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RESEARCH ARTICLE

Synthesis and *in vitro* antitumor and antimicrobial activity of some 2,3-diaryl-7-methyl-4,5,6,7-tetrahydroindazole and 3,3a,4,5,6,7-hexahydroindazole derivatives

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Abstract

The synthesis of a series of 2,3-diaryl-7-methyl-4,5,6,7-tetrahydroindazole and 3,3a,4,5,6,7-hexahydroindazole derivatives substituted with various biologically-active function groups with anticipated chemotherapeutic activity is described. 4-(7-methyl-3-aryl-3,3a,4,5,6,7-hexahydro-indazol-2-yl)benzenesulfonamides **2a–c**, which were employed as key intermediates in this study, were synthesized by cyclocondensation of 6-arylidene-2-methylcyclohexanones **1a–c** with 4-hydrazinobenzenesulfonamide hydrochloride. A detailed discussion of the reactions utilized in the preparation of the intermediate and target compounds is reported, and the structures of the newly synthesized compounds were substantiated with IR, ^{1}H and ^{13}C NMR spectral data and elementary microanalyses. Twenty of the newly synthesized compounds were selected by National Cancer Institute (NCI), Maryland, USA, to be evaluated for their antitumor activity and the results revealed that six compounds **3c**, **4d**,**e**, **5a**,**d** and **8c** exhibited broad spectrum of antitumor activity against most of the tested tumor cell lines. In addition, the *in vitro* antibacterial and antifungal activities of a number of the target compounds were also tested using the Agar-diffusion method. Some of these compounds have shown significant antibacterial and mild to moderate antifungal activities.

Keywords: Tetrahydroindazoles, hexahydroindazoles, benzenesulfonylureas, thioureas, antitumor and antimicrobial activity

Introduction

Over the past two decades, much evidence has been accumulated on the importance of pyrazole derivatives and their related fused heterocycles as chemotherapeutic agents. The pyrazole moiety is an important pharmacophore showing a multitude of biological and pharmacological properties. However, benzo-fused derivatives of pyrazole (indazole) are probably one of the least studied nuclei owing to its scarcity in nature. The indazole ring system is regarded as a bioisostere of indole. However, a large number of synthetically prepared indazoles are known to show a variety of biological activities, such as high binding affinity for estrogen receptor[1,2], inhibition of protein kinase C-β[3], 5-HT2 receptor agonism[4], human immunodeficiency virus (HIV) protease inhibition[5], anti-leukemic activity[6], and non-steroidal anti-inflammatory activity[7]. Other compounds of interest are YC-1, a benzylindazole which has been shown to activate soluble guanylate cyclase[8], nitric oxide synthase inhibition activity[9,10], to induce cellular apoptosis[11] and a MCHr1 antagonism for the treatment of obesity[12]. The most notable one is Lonidamine which was found to induce apoptosis *via* a direct effect on the mitochondrial permeability transition pore[11].

Similarly, tetrahydroindazoles are known to show activity against cancer[13] and inflammation[14] and hexahydroindazoles, a novel heterocyclic nucleus has been

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reported to have antimicrobial[15,16] and anti-inflammatory activities[17,18]. In a molecular docking study of 4,5,6,7-tetrahydro-2H-indazoles when position-2 is substituted with 4-(trifluoromethyl)phenylhydrazine group it was found to display more anti-inflammatory activity when compared with 2-Hydrazino-4-(trifluoromethyl)pyrimidine group. The difference in the anti-inflammatory activity of these two compounds may be attributed to an interplay between hydrogen bonding and hydrophobic interactions at the catalytic site of COX-2[14]. Minu *et al.* reported in a QSAR study of hexahydroindazoles as antimicrobial agents when substituted with an electron withdrawing group in position-3 of the indazole ring, the compounds were found more active than other derivatives[19]. In another study by Gokhan-Kelekci *et al.* substitution of a furan ring in position-3 of the indazole ring, produces a MAO inhibitor[20]. Compounds carrying phenyl substituents on nitrogen atom (position-2 in indazole) were found to be highly potent MAO inhibitors. Further, it was also noted that the presence of longer and voluminous groups on thiocarbamoyl nitrogen was selectively active towards MAO-A isoform while in case of MAO-B inhibitors the length of the lateral chain of the aryl diazo derivatives should not be longer than two atoms.

In view of the aforementioned findings, it was conferred that the position-2 and position-3 are crucial in the activity modulation of tetrahydro and hexahydroindazoles. Therefore, in continuation of our ongoing interest in the synthesis novel heterocycles with chemotherapeutic activities[21–30], it was considered of interest to prepare some new tetrahydro- and hexahydroindazole derivatives, bearing functionalities that are reported to contribute to potential chemotherapeutic activities such as the sulfonamide, sulfonylurea and thiourea groups. Besides incorporating an electron withdrawing group at position-3 we have also introduced a methyl substituent in position-7. Some target compounds were planned to comprise other heterocyclic rings that are known to possess significant antibacterial and anticancer activities such as the thiazole and the thiazine ring systems.

The structures of the newly synthesized compounds were confirmed by IR, ^{1}H and ^{13}C NMR data. The target compounds have been subjected to the National Cancer Institute NCI *in vitro* disease-oriented human cells screening panel assay, Maryland, USA, to screen their anticancer activity[31–33]. In addition, *in vitro* antibacterial and antifungal activities were also evaluated using the Agar-diffusion method[34]. Finally, owing to the increase of reported cases of tuberculosis even in developed countries, along with the emergence of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains, it was thought of interest to screen the *in vitro* antimycobacterial activity of the new analogs[35].

Experimental

Chemistry

Melting points were determined in open glass capillaries, on a Gallenkamp melting point apparatus, and were uncorrected (Table 1)[36]. The infrared (IR) spectra were recorded on Perkin-Elmer 297 infrared spectrophotometer, using the NaCl plate technique. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker DPX-400-FT spectrometer using $CDCl_3$ and DMSO-d_6 as a solvent and tetramethylsilane as the internal standard (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 aluminium sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254. The starting chalcone **1** was prepared according to a literature procedure.

4-(7-Methyl-3-aryl-3,3a,4,5,6,7-hexahydroindazol-2-yl) benzenesulfonamide (2a–c)

A solution of the appropriate chalcone **1a–c** (20 mmol) in ethanol (25mL) was refluxed with 4-hydrazinobenzenesulfonamide hydrochloride (4.9 g, 22 mmol) for 4 h. On concentration, the separated product was filtered, washed with cold ethanol and recrystallized from a mixture of benzene-ethanol (1:1). ^{1}H NMR data are listed in Table 2. ^{13}C NMR (DMSO/$CDCl_3$) **2a** δ = 14.0 (*C*H$_3$), 25.9, 27.6, 29.9, 33.3 (cyclohexyl-*C*), 42.6 (*C*-3a), 56.0 (*C*-3), 112.6, 125.7, 126.3, 127.7, 128.3, 139.4, 146.7, 155.6 (Ar-*C*). **2b** δ = 14.5 (*C*H$_3$), 26.9, 29.7, 32.8 (cyclohexyl-*C*), 43.2 (*C*-3a), 56.6 (*C*-3), 113.0, 126.8, 127.4, 128.2, 129.7, 131.0, 137.8, 147.3, 154.6 (Ar-C). **2c** δ = 13.9 (*C*H$_3$), 20.9 (*C*H$_3$), 25.2, 27.2, 29.4, 33.4 (cyclohexyl-*C*), 42.8 (*C*-3a), 56.8 (*C*-3), 112.2, 126.1, 127.3, 128.4, 129.0, 134.7, 135.9, 146.5, 152.8 (Ar-*C*).

4-(7-Methyl-3-aryl-4,5,6,7-tetrahydroindazol-2-yl) benzenesulfonamide (3a–c)

To a stirred suspension of the indazoline derivative **2** (10 mmol) in water (10 mL), bromine water (5%, 15 mL) was gradually added over a period of 30 min. at 25°C. After stirring for 3 h at room temperature, the pyrazole derivatives thus formed, were collected by filtration, thoroughly washed with water and dried. They were recrystallized from ethanol. ^{1}H NMR data are listed in Table 2. ^{13}C NMR (DMSO/$CDCl_3$) **3a** δ = 21.9 (*C*H$_3$), 18.1, 31.5, 32.2, 41.3 (cyclohexyl-*C*), 117.7, 119.1, 126.1, 127.0, 128.5, 129.0, 136.5, 136.8, 142.9, 145.4, 152.5 (Ar-*C*). **3c** δ = 20.2 (*C*H$_3$), 21.6 (*C*H$_3$), 18.4, 31.2, 32.6, 41.4 (cyclohexyl-*C*), 117.5, 118.8, 126.0, 126.9, 129.7, 133.5, 136.0, 137.7, 141.8, 144.6, 151.9.

N^1-[4-(7-Methyl-3-aryl-3,3a,4,5,6,7-hexahydroindazol-2-yl)benzenesulfonyl]- N^3-substituted ureas (4a-f) and N^1-[4-(7-Methyl-3-aryl-4,5,6,7-tetrahydroindazoline-2-yl) benzenesulfonyl]-N^3-substituted ureas (5a–f)

A mixture of the appropriate pyrazoline **2** or pyrazole **3** (10 mmol) and anhydrous potassium carbonate (2.8 g, 20

Table 1. Physicochemical and analytical data of compounds **2–10**.

