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Synthesis of a new class of 2-anilino substituted nicotinyl arylsulfonylhydrazides as potential anticancer and antibacterial agents

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Abstract—A series of *N*′-1-[2-anilino-3-pyridyl]carbonyl-1-benzenesulfonohydrazide derivatives (**7a–i**) was synthesized and five of them were selected by the National Cancer Institute (NCI) and evaluated for their in vitro anticancer activity. Three of the investigated compounds **7d**, **7f** and **7g** exhibited significant anticancer activity in the primary assay and further tested against a panel of 60 human tumour cell lines. Compound **7g** showed 50% growth inhibitory activity in leukaemia, melanoma, lung cancer, colon cancer, renal cancer and breast cancer cells with GI_{50} value of 3.2–9.6 μM. The synthesized compounds (**7a–i**) were also evaluated for their antibacterial activity against various Gram-positive and Gram-negative strains of bacteria. Most of these compounds showed better inhibitory activity in comparison to the standard drugs.

1. Introduction

An elaborate search of new anticancer agents has primarily been triggered by the unveiling of new molecular targets on which they intervene, followed by the discovery of novel classes of compounds that interact with such targets.[1,2] Sulfonamides constitute an important class of compounds that exhibit a broad spectrum of biological activities like antibacterial,[3] antitumour,[4–9] diuretic,[6,10] hypoglycaemic,[11] etc. A number of sulfonamides have also been screened particularly for their antitumour activity, which led to the discovery of a novel sulfonamide *N*-[2-[(4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzenesulfonamide (E7010), which inhibits tubulin polymerisation[12] (Fig. 1). This compound causes cell cycle arrest and apoptosis in M phase and is shown to exhibit microtubule assembly owing to its reversible binding to the colchicine binding site on tubulin.[13,14] Compound E7010 also exhibited good in vivo antitumour activity against various rodent tumour and human tumour xenografts[12] and presently undergoing the stages of clinical trials.

It has been shown in the literature that sulfonylhydrazide analogues have potential for cancer chemotherapy.[16] Further, sulfonamides incorporating hydrazino moieties have also been recently reported to possess carbonic anhydrase inhibition.[17] Based on these findings an attempt has been made in the present study to incorporate a sulfonylhydrazide moeity to the 2-anilino pyridyl structural component of E7010. Moreover, nicotinylarylsulfonylhydrazides potentiated barbiturate necrosis and depressed exploratory behaviour in mice and high dose showed analgesic activity.[18] Therefore, a new family of 2-anilinonicotinyl arylsulfonylhydrazides have been designed and synthesized to evaluate their antitumour activity. In continuation to our efforts for identifying a variety of biological targets of sulfonamides and sulfonylhydrazides, we have investigated the antibacterial activities of these compounds as well.

2. Chemistry

Acid-catalysed esterification of 2-chloronicotinic acid (**1**) in ethanol afforded 2-chloro nicotinic acid ethylester (**2**). The substituted anilines (**3a–e**) were refluxed with ethylester (**2**) in ethylene glycol to give the coupled product of 2-anilino nicotinic acid esters (**4a–e**),[19] which on treatment with hydrazine hydrate in ethanol gave 2-anilino nicotinic acidhydrazides (**5a–e**) in quantitative

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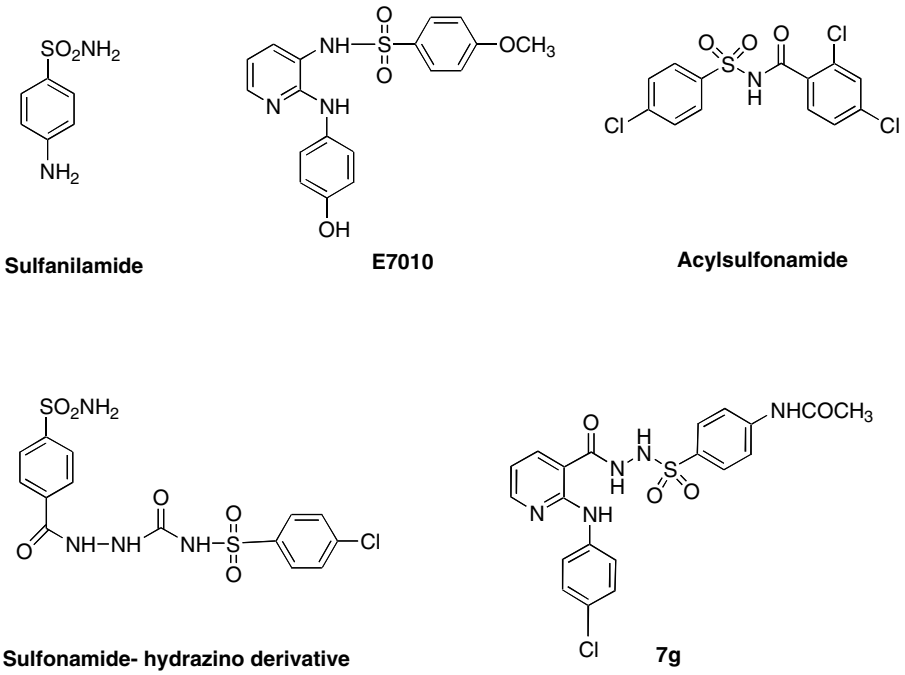


