.6	9.43	11.8	9.07	10.7	15.3	9.12
3	5.93	20.3	14.6	22.6	16.8	26.2	13.5	14.5	29.7	13.2
4	5.73	11.0	12.2	13.4	16.2	22.1	10.7	4.77	12.7	9.12
7	2.50	16.4	16.0	20.9	14.0	20.9	10.1	6.33	7.25	10.2
8	7.94	26.4	22.2	30.7	26.0	34.1	19.4	18.0	22.2	19.5
10	17.9	25.7	26.0	27.0	25.3	35.2	28.7	19.3	42.4	25.7
13	18.4	34.0	26.9	33.9	56.3	38.1	32.6	28.2	40.3	30.2
16	20.9	54.3	41.5	68.0	52.3	47.4	45.8	36.7	60.0	41.7
S[d]	1.27	1.05	1.21	1.21	1.30	1.16	1.16	NT[e]	NT	1.19

[a] Data obtained from NCI's in vitro disease-oriented human tumor 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 toward the test agent.
[d] Sulofenur (NSC 656667): NCI cancer screen; August 2004.
[e] NT, not tested.

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

Compound	Subpanel tumor cell lines[b]									MG-MID[c]
	I	II	III	IV	V	VI	VII	VIII	IX	
2	73.3	39.7	63.3	44.9	31.1	37.4	24.0	29.6	43.8	34.7 (83.2)[d]
3	19.1	52.2	45.0	55.3	29.0	46.2	28.3	30.5	39.1	33.1 (66.1)
4	21.1	33.3	36.6	52.6	47.2	70.0	35.0	20.0	48.8	33.9 (77.6)
7	7.51	48.0	76.9	55.3	48.6	62.8	40.2	22.3	42.2	34.7 (81.3)
8	27.7	82.5	74.2	79.2	76.9	88.2	74.3	47.4	65.4	63.1 (97.7)
10	48.1	62.7	64.2	63.8	59.1	84.1	72.4	39.7	83.5	61.6 (95.5)
13	50.8	89.3	74.9	89.7	87.7	98.8	87.6	79.3	87.3	77.6 (NA)[e]
16	71.6	92.6	90.6	95.9	88.3	97.5	NA	NA	NA	91.2 (NA)
S[f]	1.98	1.50	1.60	1.66	1.69	1.55	1.59	NT[g]	NT	1.63 (1.99)

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell screen.
[b] For subpanel tumor cell lines, 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] TGI (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines toward the test agent.
[d] LC_{50} (μM) full panel mean-graph mid point (MG-MID) = the average sensitivity of all cell lines toward the test agent.
[e] NA, not active, (TGI or LC_{50} (MG-MID) values >100 μM).
[f] Sulofenur (NSC 656667): NCI cancer screen; August 2004.
[g] NT, not tested.

between 0.54–2.30 and 0.60–2.00 at the GI_{50} and TGI levels, respectively, except for compound **7** which showed moderate selectivity toward the leukemia subpanel at both the GI_{50} and TGI levels (selectivity ratios 4.06 and 4.62, respectively). However, compound **8** revealed mild selectivity toward the leukemia subpanel with selectivity ratio near 3 (2.50 and 2.44, for the GI_{50} and TGI, respectively).

A close examination of the structures of the active compounds revealed that, the key intermediate indeno[1,2-*c*]pyrazoline sulfonamide **2** exhibited a broad spectrum antitumor activity as indicated from its GI_{50}, TGI, and LC_{50} (MG-MID) values (9.12, 34.7, and 83.2 μM, respectively). Oxidation of **2** resulted in the formation of the corresponding pyrazole sulfonamide **3**, which proved to be the most