Compound number	X	R	M.P. (°C)	Yield (%)	Mol. Formula (Mol. Weight)	Analysis Calculated/Found (%) C	H	N	S
2a	H		199–200	88	$C_{20}H_{23}N_3O_2S$	65.01	6.27	11.37	8.68
					(369.48)	64.95	6.33	11.45	8.59
2b	Cl		202–203	84	$C_{20}H_{22}ClN_3O_2S$	59.47	5.49	10.40	7.94
					(403.93)	59.30	5.60	10.27	8.05
2c	CH_3		204–206	85	$C_{21}H_{25}N_3O_2S$	65.77	6.57	10.96	8.36
					(383.51)	65.58	6.74	11.20	8.15
3a	H		150–152	72	$C_{20}H_{21}N_3O_2S$	65.37	5.76	11.44	8.73
					(367.46)	65.40	5.70	11.35	8.90
3b	Cl		148–149	70	$C_{20}H_{20}ClN_3O_2S$	59.77	5.02	10.46	7.98
					(401.91)	59.85	4.96	10.50	8.04
3c	CH_3		126–127	68	$C_{21}H_{23}N_3O_2S$	66.12	6.08	11.01	8.41
					(381.49)	66.12	6.08	11.01	8.41
4a	H	cyclohexyl	149–150	72	$C_{27}H_{34}N_4O_3S$	65.56	6.93	11.33	6.48
					(494.65)	65.45	7.10	11.50	6.40
4b	H	C_6H_5	138–139	75	$C_{27}H_{28}N_4O_3S$)	66.37	5.78	11.47	6.56
					(488.60)	66.15	5.92	11.55	6.77
4c	H	1-naphthyl	196–198	77	$C_{31}H_{30}N_4O_3S$	69.12	5.61	10.40	5.95
					(538.66)	69.03	5.80	10.50	5.78
4d	CH_3	cyclohexyl	206–207	74	$C_{28}H_{36}N_4O_3S$	66.11	7.13	11.01	6.30
					9508.68)	66.20	7.05	10.88	6.46
4e	CH_3	C_6H_5	158–160	75	$C_{28}H_{30}N_4O_3S$	66.91	6.02	11.15	6.38
					(502.63)	67.01	5.92	11.33	6.30
4f	CH_3	1-naphthyl	169–170	78	$C_{32}H_{32}N_4O_3S$	69.54	5.84	10.14	5.80
					(552.69)	69.48	5.96	10.20	5.76
5a	H	cyclohexyl	134–136	70	$C_{27}H_{32}N_4O_3S$	65.83	6.55	11.37	6.51
					(492.63)	66.01	6.39	11.26	6.70
5b	H	C_6H_5	162–164	72	$C_{27}H_{26}N_4O_3S$	66.65	5.39	11.51	6.59
					(486.59)	66.54	5.47	11.60	6.65
5c	H	1-naphthyl	148–149	75	$C_{31}H_{28}N_4O_3S$	69.38	5.26	10.44	5.98
					(536.64)	69.12	5.44	10.60	5.73
5d	CH_3	C_6H_5	172–173	78	$C_{28}H_{28}N_4O_3S$	67.18	5.64	11.19	6.41
					(500.61)	66.95	5.82	11.36	6.27
5e	CH_3	1-naphthyl	156–157	75	$C_{32}H_{30}N_4O_3S$	69.80	5.49	10.17	5.82
					(550.67)	69.69	5.60	10.25	5.92
6a	H	C_6H_5	162–163	89	$C_{27}H_{28}N_4O_2S_2$	64.26	5.59	11.10	12.71
					(504.67)	64.07	5.74	10.96	12.90
6b	H	$CH_2C_6H_5$	128–129	72	$C_{28}H_{30}N_4O_2S_2$	64.84	5.83	10.80	12.36
					(518.69)	65.02	5.68	10.89	12.21
6c	Cl	C_6H_5	172–174	75	$C_{27}H_{27}ClN_4O_2S_2$	60.15	5.05	10.39	11.90
					(539.11)	60.23	4.98	10.26	12.10
6d	Cl	$CH_2C_6H_5$	136–137	72	$C_{28}H_{29}ClN_4O_2S_2$	60.80	5.28	10.13	11.59
					(553.14)	60.44	5.37	10.06	11.78
6e	Cl	COC_6H_5	140–142	70	$C_{28}H_{27}ClN_4O_3S_2$	59.30	4.80	9.88	11.31
					(567.12)	59.21	4.89	10.01	11.25
6f	CH_3	C_6H_5	122–123	88	$C_{28}H_{30}N_4O_2S_2$	64.84	5.83	10.80	12.36
					(518.69)	65.00	5.67	10.90	12.25
6g	CH_3	$CH_2C_6H_5$	160–161	66	$C_{29}H_{32}N_4O_2S_2$	65.38	6.05	10.52	12.04
					(532.72)	68.18	6.22	10.44	12.13
6h	CH_3	COC_6H_5	140–142	72	$C_{29}H_{30}N_4O_3S_2$	63.71	5.53	10.25	11.73
					(546.70)	63.90	5.48	10.47	11.68

(*Continued*)

Table 1. Continued.

Compound number	X	R	M.P. (°C)	Yield (%)	Mol. Formula (Mol. Weight)	Analysis Calculated/Found (%) C	H	N	S
7a	H	C_6H_5	212–214	78	$C_{29}H_{28}N_4O_3S_2$	63.95	5.18	10.29	11.77
					(544.69)	64.04	5.10	10.06	12.01
7b	H	$CH_2C_6H_5$	126–128	65	$C_{29}H_{28}N4O_3S_2$	64.49	5.41	10.03	11.48
					(558.71)	64.22	5.60	9.89	11.63
7c	Cl	COC_6H_5	162–164	72	$C_{30}H_{27}ClN_4O_4S_2$	59.35	4.48	9.23	10.56
					(607.14)	59.52	4.21	9.44	10.10
7d	CH_3	C_6H_5	187–188	74	$C_{30}H_{30}N_4O_3S_2$	64.49	5.41	10.03	11.48
					(558.71)	64.77	5.23	10.12	11.39
7e	CH_3	$CH_2C_6H_5$	137–138	70	$C_{31}H_{32}N_4O_3S_2$	65.01	5.63	9.78	11.20
					(572.74)	64.88	5.92	9.54	11.47
7f	CH_3	COC_6H_5	150–152	72	$C_{31}H_{30}N_4O_4S_2$	63.46	5.15	9.55	10.93
					(586.72)	63.21	5.36	9.40	11.09
8a	H	C_6H_5	168–169	75	$C_{35}H_{32}N_4O_2S_2$	69.51	5.33	9.26	10.60
					(604.78)	69.86	5.18	9.14	10.82
8b	H	$CH_2C_6H_5$	112–113	62	$C_{36}H_{34}N_4O_2S_2$	69.87	5.54	9.05	10.36
					(618.81)	70.06	5.39	9.17	10.24
8c	CH_3	C_6H_5	122–123	64	$C_{36}H_{34}N_4O_2S_2$	69.87	5.54	9.05	10.36
					(618.81)	69.99	5.35	9.21	10.18
9a	H	C_6H_5	134–135	67	$C_{30}H_{30}N_4O_3S_2$	64.49	5.41	10.03	11.48
					(558.71)	64.30	5.67	9.95	11.64
9b	CH_3	C_6H_5	130–132	65	$C_{31}H_{32}N_4O_3S_2$	65.01	5.63	9.78	11.20
					(572.74)	64.86	5.99	9.90	11.03
9c	CH_3	$CH_2C_6H_5$	122–124	62	$C_{32}H_{34}N_4O_3S_2$	65.50	5.84	9.55	10.93
					(586.77)	65.77	5.60	9.37	10.74
10a	H	C_6H_5	174–176	70	$C_{30}H_{30}N_4O_3S_2$	64.49	5.41	10.03	11.48
					(558.71)	64.27	5.70	9.89	11.66
10b	H	$CH_2C_6H_5$	136–138	69	$C_{31}H_{32}N_4O_3S_2$	65.01	5.63	9.78	11.20
					(572.74)	64.85	5.81	10.01	11.14
10c	CH_3	C_6H_5	162–164	74	$C_{31}H_{32}N_4O_3S_2$	65.0164.91	5.63	9.78	11.20
					(572.74)	64.91	5.77	9.92	11.08