Figure 1. Chemical structures of sulfanilamide, E7010, acylsulfonamide, sulfonamide-hydrazino derivative and 2-anilino substituted nicotinyl arylsulfonyl hydrazide.

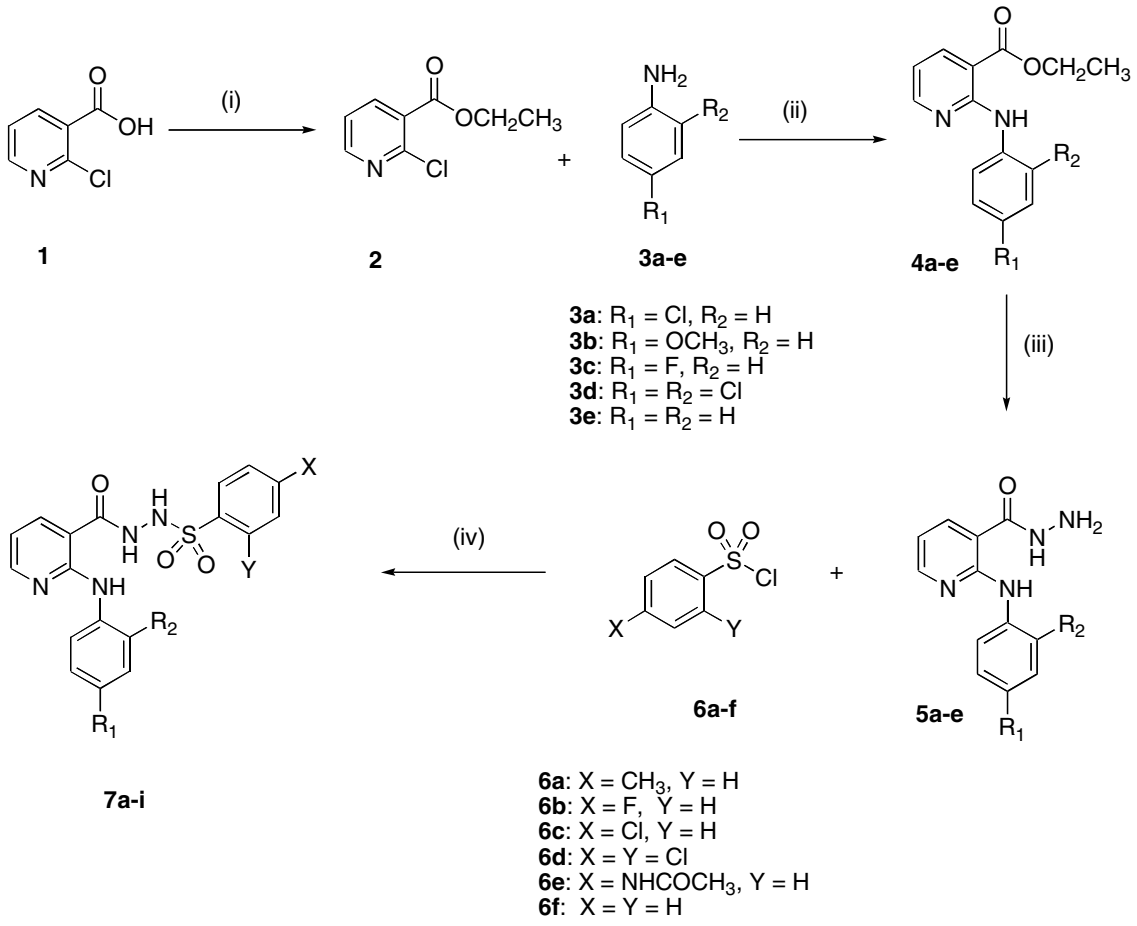
yields.[20] Substituted arylsulfonyl chlorides (**6a**–**f**) were synthesized according to the literature procedure.[21] The synthesis of the final products **7a**–**i** was carried out by the reaction of arylsulfonyl chlorides (**6a**–**f**) and hydrazides (**5a**–**e**) in pyridine[22] (Scheme 1).