active member in this series with the highst cytostatic and cytotxic potentials (TGI and LC_{50} (MG-MID) values of 33.1 and 66.1, respectively; Table 3), with special effectiveness on the leukemia subpanel at the GI_{50}, TGI, and LC_{50} (MG-MID) levels (5.93, 19.1, and 59.2 μM, respectively). These findings were substantiated by the idea that the sulfonamido functionality plays certain role in the antitumor activity.[8,42,43] Condensing the parent compound **2** with different isocyanates furnished the disubstituted sulfonylureido derivatives **4**–**6**, of which only one analog (**4**; R = cyclohexyl) exhibited remarkable antitumor activity, whereas the other congeners **5** and **6** proved to be totally inactive. Compound **4** was nearly equipotent with **2** at the GI_{50} and TGI (MG-MID) levels (9.12 vs 9.12 and 33.9 vs 34.7 μM, respectively; Tables 2 and 3), meanwhile, the cytotoxic activity of the same compound was slightly improved (LC_{50} (MG-MID) 77.6 vs 83.2 μM; Table 3). The synthesis of the oxidized pyrazole derivatives **7**–**9** gave rise to two relatively active substituted sulfonylureidopyrazole derivatives **7** and **8**. The nature of substituent in the sulfonylureido functionality seems to modulate the antitumor activity of this type of compounds as evidenced by their GI_{50}, TGI, and LC_{50} (Tables 2 and 3). The cyclohexyl moiety in compound **7** proved to be the most favorable substituent among this series with GI_{50}, TGI, and LC_{50} (MG-MID) values of 10.2, 34.7, and 81.3 μM, respectively. Shifting the substitution toward the aromatic side as in **8** (R = phenyl), resulted in a marked reduction in the antitumor activity to the half at the GI_{50} and TGI levels (19.5 and 63.1 μM, respectively), whereas its cytotoxic effect was obviously reduced (LC_{50} (MG-MID) 97.7 μM). Increasing the size and aromaticity of the substituent as in **9** (R = 1-naphthyl) led to a complete abolishment of the antitumor activity. When **7** was compared with its parent pyrazoline **4**, both displayed almost the same antitumor profile except for the activity against leukemia and breast cancer subpanels which showed about 2-fold improvement in activity at the GI_{50} level (2.50 vs 5.73 and 7.25 vs 12.7 μM, respectively). On the other hand, conversion of the sulfonamide **2** to the substituted sulfonylthioureido derivatives **10**–**12** gave only one active compound (**10**; R = phenyl). Such derivatization led to a significant reduction (2- to 3-fold) in the overall antitumor activity of **10** when compared with the parent sulfonamide **2** and the sulfonylureido isostere **7** (Tables 2 and 3). Incorporation of the thioureido moiety partly in a rigid structure resulted in two active compounds, namely, the 2-oxothiazolidine (**13**; R = phenyl) and thiazoline (**16**; R = phenyl) derivatives, with a significant reduction in the growth inhibitory, cytostatic potencies and a complete abolishment of the cytotoxic activity (Tables 2 and 3). However, the oxothiazolidine derivative **13** showed a relatively better activity (GI_{50} and TGI (MG-MID) 30.2 and 77.6 μM, respectively) when compared with the thiazoline analog **16** (GI_{50} and TGI (MG-MID) 41.7 and 91.2 μM, respectively) (Tables 2 and 3). Therefore, compound **16** could be considered the least active member in this investigation.

3. Conclusion

In conclusion, the objective of the present study was to synthesize and investigate the antitumor activity of new compounds incorporating the sulfonamido, N^1,N^3-disubstituted sulfonylurea and thiourea pharmacophores structurally related to a well-documented sulfonylurea anticancer agent sulofenur **A** and its structure congeners **B**–**D** (Fig. 1). This aim has been verified by the synthesis of hybrid compounds comprising the above-mentioned pharmacophores substituted essentially with a 3-(4-chlorophenyl)-[1,2-*c*]pyrazol(in)e counterpart at the N^3 aryl moiety having the general structures **E** and **F** (Fig. 1), for synergistic purpose. The results revealed that eight compounds namely; **2**–**4**, **7**, **8**, **10**, **13**, and **16**; showed promising broad spectrum antitumor activity. In general, the oxidized pyrazole derivatives appeared to exhibit better antitumor activity than their parent pyrazoline analogs, whereas the benzenesulfonamides and N^1, N^3-disubstituted sulfonylureas showed significant better antitumor spectrum than that of the sulfonylthioureido and derived thiazole analogs. The active compounds displayed remarkable growth inhibitory and cytostatic potencies nearly with the same order of activity (Tables 2 and 3). In addition, except for compounds **13** and **16**, the other six active compounds showed a wide range of cytotoxic activity (Table 3). The sulfonylureido derivative **7** displayed almost half the growth inhibitory and 25% of the cytostatic activities of the diarylsulfonylurea analog sulofenur (**A**) against the leukemia subpanel (Tables 2 and 3). Compound **3**, although did not show the highest growth inhibitory value (GI_{50} (MG-MID) 13.2 μM; Table 2), it could be considered the most active derivative identified in this study with the highest cytostatic and cytotoxic potentials (TGI and LC_{50} (MG-MID) concentrations of 33.1 and 66.1 μM, respectively; Table 3).

Finally, the broad spectrum antitumor activity displayed by these compounds will be of interest for future derivatization in the hope of finding more active and selective antitumor agents.