mmol) in dry acetone (25 mL) was heated under reflux with stirring with the appropriate isocyanate (11 mmol) 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 2N hydrochloric acid, the precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol. ^{1}H NMR data are listed in Table 2. ^{13}C NMR (DMSO/$CDCl_3$) **4a** δ = 14.3 (*C*H_3), 21.6, 25.7, 27.1, 27.6, 29.2, 32.7, 33.4, 46.9 (2 cyclohexyl-*C*), 41.8 (*C*-3a), 55.7 (*C*-3), 119.1, 121.2, 124.6, 126.3, 128.5, 136.7, 138.4, 142.4, 155.2 (Ar-*C*), 162.6 (*C*O). **4b** δ = 14.2 (*C*H_3), 25.6, 27.3, 29.4, 33.2 (cyclohexyl-*C*), 42.0 (*C*-3a), 56.8 (*C*-3), 119.3, 120.4, 124.1, 126.0, 127.2, 128.3, 128.7, 129.0, 129.3, 136.5, 136.7, 142.0, 155.1 (Ar-*C*), 161.3 (*C*O). **4d** δ = 14.4 (*C*H_3), 20.4 (*C*H_3), 21.2, 25.6, 26.9, 27.2, 29.4, 32.3, 33.2, 46.4 (2 cyclohexyl-*C*), 42.6 (*C*-3a), 55.8 (C-3), 118.8, 124.8, 126.4, 126.9, 129.7, 133.2, 137.0, 142.9, 155.6 (Ar-*C*), 163.1 (*C*O). **5a** δ = 15.2 (*C*H_3), 18.1, 22.0, 27.3, 31.6, 32.2, 33.5, 41.6, 44.4 (2 cyclohexyl-*C*), 117.7 (*C*-3a), 119.7, 126.8, 127.1, 128.5, 129.3, 135.9, 136.3, 145.2 (*C*-3), 145.4 (Ar-*C*), 150.6 (*C*-7a), 163.0 (CO). **5b** δ = 21.6 (*C*H_3), 18.4, 31.6, 32.8, 41.2 (cyclohexyl-*C*), 117.2 (*C*-3a), 119.3, 120.2, 124.6, 126.1, 127.2, 128.3, 128.7, 129.1, 136.4, 136.6, 139.0, 142.4, 145.0 (*C*-3), 152.5 (*C*-7a), 159.3 (*C*O). **5d** δ = 14.6 (*C*H_3), 20.7 (*C*H_3), 18.6, 31.2, 32.1, 40.9 (cyclohexyl-*C*), 118.3 (*C*-3a), 119.0, 126.1, 126.8, 127.0, 128.2, 129.0, 130.1, 133.1, 135.8, 136.7, 137.9, 142.6, 144.2 (*C*-3), 152.0 (*C*-7a), 160.3 (*C*O).

N^1-[4-(7-Methyl-3-aryl-3,3a,4,5,6,7-hexahydroindazol-2-yl) benzenesulfonyl]- N^3-substituted thioureas (6a–h)

A solution of the appropriate isothiocyanate (11 mmol) in dry acetone (5 mL) was added to a stirred mixture of the suitable indazoline **2** (10 mmol) and anhydrous potassium carbonate (2.8 g, 20 mmol) in dry acetone (25 mL), and the 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 2N hydrochloric acid. The precipitated product was filtered, washed with water, dried and recrystallized from ethanol. ^{1}HNMR data are listed in Table 2. ^{13}C NMR (DMSO/$CDCl_3$) **6a** δ = 14.8

Table 2. ^{1}H-NMR spectral data of compounds[a] (**2**–**10**).

Compound number	X	R	H-4,5,6 cyclohexyl (m, 6H)	C*H*$_3$ (d, 3H)	H-7 (m, 1H)	H-3a (m, 1H)	H-3 (d, 1H)	Ar-H (m)	Others
2a	H		1.46–1.74	1.28	3.04	3.67	5.38	6.85–7.58 (11H)[b]	
2b	Cl		1.42–1.75	1.28	2.94	3.70	4.95	6.82–7.88 (10H)[b]	
2c	CH_3		1.38–1.69	1.27	3.03	3.64	5.31	6.76–7.92 (10H)[b]	2.31 (s, 3H, Tol-C*H*$_3$)
3a	H		1.52–2.35	1.26	3.08	–	–	6.82–7.68 (11H)[b]	
3b	Cl		1.45–1.62	1.27	3.10	–	–	6.77–7.82 (10H)[b]	
3c	CH_3		1.62–2.28	1.30	3.12	–	–	6.90–7.82 (10H)[b]	2.35 (s, 3H, Tol-C*H*$_3$)
4a	H	cyclohexyl	1.14–2.74[c]	1.24	3.10	3.78	5.44	7.14–8.11 (9H)	9.1 (s, 1H, N*H*), 9.6 (s, 1H, N*H*)
4b	H	C_6H_5	1.36–1.72	1.27	2.98	3.62	5.32	6.83–7.55 (14H)	8.93 (s, 1H, N*H*), 9.25 (s, 1H, N*H*)
4c	H	1-naphthyl	1.38–1.80	1.25	3.02	3.71	5.38	6.98–8.02 (16H)	9.11 (s, 1H, N*H*), 9.72 (s, 1H, N*H*)
4d	CH_3	cyclohexyl	1.22–2.68[c]	1.23	3.14	3.65	5.52	7.23–7.92 (8H)	2.32 (s, 3H, Tol-C*H*$_3$), 9.23 (s, 1H, N*H*), 9.58 (s, 1H, N*H*)
4e	CH_3	C_6H_5	1.39–1.79	1.27	3.06	3.63	5.42	7.02–7.98 (13H)	2.35 (s, 3H, Tol-C*H*$_3$), 9.32 (s, 1H, N*H*), 9.63 (s, 1H, N*H*)
5a	H	cyclohexyl	1.18–2.60[c]	1.27	3.11	–	–	7.04–7.99 (8H)	9.25 (s, 1H, N*H*), 9.48 (s, 1H, N*H*)
5b	H	C_6H_5	1.35–2.28	1.28	3.05	–	–	7.02–8.05 (13H)	9.32 (s, 1H, N*H*), 9.43 (s, 1H, N*H*)
5c	H	1-naphthyl	1.38–2.17	1.28	3.07	–	–	7.18–8.06 (15H)	9.04 (s, 1H, N*H*), 9.65 (s, 1H, N*H*)
5d	CH_3	C_6H_5	1.42–2.30	1.27	3.10	–	–	7.12–8.15 (13H)	2.38 (s, 3H, Tol-C*H*$_3$), 9.26 (s, 1H, N*H*), 9.42 (s, 1H, N*H*)
5e	CH_3	1-naphthyl	1.29–2.26	1.26	3.18	–	–	7.24–8.12 (15H)	2.34 (s, 3H, Tol-C*H*$_3$), 9.15 (s, 1H, N*H*), 9.61 (s, 1H, N*H*)
6a	H	C_6H_5	1.48–1.75	1.24	3.68	3.65	5.24	6.92–7.80 (14H)	9.02 (s, 1H, N*H*), 9.78 (s, 1H, N*H*)
6b	H	$CH_2C_6H_5$	1.39–1.72	1.26	3.58	3.92	5.25	7.02–7.82 (14H)	4.75 (d, 2H, C*H*$_2$- C_6H_5), 9.13 (s, 1H, N*H*), 9.92 (s, 1H, N*H*)
6c	Cl	C_6H_5	1.41–1.78	1.24	3.42	3.70	5.21	6.87–7.84 (13H)	9.24 (s, 1H, N*H*), 9.63 (s, 1H, N*H*)
6d	Cl	$CH_2C_6H_5$	1.39–1.67	1.23	3.38	3.62	5.14	7.11–7.89 (13H)	4.72 (d, 2H, C*H*$_2$), 9.08 (s, 1H, N*H*), 9.52 (s, 1H, N*H*)
6e	Cl	COC_6H_5	1.40–1.82	1.25	3.02	3.74	5.16	7.16–7.79 (13H)	9.18 (s, 1H, N*H*), 9.63 (s, 1H, N*H*)
6f	CH_3	C_6H_5	1.37–1.67	1.25	3.00	3.72	5.32	6.78–7.90 (13H)	2.32 (s, 3H, Tol-C*H*$_3$), 9.21 (s, 1H, N*H*), 9.78 (s, 1H, N*H*)
6g	CH_3	$CH_2C_6H_5$	1.34–1.69	1.28	3.14	3.66	5.04	7.13–8.11 (13H)	2.29 (s, 3H, Tol-C*H*$_3$), 4.29 (d, 2H, C*H*$_2$), 9.03 (s, 1H, N*H*), 9.49 (s, 1H, N*H*)
6h	CH_3	COC_6H_5	1.44–1.78	1.31	3.06	3.72	5.12	7.16–8.03 (13H)	2.34 (s, 3H, Tol-C*H*$_3$), 9.14 (s, 1H, N*H*), 9.55 (s, 1H, N*H*)
7a	H	C_6H_5	1.42–1.72	1.28	3.10	3.64	5.34	6.92–7.85 (14H)	4.30 (s, 2H, C*H*$_2$)
7b	H	$CH_2C_6H_5$	1.45–1.75	1.29	3.03	3.78	5.22	7.01–8.10 (14H)	4.16 (s, 2H, C*H*$_2$), 4.82 (s, 2H, C*H*$_2$- C_6H_5)
7c	Cl	COC_6H_5	1.40–1.74	1.28	3.06	3.77	5.25	6.88–7.82 (13H)	4.33 (s, 2H, C*H*$_2$)
7d	CH_3	C_6H_5	1.43–1.69	1.26	3.12	3.69	5.43	7.14–7.92 (13H)	2.31 (s, 3H, Tol-C*H*$_3$), 4.13 (s, 2H, C*H*$_2$)
7e	CH_3	$CH_2C_6H_5$	1.39–1.72	1.24	3.34	3.84	5.68	7.12–7.85 (13H)	2.34 (s, 3H, Tol-C*H*$_3$), 4.24 (s, 2H, C*H*$_2$), 4.80 (s, 2H, C*H*$_2$)
7f	CH_3	COC_6H_5	1.40–1.75	1.25	3.24	3.75	5.52	7.09–7.88 (13H)	2.30 (s, 3H, Tol-C*H*$_3$), 4.19 (s, 2H, C*H*$_2$)
8a	H	C_6H_5	1.38–1.85	1.29	2.98	3.65	5.28	6.80–7.75 (19H)	5.78 (s, 1H, *H*-5 thiazole)
8b	H	$CH_2C_6H_5$	1.37–1.75	1.26	3.01	3.68	5.10	6.70–7.88 (19H)	4.75(s, 2H,C*H*$_2$), 5.89 (s, 1H, *H*-5 thiazole)
8c	CH_3	C_6H_5	1.36–1.78	1.28	3.05	3.72	5.15	6.80–7.95 (18H)	2.35 (s, 3H, Tol-C*H*$_3$), 5.74 (s, 1H, *H*-5 thiazole)
9a	H	C_6H_5	1.38–1.85	1.26	3.25[d]	3.68	5.10	6.70–7.88 (14H)	
9b	CH_3	C_6H_5	1.35–1.90	1.32	3.19[d]	3.72	5.30	6.92–7.82 (13H)	2.32 (s, 3H, Tol-C*H*$_3$)
9c	CH_3	$CH_2C_6H_5$	1.41–1.72	1.23	3.09[d]	3.74	5.48	6.88–7.90 (13H)	2.31 (s, 3H, Tol-C*H*$_3$), 4.75 (d, 2H, C*H*$_2$- C_6H_5)
10a	H	C_6H_5	1.34–1.78	1.29	2.99	3.70	5.29	6.88–7.82 (14H)	4.40 (s, 2H, *H*-4 thiazine), 5.42 (s, 2H, *H*-6 thiazine)
10b	H	$CH_2C_6H_5$	1.37–1.79	1.20	3.12	3.78	5.34	6.94–7.82 (14H)	4.42 (s, 2H, *H*-4 thiazine), 5.62 (s, 2H, *H*-6 thiazine)
10c	CH_3	C_6H_5	1.40–1.68	1.29	3.20	3.69	5.21	6.86–7.78 (13H)	2.34 (s, 3H, Tol-C*H*$_3$), 4.38 (s, 2H, *H*-4 thiazine), 5.54 (s, 2H, *H*-6 thiazine)