3. Results and discussions

3.1. Antitumour activity

Amongst the substituted 2-anilino nicotinylarylsulfonylhydrazide derivatives synthesized, compounds **7b**, **7c**, **7d**, **7f** and **7g** were chosen by National Cancer Institute (NCI) as prototypes for preliminary test and were evaluated in three cell lines one dose prescreen[23–25] comprising of MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) cell lines. These have been in use by DTP (Development Therapeutic Program) for several years to evaluate combinatorial libraries and have proven to be an effective test of agents, which exhibited some capability level to inhibit the growth of human tumour cells in culture. 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 percentage test cell growth compared with untreated control cells (PTC) as illustrated in Table 1. Compounds, which reduce the growth of any one of the cell lines to 32% or less, were selected for further evaluation in the full panel of 60 human tumour cell lines. Compound **7d** has shown 0% of growth inhibition against all the three cell lines. Similarly, **7f** and **7g** presented the 1% and 31% of growth inhibition for the NCI-H460 cell line, respectively. However, compounds **7b** and **7c** have not reduced the growth of any cell lines by 32% or less. Therefore, only three compounds **7d**, **7f** and **7g** have been selected for 60-cell line panel assay. Compounds **7d**, **7f** and **7g** were further evaluated at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M against 60 different human tumour cell lines organized in subpanels representing melanoma, leukaemia and cancers of breast, prostate, lung, colon, ovary, CNS and kidney. The details of the cell lines used are shown in Table 2 and the experimental procedures have been described in the literature in detail.[24,26–28] Three dose response parameters were calculated for each experimental agent: the compound concentration required to carry 50% of net cell growth (GI_{50}) which signifies the growth inhibitory power of the test agents; the compound concentrations resulting in total growth inhibition (TGI) which signifies the cytostatic effect of the test agent; and the concentration of the compound leading to the 50% of net cell death (LC_{50}) which signifies the cytotoxic effect of the test agent. The $\log_{10}$ GI_{50}, $\log_{10}$ TGI and $\log_{10}$ LC_{50} were then determined defined as the means of the $\log_{10}$'s of the individual GI_{50}, TGI and LC_{50} value as shown in Table 2, respectively. Compounds having $\log_{10}$ GI_{50} values -4 and <-4 were declared to be active. The mean graph points (MG_MID) represent average values for each of the mentioned parameters and indicate the average sensitivity of all cell lines to each tested compound.

From Table 2, we can conclude that, all the active compounds in this test showed broad-spectrum antitumour activity against the nine tumour subpanels tested, and demonstrated significant activity in the in vitro antitumour screening expressed by MG-MID $\log_{10}$ GI_{50} value



Scheme 1. Reagents and conditions: (i) H_2SO_4, C_2H_5OH, reflux, 3 h; (ii) ethylene glycol, 160 °C, 6 h; (iii) $N_2H_4 \cdot H_2O$, C_2H_5OH, reflux, 2 h; (iv) pyridine, 0 °C, rt.

Table 1. Primary in vitro growth inhibition assay results at 10^{-4} M concentration

Compound	PTC[a]			60-Tumour cell line selection
	MCF-7[b]	NCI-H460[c]	SF-268[d]	
7b (NSC: 736601)	102	84	106	N
7c (NSC: 736600)	111	62	123	N
7d (NSC: 736599)	0	0	0	Y
7f (NSC: 736602)	44	1	34	Y
7g (NSC: 737147)	52	31	60	Y

Y, yes selected; N, not selected.
[a] PTC, percent test cell growth compared with untreated control cells.
[b] Breast cell line.
[c] Lung cell line.
[d] CNS cell line.