4. Experimental

4.1. Chemistry

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Perkin-Elmer 297 infrared spectrophotometer using the KBr plate technique. The ^{1}H NMR spectra were recorded on a Varian EM 360 spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as the solvent (Chemical shifts in δ, 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. Follow-up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum

sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

4.1.1. 4-(3-(4-Chlorophenyl)-3a,4-dihydro-3*H*-indeno [1,2-*c*]pyrazol-2-yl)-benzenesulfonamide (2). A solution of the chalcone **1**[37] (5.1 g, 0.02 mol) in ethanol was refluxed with 4-hydrazino-benzenesulfonamide hydrochloride (4.9 g, 0.022 mol) for 4 h. The volume of the reaction mixture was concentrated to the half and the separated product was filtered, washed with cold ethanol, and recrystallized from benzene/ethanol (1:1). Yield: 85%, mp: 203–204 °C. IR (cm^{-1}): 3350 and 3210 (NH_2), 1335 and 1180 (SO_2N); ^1H NMR (δ, ppm): 2.25 (d, $J = 9$ Hz, 2H, C_4–2H), 2.64 (m, 1H, C_{3a}–H), 5.75 (d, 1H, C_3–H), 6.85–8.4 (m, 12H, Ar-H), 10.64 (s, 2H, NH_2). Anal. Calcd for $C_{22}H_{18}ClN_3O_2S$ (423.92): C, 62.33; H, 4.28; N, 9.91; S, 7.56. Found: C, 61.95; H, 4.36; N, 9.72; S, 7.78.

4.1.2. 4-(3-(4-Chlorophenyl)-4*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonamide (3). To a stirred suspension of the pyrazoline **2** (4.2 g, 0.01 mol) in water (10 mL), bromine water (5%, 15 mL) was gradually added over a period of 30 min at room temperature. After stirring for further 3 h, the pyrazole derivative thus formed was collected by filtration, washed with water, and dried. It was recrystallized from ethanol. Yield: 82%, mp: 184–185 °C. IR (cm^{-1}): 3340 and 3250 (NH_2), 1320 and 1185 (SO_2N); ^1H NMR (δ, ppm): 4.12 (s, 2H, C_4–2H), 7.47–8.01 (m, 12H, Ar-H). Anal. Calcd for $C_{22}H_{16}ClN_3O_2S$ (421.90): C, 62.63; H, 3.82; N, 9.96; S, 7.60. Found: C, 62.91; H, 3.71; N, 10.08; S, 7.75.

4.1.3. N^1-substituted N^3-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-ureas (4–6). A mixture of the pyrazoline **2** (2.1 g, 0.005 mol) and anhydrous potassium carbonate (1.4 g, 0.01 mol) in dry acetone (20 mL) was heated under reflux with stirring, with the appropriate isocyanate (0.0055 mol) for 18 h. The solvent was removed under reduced pressure and the remaining solid residue was dissolved in water (30 mL). After acidification of the resulting solution with 2 N hydrochloric acid, the precipitated crude product was filtered, washed with water, dried, and recrystallized.

4.1.3.1. N^1-Cyclohexyl N^3-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-urea (4). Yield: 72%, mp: 185–187 °C (ethanol). IR (cm^{-1}): 1300 and 1165 (SO_2N), 1640 (C=O); ^1H NMR (δ, ppm): 1.57–2.16 (m, 11H, cyclohexyl-H), 2.20 (d, $J = 9$ Hz, 2H, C_4–2H), 2.81 (m, 1H, C_{3a}–H), 5.60 (d, 1H, C_3–H), 6.98–8.12 (m, 12H, Ar-H), 10.40 (s, 1H, NH), 10.52 (s, 1H, NH). Anal. Calcd for $C_{29}H_{29}ClN_4O_3S$ (549.08): C, 63.43; H, 5.32; N, 10.20; S, 5.84. Found: C, 63.49; H, 5.29; N, 9.98; S, 5.98.

4.1.3.2. N^1-Phenyl N^3-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-urea (5). Yield: 78%, mp: 192–193 °C (ethanol). IR (cm^{-1}): 1310 and 1175 (SO_2N), 1650 (C=O); ^1H NMR (δ, ppm): 2.18 (d, $J = 9$ Hz, 2H, C_4–2H), 2.57 (m, 1H, C_{3a}–H), 5.68 (d, 1H, C_3–H), 6.90–8.35 (m, 17H, Ar-H), 10.24 (s, 1H, NH), 10.35 (s, 1H, NH). Anal. Calcd for $C_{29}H_{23}ClN_4O_3S$ (543.04): C, 64.14; H, 4.27; N, 10.32; S, 5.90. Found: C, 64.02; H, 4.40; N, 10.45; S, 5.85.