[a]Solutions in a mixture of C*D*Cl$_3$ and DMSO-d_6; [b](Ar-*H* + N*H*$_2$); [c](m, 16H, 2cyclohexyl); [d](m, 5H, H-7 and H-5 & H-6 of thiazine).

(CH_3), 25.6, 27.4, 29.7, 33.1 (cyclohexyl-*C*), 42.6 (*C*-3a), 55.8 (*C*-3), 119.1, 124.5, 125.3, 126.0, 127.2, 128.4, 128.8, 129.0, 136.5, 137.2, 140.0, 143.1, 155.2 (Ar-*C*), 186.3 (*C*S). **6b** δ = 14.2 (CH_3), 25.9, 27.9, 29.7, 33.2 (cyclohexyl-*C*), 42.6 (*C*-3a), 56.0 (*C*-3), 55.5 (CH_2), 119.3, 126.1, 126.5, 127.2, 127.5, 128.3, 128.6, 129.0, 136.8, 137.1, 141.3, 142.9, 154.8 (Ar-*C*), 188.2 (*C*S).

2-[4-(7-Methyl-3-aryl-3,3a,4,5,6,7-hexahydroindazol-2-yl) benzenesulfonylimino]-3-substituted thiazolidin-4-ones (7a–f)

To a solution of the appropriate thiourea derivative **6** (10 mmol) in absolute ethanol (20 mL) was added ethyl bromoacetate (1.84 g, 11 mmol) and anhydrous sodium acetate (1.64 g, 20 mmol), and the reaction mixture was heated under reflux for 2 h. The mixture was left to attain room temperature then poured into ice-cold water (30 mL), and the solid product thus formed was filtered, washed with water, dried and recrystallized from ethanol-benzene mixture (1:1). ^{1}H NMR data are listed in Table 2. ^{13}C NMR (DMSO/$CDCl_3$) **7a** δ = 14.0 (CH_3), 32.8 (CH_2), 26.0, 27.7, 30.1, 33.5 (cyclohexyl-*C*), 42.6 (*C*-3a), 56.0 (*C*-3), 113.3, 120.4, 124.2, 125.7, 126.9, 127.1, 128.3, 128.9, 139.4, 140.8, 148.5, 155.8 (Ar-C), 163.3 (*C*=N), 168.8 (*C*O). **7b** δ = 14.1 (CH_3), 33.0 (CH_2), 25.8, 27.6, 29.9, 33.2 (cyclohexyl-*C*), 41.8 (*C*-3a), 55.9 (*C*-3), 45.5 (Ph-CH_2), 112.9, 125.5, 126.4, 126.9, 127.1, 127.4, 128.3, 128.5, 128.7, 138.8, 142.4, 146.9, 154.2 (Ar-*C*), 164.0 (*C*=N), 169.9 (*C*O). **7c** δ = 14.6 (CH_3), 32.8 (CH_2), 25.7, 27.8, 29.6, 33.1 (cyclohexyl-*C*), 42.0 (*C*-3a), 56.2 (*C*-3), 113.4, 125.6, 126.8, 127.3, 128.2, 128.3, 128.6, 129.7, 131.6, 133.5, 137.5, 139.2, 148.4, 155.6 (Ar-C), 163.0 (*C*=N), 167.7 (*C*O-Ph), 172.3 (*C*O).

2-[4-(7-Methyl-3-aryl-3,3a,4,5,6,7-hexahydroindazol-2-yl) benzenesulfonylimino]- 4-phenyl-3-substituted thiazolines (8a–c)

A solution of the appropriate thiourea derivative **6** (10 mmol) in absolute ethanol (20 mL) was refluxed with ω-bromoacetophenone (2.2 g, 11 mmol) and anhydrous sodium acetate (1.64 g, 20 mmol) for 3 hr 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 from ethanol. ^{1}H NMR data are listed in Table 2. ^{13}C NMR (DMSO/$CDCl_3$) **8c** δ = 14.3 (CH_3), 20.7 (CH_3), 25.8, 27.7, 29.4, 33.3 (cyclohexyl-*C*), 42.3 (*C*-3a), 56.0 (*C*-3), 88.4 (*C*-2, thiazoline), 115.9, 120.4, 124.8, 125.7, 126.2, 126.8, 127.3, 127.8, 128.3, 128.5, 128.6, 134.9, 136.2, 140.2, 141.3, 148.5, 152.8 (*C*-3, thiazoline), 155.4 (Ar-C), 163.8 (*C*=N).

2-[4-(7-Methyl-3-aryl-3,3a,4,5,6,7-hexahydroindazol-2-yl) benzenesulfonylimino]-3-substituted-[1,3]thiazinan-4-ones (9a–c)

To a solution of the appropriate thiourea derivative **6** (10 mmol) in absolute ethanol (20 mL) was added ethyl 3-bromopropionate (2 g, 11 mmol) and anhydrous sodium acetate (1.64 g, 20 mmol) and the reaction mixture was heated under reflux for 4 h. After the mixture was cooled to room temperature, it was 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). ^{1}H NMR data are listed in Table 2. ^{13}C NMR (DMSO/$CDCl_3$) **9a** δ = 14.2 (CH_3), 24.8 (*C*-6, thiazine), 35.7 (*C*-5, thiazine), 25.7, 27.6, 29.5, 33.2 (cyclohexyl-C), 42.6 (*C*-3a), 56.3 (*C*-3), 114.3, 120.4, 124.1, 125.7, 126.6, 127.2, 128.2, 128.3, 128.7, 139.2, 140.8, 148.5, 155.6 (Ar-*C*), 163.0 (*C*=N), 173.4 (*C*O). **9b** δ = 14.8 (CH_3), 21.2 (CH_3), 25.6 (*C*-6, thiazine), 36.2 (*C*-5, thiazine), 43.0 (*C*-3a), 55.2 (*C*-3), 114.0, 120.3, 124.6, 125.2, 126.7, 127.0, 128.2, 128.3, 128.8, 138.9, 140.6, 149.4, 154.9 (Ar-*C*), 164.8 (*C*=N), 174.3 (*C*O).

2-[4-(7-Methyl-3-aryl-3,3a,4,5,6,7-hexahydroindazol-2-yl) benzenesulfonylimino]-3-substituted-[1,3]thiazinan-5-ones (10a–c)

To a solution of the appropriate thiourea derivative **6** (10 mmol) in absolute ethanol (20 mL) was added 1,3-dichloroacetone (1.4 g, 11 mmol) and anhydrous sodium acetate (2 g, 25 mmol) and the reaction mixture was heated under reflux for 5 h. After the mixture was cooled to room temperature, it was 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). ^{1}H NMR data are listed in Table 2. ^{13}C NMR (DMSO/$CDCl_3$) **10a** δ = 14.1 (CH_3), 25.8, 27.6, 29.7, 33.1 (cyclohexyl-*C*), 37.4 (*C*-6, thiazine), 59.4 (*C*-4, thiazine), 112.3, 115.8, 116.8, 126.0, 126.8, 127.0, 127.4, 128.3, 129.2, 137.4, 143.5, 148.1, 155.2 (Ar-*C*), 164.6 (*C*=N). **10c** δ = 14.3 (CH_3), 20.8 (CH_3), 25.7, 27.8, 30.0, 33.2 (cyclohexyl-*C*), 36.9 (*C*-6, thiazine), 58.1 (*C*-4, thiazine), 113.4, 114.2, 117.1, 125.4, 126.7, 127.1, 128.1, 128.3, 129.4, 137.1, 138.2, 148.4, 155.9 (Ar-*C*), 163.3 (*C*=N).