of −4.55, −4.67 and −4.73 of compounds **7d**, **7f** and **7g**, respectively, whereas compounds **7b** and **7c** were inactive ($\log_{10}$ $GI_{50} > -4$). Substitution of para methoxy on 2-anilino ring, para methyl on aryl sulfonyl ring (**7b**, $R_1 = OCH_3$, $X = CH_3$) and para fluoro on both the 2-anilino as well as aryl sulfonyl rings (**7c**, $R_1 = F$, $X = F$) has reduced the activity. $\log_{10}$ GI_{50} value of compound **7d** is −4.50 and −4.54 in the breast cancer cell lines (MCF-7 and MDA-MB-231, respectively), whereas this value in case of E7010 is −6.14 and −5.07 for the same cell lines.[15] Substitution of chloro group on the para position of 2-anilino ring, and aryl sulfonyl ring at ortho and para positions, respectively, (**7f**, $R_1 = Cl$, $X = Y = Cl$) also exhibits similar $\log_{10}$ GI_{50} value for these cell lines, that is, −4.45 and −4.72, however the activity is enhanced particularly in case of leukaemia CCRF-CEM cell line ($\log_{10}$ GI_{50} −5.18; GI_{50} is 6.62 μM). In case of compound **7g** $\log_{10}$ GI_{50} value is −5.36 and −4.38 in the breast cancer cell lines (MCF-7 and MDA-MB-231 cell lines, respectively) and also demonstrated significant activity in other cell lines.

Table 2. Inhibition of in vitro cancer cell lines by selected 2-anilino substituted nicotinyl arylsulfonylhydrazides **7d**, **7f** and **7g**[a]