4.1.3.3. N^1-(1-Naphthyl) N^3-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-urea (6). Yield: 80%, mp: 188–189 °C (ethanol). IR (cm^{-1}): 1320 and 1190 (SO_2N), 1665 (C=O); ^1H NMR (δ, ppm): 2.26 (d, $J = 9$ Hz, 2H, C_4–2H), 2.66 (m, 1H, C_{3a}–H), 5.72 (d, 1H, C_3–H), 6.85–8.40 (m, 19H, Ar-H), 10.30 (s, 1H, NH), 10.55 (s, 1H, NH). Anal. Calcd for $C_{33}H_{25}ClN_4O_3S$ (593.10): C, 66.83; H, 4.25; N, 9.45; S, 5.41. Found: C, 67.05; H, 4.13; N, 9.70; S, 5.23.

4.1.4. N^1-substituted N^3-[4-(3-(4-chlorophenyl)-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-ureas (7–9). *Method A.* A mixture of the sulfonamide **3** (4.2 g, 0.01 mol) and anhydrous potassium carbonate (2.8 g, 0.02 mol) in dry acetone (25 mL) was heated under reflux with stirring, with the appropriate isocyanate (0.011 mol) for 24 h. Working up of the reaction mixture was carried out as described under **4–6**.

Method B. To a stirred suspension of the appropriate sulfonylurea **4–6** (0.01 mol) in water (10 mL), bromine water (5%, 15 mL) was gradually added over a period of 30 min at room temperature. After further stirring for 6 h, the oxidation product thus formed was treated as described under **3**.

4.1.4.1. N^1-Cyclohexyl N^3-[4-(3-(4-chlorophenyl)-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-urea (7). Yield: 62%, mp: 136–138 °C (ethanol). IR (cm^{-1}): 1305 and 1160 (SO_2N), 1650 (C=O); ^1H NMR (δ, ppm): 1.57–2.36 (m, 11H, cyclohexyl-H), 4.23 (s, 2H, C_4–2H), 7.08–8.05 (m, 12H, Ar-H), 9.80 (s, 1H, NH), 10.25 (s, 1H, NH). Anal. Calcd for $C_{29}H_{27}ClN_4O_3S$ (547.07): C, 63.67; H, 4.97; N, 10.24; S, 5.86. Found: C, 63.55; H, 5.07; N, 10.33; S, 5.70.

4.1.4.2. N^1-Phenyl N^3-[4-(3-(4-chlorophenyl)-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-urea (8). Yield: 68%, mp: 166–167 °C (ethanol). IR (cm^{-1}): 1320 and 1170 (SO_2N), 1660 (C=O); ^1H NMR (δ, ppm): 4.28 (s, 2H, C_4–2H), 7.12–8.17 (m, 17H, Ar-H), 9.68 (s, 1H, NH), 10.40 (s, 1H, NH). Anal. Calcd for $C_{29}H_{21}ClN_4O_3S$ (541.02): C, 64.38; H, 3.91; N, 10.36; S, 5.93. Found: C, 64.55; H, 3.76; N, 10.44; S, 5.83.

4.1.4.3. N^1-(1-Naphthyl) N^3-[4-(3-(4-chlorophenyl)-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-urea (9). Yield: 60%, mp: 168–169 °C (ethanol). IR (cm^{-1}): 1330 and 1210 (SO_2N), 1665 (C=O); ^1H NMR (δ, ppm): 4.31 (s, 2H, C_4–2H), 6.95–8.35 (m, 19H, Ar-H), 10.10 (s, 1H, NH), 10.36 (s, 1H, NH). Anal. Calcd for $C_{33}H_{23}ClN_4O_3S$ (591.08): C, 67.06; H, 3.92; N, 9.48; S, 5.42. Found: C, 66.89; H, 4.06; N, 9.60; S, 5.39.

4.1.5. N^1-substituted N^3-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-thioureas (10–12). A solution of the appropriate isothiocyanate (0.011 mol) in dry acetone (5 mL) was

added to a stirred mixture of the sulfonamide **2** (4.2 g, 0.01 mol) and anhydrous potassium carbonate (2.8 g, 0.02 mol) in dry acetone (25 mL), and the reaction mixture was heated under reflux with stirring for 10 h. The solvent was removed under reduced pressure and the remaining solid residue was dissolved in water (30 mL) and acidified with 2 N hydrochloric acid. The precipitated crude product was filtered, washed with water, dried, and recrystallized.