Biological activity

In vitro *antitumor screening*

Twenty of the newly synthesized compounds have been selected by the 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) according to the protocol of the Drug Evaluation Branch, NCI, Bethesda[31–33]. All the compounds that reduced the growth of any one of the cell lines to 32% or less namely **3c**, **4d**,**e**, **5a**,**d** and **8c** were passed on for evaluation in the full panel of 60 human tumor cell lines of 9 tumor subpanels including leukemia, non-small cell lung cancer, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast cancer cell lines. These cell lines were incubated with five concentrations (0.01–100 μM) for each compound. A 48h continuous drug exposure protocol was used, and a SRB protein assay was employed to estimate cell viability or growth. 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 sub-panel or the full panel, respectively.

Antimicrobial screening

Inhibition zone (IZ) measurement Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 µg/mL) was placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6051) as examples of Gram positive bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) as examples of Gram negative bacteria, *Candida albicans* (ATCC 10231) and *Aspergillus niger* (recultured) as representatives of fungi. Ampicillin trihydrate and Clotrimazole were used as standard antibacterial and antifungal agents, respectively. DMSO alone was used as control at the same above-mentioned concentration. The plates were incubated at 37°C for 24 h for bacteria and 72 h for fungi. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth around the discs in mm (Table 5).

Minimal inhibitory concentration (MIC) measurement MICs were measured for compounds that showed significant growth inhibition zones (≥ 12 mm) using the two-fold serial dilution technique[34]. The micro-dilution susceptibility test in Muller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin trihydrate and Clotrimazole were prepared in DMSO at concentration of 1600 µg/mL followed by two-fold dilution at concentrations of 800, 400, 6.25 µg/mL). The microorganism suspensions at 10^6 CFU/mL (Colony Forming Unit/mL) concentration were inoculated to the corresponding wells. Plates were incubated at 36°C for 24 h to 48 h and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also done.

Antimycobacterial screening

The available *Mycobacterium tuberculosis* strain was cultured in tubes with 10 µL of a suspension of the strain in physiological saline. The strain was collected from the slant after four weeks incubation[33]. The inoculum was prepared with 3–5 weeks old *M. tuberculosis* colonies from Loewenstein-Jensen slants, emulsified in dilution fluid containing 2% fatty acid free albumin and 0.02% Tween 80, pH 6.9. Suspensions were then diluted in saline to a turbidity of McFarland no.1 standard and then diluted to obtain inocula of 3×10^5 cells per well. The MICs measurement were determined by agar dilution technique Agar supplemented with 10% OADC (oleic acid-albumin-dextrose-catalase) enrichment, was used to prepare quadrant plates with serial DMSO two-fold dilutions of the test compounds. The following concentrations were used: 1000, 500, 250, 125, 62, 32, 16, 8, 4 µg/mL. A 100 µL sample of the mycobacterial suspension was inoculated onto each compound-containing quadrant. Control quadrant consisted of agar alone, culture medium with DMSO and culture medium with reference antimycobacterial drugs; rifampicin and 1HN was performed. All plates were then incubated at 37°C in a CO_2 (5% CO_2 / 95% humidified air) incubator, for 3–4 weeks. The MIC was defined as the lowest chemical dilution associated with at least a 99% reduction in the number of visible colonies.

Results and discussion

Chemistry

The starting compounds in this study are 6-arylidene-2-methylcyclohexanones **1a–c** could be synthesized *via* Claisen-Schmidt condensation of aromatic aldehydes with 2-methylcyclohexanone. These chalcones were reacted with 4-hydrazinobenzenesulfonamide hydrochloride to afford the desired hexahydroindazole derivatives **2a–c** (Scheme 1). Their IR spectra revealed two bands at 3365–3388 cm^{-1} and 3220–3258 cm^{-1} corresponding to the amino group and two bands at 1350–1378 cm^{-1} and 1180–1195 cm^{-1} attributed to the SO_2N function. The structures of these compounds were further confirmed by the ^{1}H-NMR which exhibited a characteristic doublet at δ 4.95–5.38 ppm attributed to the H-3 proton and a multiplet at δ 3.64–3.70 ppm due to the H-3a proton which substantiate the closure of the indazoline ring (Table 2). Mild oxidation of the indazoline **2a–c** with bromine water led to the formation of the corresponding indazoles **3a–c**. Such oxidation was confirmed by the ^{1}H-NMR spectra, which lacked the characteristic doublet and multiplet of H-3 and H-3a protons of the parent indazolines (Table 2). The ureido derivatives **4a–f** and **5a–e** were prepared in good yields by reacting the indazoline **2a–c** or the indazole **3a–c** derivatives with the appropriate isocyanate in the presence of anhydrous potassium carbonate as a mild basic catalyst. The IR spectra of these compounds showed beside the absorption bands corresponding to the NH group at 3216–3288 cm^{-1}, two bands at 1345–1372 cm^{-1} and 1177–1190 cm^{-1} for the SO_2N function, in addition to a characteristic urea carbonyl band at 1645–1665 cm^{-1}. In a similar fashion, the synthesis of the thioureido derivatives **6a–h** was achieved by refluxing **2a–c** with the appropriate isothiocyanate in dry acetone in the presence of anhydrous potassium carbonate as a mild basic catalyst (Scheme 2). Their IR spectra revealed beside the characteristic absorption bands corresponding to the amino group and the SO_2N function lying at the same range of their structurally-related derivatives, a characteristic C=S band at 1155–1165 cm^{-1}. Cyclization of the thioureido derivatives **6a–h** with ethyl bromoacetate afforded the corresponding 4-oxothiazolidines **7a–f** in good yields. The IR of these compounds was characterized by the appearance of a new band at 1720–1735 cm^{-1} due to the carbonyl group at the thiazol-C_4 and two bands at 1338–1365 cm^{-1} and 1169–1188 cm^{-1} for the SO_2N

moiety. Refluxing **6** with phenacyl bromide afforded the targeted thiazolines **8a–c**. Whereas, reacting the same compounds **6a–c** with ethyl 3-bromopropionate or 1,2-dichloroacetone resulted in the formation of the corresponding 4-oxo-5,6-dihydrothiazines **9a–c** and the 5-oxo-5,6-dihydrothiazines **10a–c**, respectively. Their IR spectra were characterized by the presence of a carbonyl band at 1720–1730 cm^{-1} as well as two bands at 1340–1373 cm^{-1} and 1174–1185 cm^{-1} for the SO_2N group. In addition, their ^{1}H-NMR spectra showed the appearance of new methylene groups of the thiazine rings at a range of δ 3.19–5.42 ppm (Table 2).

In vitro antitumor evaluation

Primary in vitro *3-cell line assay*

Twenty of the newly synthesized compounds were chosen by the NCI to be evaluated for their in vitro antitumor activity. Primary *in vitro* 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, National Cancer Institute, Bethesda[31–33]. The compounds were added at a single concentration (10^{-4} M) and the culture was incubated for 48 h. Compounds which reduced the growth of any one of the cell lines to 32% or less, were passed on for the full panel 60-cell lines assay. Six compounds **3c**, **4d**, **4e**, **5a**, **5d** and **8c** out of the 16 tested compounds have been selected for a 60-cell panel assay.

In vitro *full panel 60-cell line assay*

About 60 cell lines of nine tumor subpanels including leukemia, non-small cell lung cancer, colon, central nervous system, 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 corresponds to the concentration of the compounds causing 50% decrease in net cell growth, the TGI value is the concentration of the compounds resulting in total growth inhibition and the LC_{50} value 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[31–33].

From the results obtained it could be noticed that, only six compounds, namely; **3c**, **4d**,**e**, **5a**,**d** and **8c** exhibited remarkable antitumor activity against most of the tested subpanel tumor cell lines (GI_{50} and TGI values < 100 μM), whereas the rest of the compounds proved to be totally inactive. Those six compounds showed an obvious sensitivity against some individual cell lines (Table 3). Compound **3c** proved to be significantly active towards leukemia CCRF-CEM, colon SW-620 and renal A498 cell lines with GI_{50} values of 3.65, 4.82 and 6.28 μM respectively. However, compound **4d** was found to be active against leukemia RPMI-8226 and non-small cell lung NCI-H322 cell lines with GI_{50} values of 5.56 and 4.98 μM, respectively and compound **4e** exhibited distinct activity against the colon HCT-116 cancer cells (GI_{50} 3.40 μM). Furthermore, **5d** displayed potential activity against all the leukemia cell lines with a GI_{50} values range of 2.46–24.62 μM. A special activity pattern was shown by compound **5a** which was remarkably active against most of the tested 60 cell lines with GI_{50} values range of 0.02–28.4 μM. It revealed super potent activity towards leukemia CCRF-CEM, SR, melanoma SK-MEL-5 and renal A498, TK-10, UO-31 with GI_{50} values of 0.55, 0.80, 1.36, 0.02, 1.72 and 1.35 μM, respectively (Figures 1–3).

Concerning the broad spectrum of antitumor activity, the active compounds displayed effective growth inhibition GI_{50} (MG-MID), total growth inhibitory TGI (MG-MID) and cytotoxic LC_{50} (MG-MID) activities (<100 μM) (Table 4). Compound **5a** having GI_{50}, TGI, and LC_{50} MG-MID values of 9.22, 26.61 and 69.80 μM, respectively, proved to be the most active member in this study. It revealed potential activity against all the tested subpanel tumor cell lines with special high potency on the leukemia subpanel at the GI_{50} level (2.45 μM) (Table 4). Furthermore, the compound showed almost the same level of antitumor activity against the non-small cell lung, colon, melanoma, renal and prostate cancer subpanels (GI_{50} range 10.41–14.20 μM). Moderate activity has been shown by this compound against the CNS, ovarian and breast subpanel tumor cell lines (Table 4). On the other hand, compounds **3c** and **4d** were almost equipotent at the GI_{50} level (15.60 and 15.91 μM, respectively), while they showed some difference in the TGI and LC_{50} MG-MID values (Table 4). Moreover, compounds **4e**, **5d** and **8c** displayed appreciable antitumor activity towards most of the tested tumor cell lines at the GI_{50}, TGI, and LC_{50} MG-MID levels (Table 4).