Panel cell line	Response parameters: (A) $\log_{10}$ GI_{50}[b] (M), (B) $\log_{10}$ TGI[c] (M), (C) $\log_{10}$ LC_{50}[d] (M) and MG_MID[e]								
	Compound **7d**			Compound **7f**			Compound **7g**		
	A	B	C	A	B	C	A	B	C
Leukaemia									
CCRF-CEM	−4.68	>−4.00	>−4.00	−5.18	>−4.00	>−4.00	−4.67	>−4.00	>−4.00
HL-60 (TB)	−4.60	−4.21	>−4.00	−4.62	−4.35	−4.08	−5.01	>−4.00	>−4.00
K-562	−4.70	−4.08	>−4.00	−4.89	−4.59	−4.30	−5.07	>−4.00	>−4.00
MOLT-4	−4.64	>−4.00	>−4.00	−4.71	−4.24	>−4.00	−4.30	>−4.00	>−4.00
RPMI-8226	nt	nt	nt	−4.75	−4.27	>−4.00	−4.54	>−4.00	>−4.00
SR	−4.64	−4.17	>−4.00	−4.83	−4.42	−4.01	−5.20	>−4.00	>−4.00
Non-small cell lung cancer									
A549/ATCC	−4.76	−4.39	−4.01	−4.61	−4.15	>−4.00	−4.49	>−4.00	>−4.00
EKVX	−453	>−4.00	>−4.00	4.58	>−4.00	>−4.00	−4.31	>−4.00	>−4.00
HOP-62	−4.51	−4.07	>−4.00	−4.78	−4.42	−4.07	>−4.00	>−4.00	>−4.00
HOP-92	−4.81	−4.44	−4.07	−4.83	−4.39	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H226	−4.53	−4.01	>−4.00	−4.62	−4.23	>−4.00	−4.37	>−4.00	>−4.00
NCI-H23	−4.44	>−4.00	>−4.00	−4.76	−4.37	>−4.00	nt	nt	nt
NCI-H322M	−4.51	>−4.00	>−4.00	−4.83	−4.39	>−4.00	>−4.00	>−4.00	>−4.00
NCI-H460	−4.50	>−4.00	>−4.00	−4.45	−4.00	>−4.00	−5.33	>−4.00	>−4.00
NCI H522	nt	nt	nt	nt	nt	nt	−5.19	>−4.00	>−4.00
Colon cancer									
COLO 205	−4.79	−4.29	>−4.00	−4.92	4.61	−4.31	−4.99	−4.24	>−4.00
HCC-2998	−4.52	>−4.00	>−4.00	−4.87	−4.54	−4.21	−5.08	>−4.00	>−4.00
HCT-116	−4.52	>−4.00	>−4.00	−4.81	−4.54	−4.27	−5.14	>−4.00	>−4.00
HCT-15	−4.57	>−4.08	>−4.00	−4.48	>−4.00	−4.00	−4.86	>−4.00	>−4.00
HT29	−4.66	−4.24	>−4.00	−4.64	−4.27	>−4.00	−5.40	>−4.00	>−4.00
KM12	−4.64	−4.31	>−4.00	−4.86	−4.54	−4.23	−5.38	>−4.00	>−4.00
SW-620	−4.54	>−4.00	>−4.00	−4.55	>−4.00	>−4.00	−5.12	>−4.00	>−4.00
CNS cancer									
SF-268	−4.34	>−4.00	>−4.00	−4.44	4.00	>−4.00	−4.66	>−4.00	>−4.00
SF-295	−4.43	>−4.00	>−4.00	−4.52	4.11	>−4.00	−5.46	>−4.00	>−4.00
SF-539	−4.58	−4.13	>−4.00	−4.72	4.35	>−4.00	−4.81	−4.12	>−4.00
SNB-19	−4.53	>−4.00	>−4.00	−4.57	4.00	>−4.00	−4.85	>−4.00	>−4.00
SNB-75	−4.59	>−4.00	>−4.00	−4.65	4.07	>−4.00	−5.26	>−4.00	>−4.00
U251	−4.54	>−4.00	>−4.00	−4.46	−4.00	>−4.00	−4.62	>−4.00	>−4.00
Melanoma									
LOX IMVI	−4.49	>−4.00	>−4.00	−4.68	−4.33	>−4.00	−4.62	>−4.00	>−4.00
MALME 3M	−4.46	>−4.00	>−4.00	−4.95	−4.46	>−4.00	>−4.00	>−4.00	>−4.00
M14	−4.33	>−4.00	>−4.00	−4.72	−4.41	−4.10	−4.94	>−4.00	>−4.00
SK-MEL-2	−4.37	>−4.00	>−4.00	−4.73	−4.37	−4.01	nt	nt	nt
SK-MEL-28	−4.05	>−4.00	>−4.00	−4.45	>−4.00	>−4.00	−4.63	>−4.00	>−4.00
SK-MEL-5	−4.60	−4.07	>−4.00	−4.85	−4.53	−4.21	−5.25	>−4.00	>−4.00
UACC-257	−4.93	−4.28	>−4.00	−4.64	−4.22	>−4.00	−4.05	>−4.00	>−4.00
UACC-62	−4.34	>−4.00	>−4.00	−4.66	−4.12	>−4.00	−5.05	>−4.00	>−4.00
Ovarian cancer									
IGROV1	nt	nt	nt	nt	nt	nt	−4.56	>−4.00	>−4.00
OVCAR-3	−4.53	−4.02	>−4.00	−4.69	>−4.00	>−4.00	−5.43	>−4.00	>−4.00
OVCAR-4	−4.42	>−4.00	>−4.00	−4.54	>−4.00	>−4.00	−4.35	>−4.00	>−4.00
OVCAR-5	−4.28	>−4.00	>−4.00	−4.58	−4.11	>−4.00	nt	nt	nt
OVCAR-8	−4.57	>−4.00	>−4.00	−4.57	−4.13	>−4.00	−4.44	>−4.00	>−4.00
SK-OV-3	−4.42	>−4.00	>−4.00	−4.77	−4.28	>−4.00	−4.24	>−4.00	>−4.00
Renal cancer									
786-0	−4.64	−4.27	>−4.00	−4.71	−4.31	>−4.00	−4.32	>−4.00	>−4.00
A498	−4.76	−4.44	−4.11	−4.60	−4.32	−4.04	−4.73	>−4.00	>−4.00
ACHN	−4.34	>−4.00	>−4.00	−4.53	−4.03	>−4.00	−4.28	−4.12	>−4.00
CAKI-1	−4.51	>−4.00	>−4.00	−4.70	−4.23	>−4.00	−5.04	>−4.00	>−4.00
RXF 393	−4.94	−4.56	−4.18	−4.77	−4.36	>−4.00	−4.43	>−4.00	>−4.00
SN12C	−4.75	−4.50	−4.24	−4.50	>−4.00	>−4.00	−4.23	>−4.00	>−4.00
TK-10	−4.47	>−4.00	>−4.00	−4.54	−4.07	>−4.00	−4.60	>−4.00	>−4.00
UO-31	nt	nt	nt	−4.43	>−4.00	>−4.00	−4.04	>−4.00	>−4.00