4.1.5.1. N^1-Phenyl N^3-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-thiourea (10). Yield: 85%, mp: 155–157 °C (ethanol). IR (cm^{-1}): 1310 and 1175 (SO_2N), 1155 (C=S); ^1H NMR (δ, ppm): 2.25 (d, $J = 9$ Hz, 2H, C_4–2H), 2.54 (m, 1H, C_{3a}–H), 5.60 (d, 1H, C_3–H), 6.85–8.23 (m, 17H, Ar-H), 9.82 (s, 1H, NH), 10.12 (s, 1H, NH). Anal. Calcd for $C_{29}H_{23}ClN_4O_2S_2$ (559.10): C, 62.30; H, 4.15; N, 10.02; S, 11.47. Found: C, 62.10; H, 4.22; N, 9.86; S, 11.70.

4.1.5.2. N^1-Benzyl N^3-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-thiourea (11). Yield: 78%, mp: 136–138 °C (ethanol). IR (cm^{-1}): 1315 and 1190 (SO_2N), 1150 (C=S); ^1H NMR (δ, ppm): 2.29 (d, $J = 9$ Hz, 2H, C_4–2H), 2.61 (m, 1H, C_{3a}–H), 4.73 (d, 2H, benzyl CH_2), 5.54 (d, 1H, C_3–H), 6.92–8.34 (m, 17H, Ar-H), 9.90 (s, 1H, NH), 10.22 (s, 1H, NH). Anal. Calcd for $C_{30}H_{25}ClN_4O_2S_2$ (573.13): C, 62.87; H, 4.40; N, 9.78; S, 11.19. Found: C, 62.56; H, 4.58; N, 9.90; S, 11.35.

4.1.5.3. N^1-Benzoyl N^3-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonyl]-thiourea (12). Yield: 71%, mp: 160–162 °C (ethanol). IR (cm^{-1}): 1115 (C=S), 1330 and 1220 (SO_2N), 1660 (C=O); ^1H NMR (δ, ppm): 2.22 (d, $J = 9$ Hz, 2H, C_4–2H), 2.51 (m, 1H, C_{3a}–H), 5.49 (d, 1H, C_3–H), 6.88–8.29 (m, 17H, Ar-H), 10.05 (s, 1H, NH), 10.28 (s, 1H, NH). Anal. Calcd for $C_{30}H_{23}ClN_4O_3S_2$ (587.12): C, 61.36; H, 3.95; N, 9.54; S, 10.92. Found: C, 61.03; H, 4.11; N, 9.81; S, 11.05.

4.1.6. 3-Substituted-2-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-thiazolidin-4-ones (13–15). To a solution of the appropriate sulfonylthioureido derivative **6–8** (0.005 mol) in absolute ethanol (20 mL) were added ethyl bromoacetate (1 g, 0.0055 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) and the reaction mixture was heated under reflux for 2 h. The mixture was left to attain room temperature then poured into an ice-cold water (30 mL), and the solid product thus formed was filtered, washed with water, dried, and recrystallized.

4.1.6.1. 3-Phenyl-2-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-thiazolidin-4-one (13). Yield: 68%, mp: 184–186 °C (ethanol/benzene; 1:1). IR (cm^{-1}): 1730 (C=O); ^1H NMR (δ, ppm): 2.26 (d, $J = 9$ Hz, 2H, C_4–2H), 2.76 (m, 1H, C_{3a}–H), 4.15 (s, 2H, thiazol-CH_2), 5.39 (d, 1H, C_3–H), 7.20–8.15 (m, 17H, Ar-H). Anal. Calcd for $C_{31}H_{23}ClN_4O_3S_2$ (599.12): C, 62.15; H, 3.87; N, 9.35; S, 10.70. Found: C, 61.97; H, 4.00; N, 9.30; S, 10.82.

4.1.6.2. 3-Benzyl-2-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-thiazolidin-4-one (14). Yield: 66%, mp: 154–156 °C (ethanol/benzene; 1:1). IR (cm^{-1}): 1720 (C=O); ^1H NMR (δ, ppm): 2.26 (d, $J = 9$ Hz, 2H, C_4–2H), 2.61 (m, 1H, C_{3a}–H), 4.21 (s, 2H, thiazol-CH_2), 4.67 (s, 2H, benzyl-CH_2), 5.44 (d, 1H, C_3–H), 7.05–8.30 (m, 17H, Ar-H). Anal. Calcd for $C_{32}H_{25}ClN_4O_3S_2$ (613.15): C, 62.68; H, 4.11; N, 9.14; S, 10.46. Found: C, 62.44; H, 4.25; N, 9.02; S, 10.51.