A close examination of the structures of the active compounds revealed that, while the hexahydroindazole derivative **2c** was totally inactive, its corresponding tetrahydroindazole analog **3c** showed significant antitumor activity against most of the tested subpanel tumor cell lines with GI_{50}, TGI and LC_{50} MG-MID values of 15.6, 39.6 and 78.3 μM, respectively. Condensation of **3c** with different isocyanates to produce the dihydrosulfonylureas **4d** (X = CH_3, R^1 = cyclohexyl) and **4e** (X = CH_3, R^1 = phenyl), did not significantly improve the anticancer activity at the GI_{50} MG-MID (15.91 and 14.40 μM, respectively), TGI MG-MID (38.4 and 49.62 μM, respectively) and the LC_{50} MG-MID levels (77.80 and 88.23 μM, respectively). On the contrary, oxidation of compound **4d** resulted in a highly active indazolesulfonylurea **5a** (X = H, R^1 = cyclohexyl), which is proved to be the most active member in this study with potential activity against all the tested subpanel tumor cell lines (GI_{50}, TGI and LC_{50} MG-MID values 9.22, 26.61 and 69.80 μM, respectively). In addition, **5a** was able to exert potential inhibitory activity against the leukemia subpanel (GI_{50} 2.45 μM, Table 4), when compared with

Table 3. Growth inhibitory concentration (GI_{50}, μM) of the active compounds.

Cell Lines	3c	4d	4e	5a	5d	8c
Leukaemia						
CCRF-CEM	3.65	18.43	18.67	0.55	2.46	16.71
HL-60 (TB)	NT[a]	23.02	18.51	6.20	24.62	15.50
K-562	17.4	13.36	12.70	3.23	12.24	16.01
MOLT-4	10.2	7.70	8.23	1.90	6.11	13.72
RPMI-8226	18.2	5.56	15.41	3.20	7.43	13.63
SR	14.5	6.53	3.01	0.80	2.35	12.06
Non-Small Cell Lung Cancer						
A549/ATCC	24.74	13.91	13.21	22.27	27.90	19.81
EKVX	20.21	21.10	13.92	11.62	18.52	22.00
HOP-62	21.41	46.75	18.95	17.55	33.15	42.22
HOP-92	14.60	13.33	15.30	2.79	18.83	19.21
NCI-H226	17.12	20.82	16.01	21.81	16.46	22.22
NCI-H23	23.12	14.85	14.72	11.90	16.90	NT
NCI-H322	19.80	4.98	20.62	4.95	15.84	29.01
NCI-H460	21.32	21.81	22.03	8.93	19.91	16.20
NCI-H522	11.75	6.80	12.66	12.84	11.22	24.61
Colon Cancer						
COLO 205	22.14	13.51	19.01	11.81	20.00	24.32
HCC-2998	8.62	11.82	NT	NT	13.81	NT
HCT-116	11.53	16.52	3.40	4.02	20.92	17.90
HCT-15	20.62	20.83	15.52	17.42	16.45	24.32
HT29	22.15	23.86	25.35	24.94	36.78	NT
KM12	16.40	11.92	18.03	13.52	17.51	23.20
SW-620	4.82	16.60	12.92	2.82	12.40	15.9
CNS Cancer						
SF-268	21.31	23.24	18.54	12.07	19.77	15.47
SF-295	21.14	18.54	13.07	17.62	33.22	18.92
SF-539	19.01	21.21	13.92	10.06	18.87	18.35
SNB-19	384	22.32	25.78	20.92	31.32	66.77
SNB-75	17.94	44.73	18.36	21.64	23.48	NT
U251	20.14	12.85	13.94	11.57	18.50	19.64
Melanoma						
LOX IMVI	19.35	18.43	12.54	1.95	11.37	18.40
M14	20.20	35.70	17.41	19.47	23.74	32.52
SK-MEL-2	12.67	17.56	15.32	15.67	17.15	22.01
SK-MEL-28	18.67	18.32	23.5	17.30	20.44	NT
SK-MEL-5	12.78	14.97	14.26	1.36	13.23	14.1
UACC-257	21.22	22.44	18.49	24.72	17.70	21.43
UACC-62	13.74	11.94	12.28	18.08	11.82	15.05
Ovarian Cancer						
IGROV1	3.3	5.8	11.7	11.3	3.8	26.9
OVCAR-3	14.6	14.9	13.7	19.7	33.3	16.2
OVCAR-4	16.4	18.6	16.5	16.8	20.4	NT
OVCAR-5	26.2	28.3	13.1	28.4	31.9	NT
OVCAR-8	35.4	19.7	22.4	27.0	18.9	22.3
SK-OV-3	48.2	21.2	17.9	19.5	17.7	35.1
Renal Cancer						
786-0	16.12	29.40	12.61	2.33	22.84	32.57
A498	6.28	22.51	16.0	0.02	19.70	14.76
ACHN	19.89	14.77	13.81	15.6	14.8	21.82
CAKI-1	18.17	17.40	13.66	19.50	16.04	17.16
RXF 393	17.80	18.78	16.70	22.73	16.59	23.80
SN12C	18.11	17.06	14.10	18.94	17.00	16.76
TK-10	20.12	12.34	16.60	1.72	11.32	19.19

(*Continued*)

Table 3. Continued.

Cell Lines	3c	4d	4e	5a	5d	8c
UO-31	6.64	11.16	11.72	1.35	8.75	17.74
Prostate Cancer						
PC-3	17.12	10.66	14.95	12.61	17.10	22.53
DU-145	15.26	24.61	14.22	15.53	15.93	23.79
Breast Cancer						
MCF7	32.43	11.35	18.11	5.94	21.54	5.67
NCI/ADR-RES	22.44	12.74	16.17	17.00	33.81	NT
MDA-MB-231/ATCC	13.85	28.52	11.58	21.89	17.55	15.19
HS 578T	14.57	24.76	18.74	21.15	23.81	19.02
MDA-MB-435	17.92	15.06	17.17	19.54	28.22	18.16
BT-549	NT	17.78	15.47	12.14	18.09	19.28
T-47D	72.23	31.45	35.98	26.47	31.05	36.85

[a]NT: Not Tested.

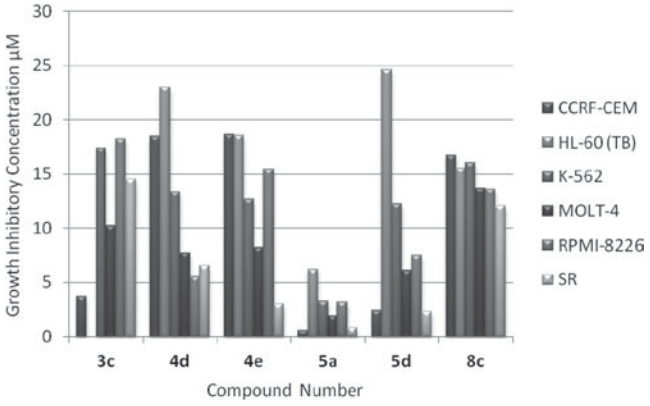


Figure 1. Leukaemia Growth inhibitory concentration (GI_{50}, µM) of the active compounds.

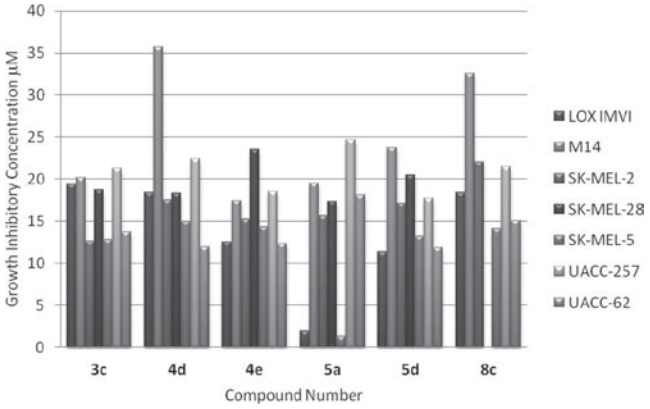


Figure 2. Melanoma Growth inhibitory concentration (GI_{50}, µM) of the active compounds.

sulofenur; (NSC 656667; GI_{50} 1.27 µM, Table 4); a known sulfonylurea derivative with potential antineoplastic activity (Figure 4).Replacing the cyclohexyl moiety in **5a** with a phenyl moiety and introduction of a methyl group as in **5d** (X = CH_3, R^1 = phenyl), resulted in about 2-fold decrease in activity at the GI_{50} and TGI levels (16.64 and 54.22, respectively). However, to substantiate the postulate a detailed protein assay is required

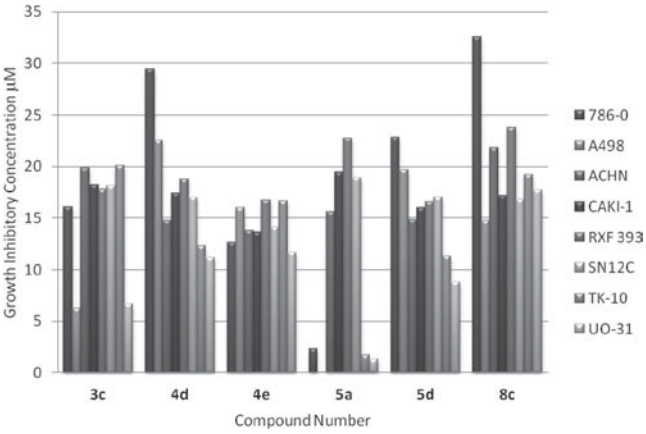


Figure 3. Renal Cancer Growth inhibitory concentration (GI_{50}, µM) of the active compounds.