(*continued on next page*)

Table 2 *(continued)*

Panel cell line	Response parameters: (A) $\log_{10}$ GI_{50}[b] (M), (B) $\log_{10}$ TGI[c] (M), (C) $\log_{10}$ LC_{50}[d] (M) and MG_MID[e]								
	Compound **7d**			Compound **7f**			Compound **7g**		
	A	B	C	A	B	C	A	B	C
Prostate cancer									
PC-3	−4.83	−4.49	−4.16	−4.63	−4.09	>−4.00	−4.53	>−4.00	>−4.00
DU-145	−4.64	−4.26	>−4.00	−4.38	>−4.00	>−4.00	−4.46	>−4.00	>−4.00
Breast cancer								>−4.00	>−4.00
MCF-7	−4.50	>−4.00	>−4.00	−4.45	>−4.00	>−4.00	−5.36	>−4.00	>−4.00
NCI/ADR-RES	−4.40	>−4.00	>−4.00	−4.63	−4.12	>−4.00	nt	nt	nt
MDA-MB-231/ATCC	−4.54	>−4.00	>−4.00	−4.72	−4.30	>−4.00	−4.38	>−4.00	>−4.00
HS 578T	−4.55	>−4.00	>−4.00	−4.75	−4.38	−4.01	−4.62	>−4.00	>−4.00
MDA-MB-435	−4.07	>−4.00	>−4.00	−4.56	−4.09	>−4.00	−5.49	>−4.00	>−4.00
BT-549	−4.44	−4.00	>−4.00	−4.78	−4.46	−4.14	−4.60	>−4.00	>−4.00
T-47D	−4.73	>−4.01	>−4.00	−4.97	−4.29	>−4.00	−5.20	>−4.00	>−4.00
MG_MID	−4.55	−4.10	−4.01	−4.67	−4.23	−4.03	−4.73	−4.01	−4.00

[a] Data obtained from the NCI's in vitro disease-oriented human tumour cells screen.
[b] $Log_{10}GI_{50}$ = log of molar concentration that inhibits 50% net cell growth.
[c] $Log_{10}TGI$ = log of molar concentration that produces a total growth inhibition.
[d] $Log_{10}LC_{50}$ = log of molar concentration that leads to 50% net cell death.
[e] MG-MID = mean graph midpoint = arithmetical mean value for all tested cell lines.

Table 3. 2-Anilino nicotinyl arylsulfonylhydrazide derivatives (**7a–i**) and related log *P* and log *C* values[a]

Compound	R_1	R_2	X	Y	log *P*	Log *S* (mg/L)
7a	Cl	H	CH_3	H	2.88	5.70
7b	OCH_3	H	CH_3	H	2.41	8.81
7c	F	H	F	H	3.11	9.90
7d	Cl	Cl	F	H	4.11	2.64
7e	F	H	Cl	H	3.23	5.97
7f	Cl	H	Cl	Cl	4.33	2.72
7g	Cl	H	$NHCOCH_3$	H	2.79	11.68
7h	F	H	$NHCOCH_3$	H	2.50	21.19
7i	H	H	H	H	1.47	22.00

[a] Determination of log P and log C values described in text.

Table 4. In vitro antibacterial activity of 2-anilino substituted nicotinylsulfonylhydrazides (**7a-i**)

Compound	MIC (μg/mL)				
	E. coli[a] MTCC 448	*P.aeruginosa*[a] MTCC 424	*S. epidermidis*[b] MTCC 435	*B. subtilis*[b] MTCC 441	*Vibrio species*[a]
7a	16	>25	14	9	23
7b	15	>25	17	8	19
7c	13	>25	20	10	22
7d	15	>25	21	9	20
7e	18	>25	16	10	21
7f	20	>25	17	15	14
7g	18	>25	22	17	18
7h	20	>25	19	16	22
7i	20	>25	21	17	20
SA	>128	>512	>512	>128	>512
SZ	15	22	20	18	13

SA, sulfanilamide; SZ, sulfadiazine.
[a] Gram-negative.
[b] Gram-positive.