4.1.6.3. 3-Benzoyl-2-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-thiazolidin-4-one (15). Yield: 60%, mp: 177–178 °C (ethanol/benzene; 1:1). IR (cm^{-1}): 1690 (C=O), 1735 (C=O); ^1H NMR (δ, ppm): 2.19 (d, $J = 9$ Hz, 2H, C_4–2H), 2.54 (m, 1H, C_{3a}–H), 4.28 (s, 2H, thiazol-CH_2), 5.50 (d, 1H, C_3–H), 6.93–8.25 (m, 17H, Ar-H). Anal. Calcd for $C_{32}H_{23}ClN_4O_4S_2$ (627.19): C, 61.28; H, 3.69; N, 8.93; S, 10.22. Found: C, 60.93; H, 3.95; N, 9.11; S, 10.07.

4.1.7. 3-Substituted-4-phenyl-2-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-1,3-thiazolines (16–18). A solution of the appropriate sulfonyl-thioureido derivative **6–8** (0.005 mol) in absolute ethanol (20 mL) was refluxed with phenacyl bromide (1.1 g, 0.0055 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) for 3 h during which the solid product was partially crystallized out. The mixture was left to attain room temperature then filtered, washed with cold ethanol, dried, and recrystallized.

4.1.7.1. 3,4-Diphenyl-2-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-1,3-thiazoline (16). Yield: 72%, mp: 146–148 °C (ethanol). IR (cm^{-1}): 1600 (C=N); ^1H NMR (δ, ppm): 2.18 (d, $J = 9$ Hz, 2H, C_4–2H), 2.61 (m, 1H, C_{3a}–H), 5.39 (d, 1H, C_3–H), 6.11 (s, 1H, thiazol-H), 7.03–8.32 (m, 22H, Ar-H). Anal. Calcd for $C_{37}H_{27}ClN_4O_2S_2$ (659.22): C, 67.41; H, 4.13; N, 8.50; S, 9.73. Found: C, 67.30; H, 4.25; N, 8.66; S, 9.48.

4.1.7.2. 3-Benzyl-4-phenyl-2-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-1,3-thiazoline (17). Yield: 69%, mp: 154–156 °C (ethanol). IR (cm^{-1}): 1560 (C=N); ^1H NMR (δ, ppm): 2.24 (d, $J = 9$ Hz, 2H, C_4–2H), 2.55 (m, 1H, C_{3a}–H), 4.69 (s, 2H, benzyl-CH_2), 5.48 (d, 1H, C_3–H), 6.15 (s, 1H, thiazol-H), 6.89–8.18 (m, 22H, Ar-H). Anal. Calcd for $C_{38}H_{29}ClN_4O_2S_2$ (673.25): C, 67.79; H, 4.34; N, 8.32; S, 9.53. Found: C, 67.90; H, 4.30; N, 8.12; S, 9.69.

4.1.7.3. 3-Benzoyl-4-phenyl-2-[4-(3-(4-chlorophenyl)-3a,4-dihydro-3*H*-indeno[1,2-*c*]pyrazol-2-yl)-benzenesulfonylimino]-1,3-thiazoline (18). Yield: 55%, mp: 191–193 °C (ethanol). IR (cm^{-1}): 1740 (C=O); ^1H NMR (δ, ppm): 2.20 (d, $J = 9$ Hz, 2H, C_4–2H), 2.56 (m, 1H, C_{3a}–H), 5.55 (d, 1H, C_3–H), 6.19 (s, 1H, thiazol-H), 7.01–8.24 (m, 22H, Ar-H). Anal. Calcd for $C_{38}H_{27}ClN_4O_3S_2$ (687.29): C, 66.41; H, 3.96; N, 8.15; S, 9.33. Found: C, 66.12; H, 4.16; N, 8.36; S, 9.09.

4.2. In vitro antitumor screening

Compounds **2**–**4**, **7**, **8**, **10**, **13**, and **16** were subjected to the NCI in vitro disease-oriented human cells screening panel assay to screen their antitumor activities. About 60 cell lines of nine tumor subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines, were utilized. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 mL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg mL^{-1} gentamicin. Additional four 10-fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 mL of these different drug dilutions were added to the appropriate microtiter wells already containing 100 mL of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 mL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 mL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 mL of 80% TCA (final concentration, 16% TCA). Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line.[38–40]

Acknowledgments

The author is very grateful to the staff members of the Department of Health and Human Services, National Cancer Institute (NCI), Bethesda, Maryland, USA, for carrying out the anticancer screening of the newly synthesized compounds.