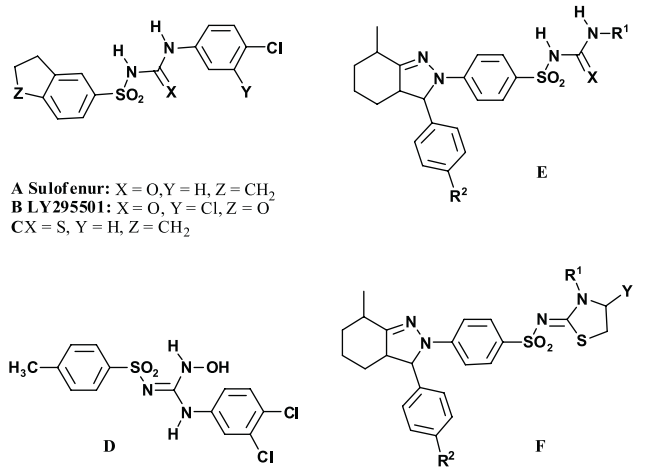


Figure 4. Structures of sulofenur **A**, its congeners **B–D** and the general structures of the new compounds **E** and **F**. (See colour version of this figure online at www.informahealthcare.com/enz)

in order to outline the mechanism of their anticancer activity. Moreover, in a related study 3-substituted-phenyl-1H-indole-5-sulfonamides[37] have shown inhibitions of some of the isoforms of carbonic anhydrase enzyme. This involves interfering with pH regulations of various

Table 4. Median growth inhibitory concentrations (GI_{50}, μM), total growth inhibitory concentrations (TGI, μM) and lethal concentrations (LC_{50}, μM) of *in vitro* subpanel tumor cell lines.

Compound number	Subpanel Tumor Cell Lines[a]									MG-MID[b]		
	I	II	III	IV	V	VI	VII	VIII	IX	GI_{50}	TGI	LC_{50}
3c	13.08	18.13	15.38	22.2	15.5	24.02	14.81	16.84	30.0	15.60	39.61	78.30
4d	10.43	16.54	16.40	23.6	19.6	18.14	17.42	16.40	20.5	15.91	38.40	77.80
4e	12.55	14.37	15.25	17.01	15.72	16.05	15.03	13.84	18.6	14.40	49.62	88.23
5a	2.45	11.21	12.00	15.82	14.20	20.51	10.41	12.21	17.1	9.22	26.61	69.80
5d	8.70	18.29	19.41	24.71	16.67	20.65	16.51	15.65	21.8	16.64	54.22	91.23
8c	14.19	23.61	20.67	18.58	20.62	25.52	20.82	23.00	18.2	20.04	51.63	90.11
S[c]	1.27	1.05	1.21	1.21	1.30	1.163	1.16	NT[d]	NT	1.19	1.63	1.99

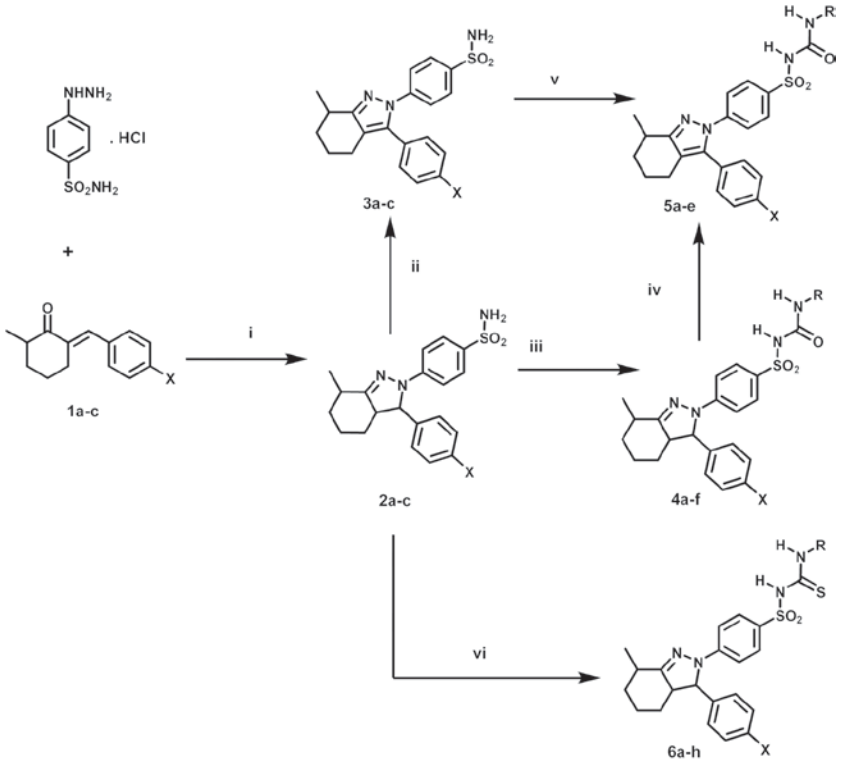
[a]GI_{50} values against: I, Leukaemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.
[b]GI_{50}, TGI and LC_{50} (μM) full panel mean-graph midpoint (MG-MID) = the average sensitivity of all cell lines towards the test agent.
[c]Sulofenur; (NSC 656667): NCI cancer screen; August 2004.
[d]NT = Not tested.

Table 5. Antimicrobial activity of the target compounds **2–10.**

Compound number	S. aureus (ATCC 25923)		B. subtilis (ATCC 6051)		E.coli (ATCC 25922)		P. aeruginosa (ATCC 27853)		C. albicans (ATCC 10231)	
	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
2a	10	>200 (540)	9	...c	7	...	NA[d]	...	6	...
2b	13	100 (247.6)	14	100 (247.6)	14	200 (495.1)	10	...	12	...
2c	15	100 (260.7)	16	200 (521.5)	8	...	NA	...	14	100
3a	19	100 (272.1)	18	100 (272.1)	10	...	NA	...	9	...
3b	22	100 (248.8)	17	100 (248.8)	14	200 (497.6)	10	...	16	100 (248.8)
3c	24	50 (131)	20	50 (131)	15	200 (524)	11	...	23	100 (262)
4a	19	50 (101.1)	18	100 (202.2)	6	...	NA	...	8	...
4b	16	100 (204.7)	15	200 (409.3)	9	...	NA	...	10	...
4d	18	100 (196.6)	16	200 (393.2)	14	200 (393.2)	12	100 (196.6)	15	100 (196.6)
4e	17	100 (198.9)	15	200 (397.9)	12	200 (397.9)	10	...	9	...
5a	28	12.5 (25.4)	22	50 (101.5)	17	50 (101.5)	14	200 (406)	29	50 (101.5)
5b	20	50 (102.7)	16	100 (205.4)	12	200 (410.8)	10	...	18	50 (102.7)
5d	24	50 (100)	18	100 (200)	16	100 (200)	11	...	20	50 (100)
6a	8	...	6	...	NA	...	10	...	NA	...
6b	7	...	8	...	6	...	5	...	NA	...
6c	9	...	6	...	NA	...	NA	...	NA	...
6f	10	...	8	...	10	...	6	...	9	...
7a	12	>200 (367.2)	10	...	8	...	NA	...	10	...
7b	14	100 (179)	12	200 (358)	15	200 (358)	10	...	12	100 (179)
7c	16	100 (164.7)	14	200 (329.4)	12	200 (329.4)	10	...	9	...
7d	18	100 (179)	15	200 (358)	14	200 (358)	10	...	12	100 (179)
8a	16	100 (165.3)	18	100 (165.3)	14	200 (330.6)	16	200 (330.6)	8	...
8c	18	100 (161.6)	17	100 (161.6)	16	200 (323.2)	NA	...	6	...
9a	12	>200 (358)	9	...	8	...	NA	...	NA	...
9b	18	100 (174.6)	17	50 (87.3)	14	200 349.2)	NA	...	NA	...
10a	14	200 (358)	12	200 (358)	12	...	13	...	NA	...
10b	16	100 (174.6)	14	200 (349.2)	10	...	NA	...	6	...
A*	36	12.5 (31)	30	25 (62)	32	25 (62)	27	50 (124)	...	...
C**	...	...	...	...	...	...	...	...	42	12.5 (36.2)