References and notes

1. Eckhardt, S. *Curr. Med. Chem.—Anti-Cancer Agents* **2002**, *2*, 419–439.
2. Lee, C. W.; Hong, D. H.; Han, S. B.; Jong, S.-H.; Kim, H. C.; Fine, R. L.; Lee, S.-H.; Kim, H. M. *Biochem. Pharmacol.* **2002**, *64*, 473–480.
3. Mohamadi, F.; Spees, M. M.; Grindey, G. B. *J. Med. Chem.* **1992**, *35*, 3012–3016.
4. Chern, J. W.; Leu, Y. L.; Wang, S. S.; Jou, R.; Lee, C. F.; Tsou, P. C.; Hsu, S. C.; Liaw, Y. C.; Lin, H. M. *J. Med. Chem.* **1997**, *40*, 2276–2286.
5. Toth, J. E.; Grindey, G. B.; Ehlhardt, W. J.; Ray, J. E.; Boder, G. B.; Bewley, J. R.; Klingerman, K. K.; Gates, S. B.; Rinzel, S. M.; Schultz, R. M.; Weir, L. C.; Worzalla, J. F. *J. Med. Chem.* **1997**, *40*, 1018–1025.
6. Medina, J. C.; Shan, B.; Beckmann, H.; Farrell, R. P.; Clark, D. L.; Learned, R. M.; Roche, D.; Li, A.; Baichwal, V.; Case, C.; Baeuerle, P. A.; Rosen, T.; Jaen, J. C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2653–2656.
7. Medina, J. C.; Roche, D.; Shan, B.; Learned, R. M.; Frankmoelle, W. P.; Clark, D. L.; Rosen, T.; Jaen, J. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1843–1846.
8. Mastrolorenzo, A.; Scozzafava, A.; Supuran, C. T. *Eur. J. Pharm. Sci.* **2000**, *11*, 325–332.
9. Howbert, J. J.; Grossman, C. S.; Crowell, T. A.; Rieder, B. J.; Harper, R. W.; Grindey, G. B. *J. Med. Chem.* **1990**, *33*, 2393.
10. Supuran, C. T.; Briganti, F.; Tilli, S.; Chegwidden, W. R.; Scozzafava, A. *Bioorg. Med. Chem.* **2001**, *9*, 703–714.
11. Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib.* **1999**, *14*, 343–363.
12. Scozzafava, A.; Supuran, C. T. *Eur. J. Pharm. Sci.* **2000**, *10*, 29–41.
13. Casini, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Cancer Drug Targets* **2002**, *2*, 55–75.
14. Guan, X.; Hoffman, B. N.; McFarland, D. C.; Gilkerson, K. K.; Dwivedi, C.; Erickson, A. K.; Bebensee, S.; Pellegrini, J. *Drug Metab. Dispos.* **2002**, *30*, 331–335.
15. Forouzesh, B.; Takimoto, C. H.; Goetz, A.; Diab, S.; Hammond, L. A.; Smetzer, L.; Schwartz, G.; Gazak, R.; Callaghan, J. T.; Von Hoff, D. D.; Rowinsky, E. K. *Clin. Cancer Res.* **2003**, *9*, 5540–5549.
16. Foks, H.; Pancechowska-Ksepko, D.; Kedzia, A.; Zwolska, Z.; Janowiec, M.; Augustynowicz-Kopec, E. *Il Farmaco* **2005**, *60*, 513–517.
17. Kumar, V.; Aggarwal, R.; Tyagi, P.; Singh, S. P. *Eur. J. Med. Chem.* **2005**, *40*, 922–927.
18. Brana, M. F.; Gradillas, A.; Ovalles, A. G.; Lopez, B.; Acero, N.; Llinares, F.; Mingarro, D. M. *Bioorg. Med. Chem.* **2006**, *14*, 9–16.
19. Storer, R.; Ashton, C. J.; Baxter, A. D.; Hann, M. M.; Marr, C. L. P.; Mason, A. M.; Mo, C.-L.; Myers, P. L.; Noble, S. A.; Penn, H. R.; Wier, N. G.; Niall, G.; Woods, J. M.; Coe, P. L. *Nucleosides Nucleotides* **1999**, *18*, 203–216.
20. Moukha-chafiq, O.; Taha, M. L.; Lazrek, H. B.; Vasseur, J.-J.; Pannecouque, C.; Witvrouw, M.; De Clercq, E. *Il Farmaco* **2002**, *57*, 27–32.
21. Allen, S. H.; Johns, B. A.; Gudmundsson, K. S.; Freeman, G. A.; Boyd, F. L., Jr.; Sexton, C. H.; Selleseth, D. W.; Creech, K. L.; Moniri, K. R. *Bioorg. Med. Chem.* **2006**, *14*, 944–954.

22. Poreba, K.; Opolski, A.; Wietrzyk, J.; Kowalska, M. *Arch. Pharm. Pharm. Med. Chem.* **2001**, *334*, 219–223.
23. Baraldi, P. G.; Beria, I.; Cozzi, P.; Geroni, C.; Espinosa, A.; Gallo, M. A.; Entrena, A.; Bingham, J. P. *Bioorg. Med. Chem.* **2004**, *12*, 3911–3921.
24. Park, H.-J.; Lee, K.; Park, S.-J.; Ahn, B.; Lee, J.-C.; Cho, H. Y.; Lee, K.-I. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3307–3312.
25. Ortega, M. A.; Montoya, M. E.; Zarranz, B.; Jaso, A.; Aldana, I.; Leclerc, S.; Meijerc, L.; Monge, A. *Bioorg. Med. Chem.* **2002**, *10*, 2177–2184.
26. Witherington, J.; Bordas, V.; Garland, S. L.; Hickey, D. M. B.; Ife, R. J.; Liddle, J.; Saunders, M.; Smith, D. G.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1577–1580.
27. Witherington, J.; Bordas, V.; Haigh, D.; Hickey, D. M. B.; Ife, R. J.; Rawlings, A. D.; Slingspy, B. P.; Smith, D. G.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1581–1584.
28. Fahmy, H. T. Y.; Rostom, Sh. A. F.; Bekhit, A. A. *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 213–222.
29. Fahmy, H. T. Y.; Rostom, Sh. A. F.; Saudi, M. N. S.; Zjawiony, J. K.; Robins, D. J. *Arch. Pharm. Pharm. Med. Chem.* **2003**, *336*, 216–225.
30. Rostom, Sh. A. F.; Shalaby, M. A.; El-Demellawy, M. A. *Eur. J. Med. Chem.* **2003**, *38*, 959–974.
31. Rostom, Sh. A. F.; Fahmy, H. T. Y.; Saudi, M. N. S. *Sci. Pharm.* **2003**, *71*, 57–74.
32. Al-Saadi, M. S. M.; Rostom, Sh. A. F.; Faid Allah, H. M. *Saudi Pharm. J.* **2005**, *13*, 89–96.
33. Al-Saadi, M. S. M.; Rostom, Sh. A. F.; Faid Allah, H. M. *Alex. J. Pharm. Sci.* **2005**, *19*, 15–21.
34. Tovari, J.; Bocsi, J.; Ladenyi, A.; Lapis, K.; Timar, J. *Anticancer Res.* **1996**, *16*, 3307–3312.
35. El-Subbagh, H. I.; Abadi, A. H.; Lehmann, J. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 137–142.
36. Ryabinin, V. A.; Sinyakov, A. N.; de Soultrait, V. R.; Caumont, A.; Parissi, V.; Zakharova, O. D.; Vasyutina, E. L.; Yurchenko, E.; Bayandin, R.; Litvak, S.; Tarrago-Litvak, L.; Nevinsky, G. A. *Eur. J. Med. Chem.* **2000**, *35*, 989–1000.
37. Makki, M. S.; Faidallah, H. M. *Int. J. Chem.* **1993**, *4*, 117.
38. Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Sem. Oncol.* **1992**, *19*, 622–638.
39. Boyd, M. R.; Paull, K. D. *Drug Rev. Res.* **1995**, *34*, 91–109.
40. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Jangley, J.; Cronisie, P.; Viagro-Wolff, A.; Gray-Goodrich, M.; Campell, H.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
41. Acton, E. M.; Narayanan, V. L.; Risbood, P. A.; Shoemaker, R. H.; Vistica, D. T.; Boyd, M. R. *J. Med. Chem.* **1994**, *37*, 2185–2189.
42. Rigas, J. R.; Francis, P. A.; Miller, V. A.; Tong, W. P.; Roistacher, N.; Kris, M. G.; Orazem, J. P.; Young, C. W., Jr.; Warrell, R. P. *Cancer Chemother. Pharmacol.* **1995**, *35*, 483–488.
43. Almajan, G. L.; Innocenti, A.; Puccetti, L.; Manole, G.; Barbuceanu, S.; Saramet, I.; Scozzafava, A.; Supuran, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2347–2352.