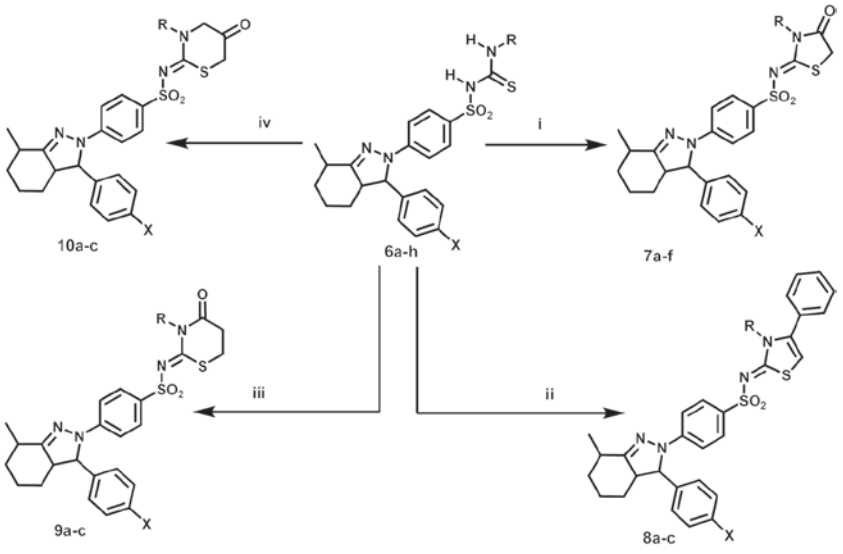
[a]Inhibition zone (mm).
[b]Minimal Inhibitory Concentration: μg/mL(μM).
[c]Not tested.
[d]Not active.
A*: Ampicillin trihydrate; C**: Clotrimazole.

forms of tumours and represents a new anticancer drug discovery strategy. Finally, although the thiourea derivatives **6f** and **6g** were inactive in this test, one of the cyclized products namely **8c** showed some moderate antitumor activity (GI_{50}, TGI and LC_{50} MG-MID values 20.04, 51.63 and 90.11 μM, respectively).



1, 2, 3: X = **a:** H, **b:** Cl, **c:** CH_3
4: a: X = H, R = cyclo-C_6H_{11}; **b:** X = H, R = C_6H_5; **c:** X = H, R = 1-naphthyl;
d: X = CH_3, R = cyclo-C_6H_{11}; **e:** X = CH_3, R = C_6H_5; **f:** X = CH_3, R = 1-naphthyl;
5: a: X = H, R = cyclo-C_6H_{11}; **b:** X = H, R = C_6H_5; **c:** X = H, R = 1-naphthyl;
d: X = CH_3, R = C_6H_5; **e:** X = CH_3, R = 1-naphthyl;
6: a: X = H, R = C_6H_5; **b:** X = H, R = $CH_2C_6H_5$;
c: X = Cl, R = C_6H_5; **d:** X = Cl, R = $CH_2C_6H_5$; **e:** X = Cl, R = COC_6H_5;
f: X = CH_3, R = C_6H_5; **g:** X = CH_3, R = $CH_2C_6H_5$; **h:** X = CH_3, R = COC_6H_5;

Scheme 1. Scheme 1 Reagents and reaction conditions: (i) ethanol, reflux, 4 h; (ii, iv) Br_2 water, r.t., 3 h; (iii, v) RNCO, anhyd.K_2CO_3, acetone, reflux, 18 h; (vi) RNCS, anhyd. K_2CO_3, acetone, reflux, 10 h.



7: a: X = H, R = C_6H_5; **b:** X = H, R = $CH_2C_6H_5$; **c:** X = Cl, R = COC_6H_5;
d: X = CH_3, R = C_6H_5; **e:** X = CH_3, R = $CH_2C_8H_5$; **f:** X = CH_3, R = COC_6H_5;
8: a: X = H, R = C_6H_5; **b:** X = H, R = $CH_2C_6H_5$; **c:** X = CH_3, R = C_6H_5;
9: a: X = H, R = C_6H_5; **b:** X = CH_3, R = C_6H_5; **c:** X = CH_3, R = $CH_2C_6H_5$;
10: a: X = H, R = C_6H_5; **b:** X = H, R = $CH_2C_6H_5$; **c:** X = CH_3, R = C_6H_5

Scheme 2. Reagents and reaction conditions: (i) ethyl bromoacetate, anhyd. Na acetate, ethanol, reflux, e h; (ii) phenacyl bromide, anhyd. Na acetate, ethanol, reflux, 3 h; (iii) ethyl 3-bromopropionate, anhyd. Na acetate, ethanol, reflux, 4 h; (iv) 1,3-dichloroacetone, anhyd. Na acetate, ethanol, areflux, 5 h.

Antimicrobial screening

Compounds **2a-c**, **3a-c**, **4a**,**b**,**d**,**e**, **5a**,**b**,**d**, **6a-c**,**f**, **7a-d**, **8a**,**c**, **9a**,**b** and **10a**,**c** were evaluated for their in *vitro* antimicrobial activity[34] against *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6051) as examples of Gram positive bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) as examples of Gram negative bacteria, *Candida albicans* (ATCC 10231) and *Aspergillus niger* (recultured) as representatives of fungi.

The results revealed that most of the tested compounds displayed greater inhibitory effect on the growth of the tested Gram positive strain compared to Gram negative ones. Most of the compounds showed weak or no antibacterial activity against the Gram negative, *P. aeruginosa*. Moreover, few compounds were able to exert mild to moderate antifungal activity against *C. albicans,* while, all the tested compounds lacked antifungal activity against *Aspergillus niger*. A close examination of the structures of the active compounds revealed that the antimicrobial profile of the tetrahydroindazole compounds **3** seemed to be more interesting than their corresponding hexahydroindazole derivatives **2**, as evidenced by their IZ diameters and MIC values recorded in Table 5.

Among the hexahydroindazole series, compound **2a** showed weak antimicrobial activity against all the tested microbial strains (IZ ≤ 12 mm). Introduction of chlorine atom or methyl group in the phenyl ring in position 3 of the hexahydroindazole derivative as in **2b** and **2c** resulted in a slight improvement in the activity against the Gram positive *S. aureus* and *B. subtilis* (MIC values 100 and 200 μg/mL, respectively). Whereas, replacement of the hexahydroindazole moiety in **2a–c** with tetrahydroindazole resulted in more potent compounds **3a–c**, with an appreciable broad spectrum of antibacterial activity against the tested Gram positive, Gram negative bacteria (MIC values 100–200 μg/mL), and moderate antifungal activity towards *C. albicans* (MIC 100 μg/mL). The replacement of amino group in **3a** with a urea moiety as in **5a** produced the most potent antimicrobial activity in the current series of compounds which was as potent as ampicillin (MIC 12.5 μg/mL) against *S. aureus,* whereas its activity against *B. subtilis* and *E.Coli* was 50% lower than that of ampicillin (MIC 50 *vs* 25 μg/mL, respectively). It displayed a moderate antifungal activity towards *C. albicans*, which was about 50% of that of Clotrimazole (MIC 25 *vs* 12.5 μg/mL, respectively). However, it is worthy to mention that, structure modification of the urea derivatives to thiourea ones led to almost complete abolishment of the antimicrobial activity (IZ ≤ 10 mm).

Antimycobacterial screening

Determination of *in vitro* antimycobacterial activity of the target compounds **2a–c**, **3a–c**, **4a**,**b**,**d**,**e**, **5a**,**b**,**d**, **6a–c**,**f**, **7a–d**, **8a**,**c**, **9a**,**b** and **10a**,**c** was performed by employing the two-fold agar dilution method slightly modified from that described by Mamolo and Vio[34]. A strain of *Mycobacterium tuberculosis* (locally isolated, Alexandria, Egypt) was utilized in this assay. Rifampicin and isonicotinic acid hydrazide (INH) were used as reference antimycobacterial drugs. The MIC was defined as the lowest concentration of the tested compound that yielded no visible growth on the plate. Among the compounds tested, only compound **7a** was able to exert weak growth inhibitory effect (MIC 250 μg/mL) against the *Mycobacterium tuberculosis* used in this screening, while the rest of the synthesized compounds were totally inactive.

Conclusion

In conclusion, the objective of the present study was to synthesize and investigate the antitumor, antimicrobial and antimycobacterial activities 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** (Figure 1). This aim has been verified by the synthesis of hybrid compounds comprising the above mentioned pharmacophores substituted essentially with a 3-(4-toly)-[1,2-c] pyrazol(in)e counterpart at the N^3 aryl moiety having the general structure **E** and **F** (Figure 1), for synergistic purpose. The results revealed that six compounds namely, **3c**, **4d**,**e**, **5a**,**d** and **8c** have exhibited broad spectrum of antitumor activity against most of the tested tumor cell lines. Compound **5a** proved to be the most active antitumor agent in the present study with GI_{50}, TGI and LC_{50} MG-MID values of 9.22, 26.61 and 69.80 μM, respectively, with high sensitivity towards some leukemia, melanoma and renal cell lines. The other five active compounds showed variable degrees of appreciable antitumor activity (GI_{50} and TGI MG-MID values range 14.40–20.04 and 38.40–54.22 μM, respectively). In addition, the *in vitro* antibacterial and antifungal activities of a number of the target compounds revealed that some of them have significant antibacterial and mild to moderate antifungal activities. Compound **5a** produced the most potent antimicrobial activity in the current series of compounds with equipotency to ampicillin (MIC 12.5 μg/mL) against *S. aureus,* and 50% of ampicillin's activity against *B. subtilis* and *E.coli* (MIC 50 *vs* 25 μg/mL, respectively), beside a moderate antifungal activity against *C. albicans*, which was about 50% of that of Clotrimazole (MIC 25 *vs* 12.5 μg/mL, respectively).

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 and/or antimicrobial agents.

Declaration of interest

The authors report no conflicts of interest.

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