We focused on making sulfonylhydrazide compounds based upon E7010 which was found to possess interesting in vitro anticancer activity[14] (IC_{50} 0.06–0.8 μg/ml) and also in vivo studies.[12] The sulfonamide moiety located between two aromatic rings was fixed as a basic motif, and the NH group at the ortho position of the sulfonamide was considered a key functionality to afford substantial anti-proliferative activity in cell-based assay.[5,14,29] We thus planned to introduce an extra amide moiety between two aromatic rings in the basic skeleton

of E7010 with different substituents on the two aromatic rings to explore the possibility of a potential antitumour agent.

Prediction of lipophilicity ($\log P$) and aqueous solubility ($\log S$) of compounds **7a**–**i** was calculated using ALOGPS 2.1 software.[30,31] These compounds showed lipophilicity with $\log P$ values in the range of 1.47–4.33 (Table 3) and aqueous solubility with $\log S$ values in the range of 2.72–22 mg/L in comparison with E7010 and sulfadiazine with $\log P$ and $\log S$ values of, respectively, 3.4; 30 mg/L and 0.25; 600 mg/L. These studies indicate that the aqueous solubility of these compounds is almost similar to that of E7010.

3.2. Antibacterial activity

The compounds **7a**–**i** were also evaluated for their efficacy as antibacterials in vitro by disc diffusion method against various bacterial strains. The antibacterial activity has been compared to some standard antibacterial agents like sulfanilamide and sulfadiazine that contain a *p*-amino benzene sulfonamide moiety. From the results in Table 4 compound **7c** showed significant inhibition against *Escherichia coli*. This may be due to the presence of fluorine atoms on para positions of 2-anilino ring and arylsulfonyl ring of the nicotinyl hydrazide. Most of the compounds showed significant in vitro activity against *Staphylococcus epidermidis* and *Bacillus subtilis*. Compound **7b** was most active against *B. subtilis*. All other compounds exhibited mild to moderate activity compared to sulfadiazine against *vibrio* species and none of the compounds were better than sulfadiazine against *Pseudomonos aeruginosa* (Table 4).

4. Conclusion

The synthesis and screening of anticancer and antibacterial activities of a novel series of 2-anilino substituted nicotinyl arylsulfonyl hydrazides have been investigated. Compounds **7d**, **7f** and **7g** were screened against 60 human cancer cell lines and exhibited broad spectrum of activity against almost all the cancer cell lines and in case of certain cancer cell lines the activity was comparable to E7010. Furthermore, most of the compounds showed better activity than the controls in antibacterial screening except against *P. aeruginosa*.

5. Experimental

5.1. General

Melting points were determined with an electrothermal melting point apparatus and are reported uncorrected. IR spectra (KBr) were measured with a Thermo Nicolet Nexus 670 Spectrometer (ν in cm^{-1}). ^1H NMRs were recorded on a Bruker UXNMR/XWIN-NMR (200 MHz) or Varian VXR-Unity (400 MHz) with TMS (0 ppm) as an internal standard. Coupling constants are reported in Hertz (Hz). EI mass spectra were recorded on a VG-7070H Micromass mass spectrometer at 200 °C, 70 eV, with a trap current of 200 μA and 4 kV of acceleration voltage. FAB mass spectra were recorded on a LSIMS-VG-AUTOSPEC Micromass spectrometer. LC–MS were recorded on the instrument LC-MSD-Trap-SL. Analytical TLC of all reactions was performed on Merck Prepared plates (silica gel 60 F-254 on glass). Column chromatography was performed using Acme silica gel. Yields were not optimized. Substituted arylsulfonylchlorides were synthesized from the reported procedures.[21] Starting materials were purchased from Sigma–Aldrich. All solvents and reagents were used without further purification unless otherwise specified. Micro analytical data (C, H and N) agreed with the proposed structures within ±0.4% of the theoretical values. All the standard organisms used in the antibacterial screening were obtained from Hi-media Laboratories, India.

6. General procedures

6.1. Synthesis of ethylester of 2-chloronicotinic acid (*2*)

2-Chloronicotinic acid (4 g, 0.025 mol) and 2 mL H_2SO_4 were taken in absolute ethanol and refluxed for 3 h. Then the reaction mixture was cooled to room temperature and the solvent was evaporated and extracted in ethyl acetate, washed with brine solution and then concentrated in vacuo.

6.2. General procedure for the synthesis of substituted 2-anilino nicotino ester (4a–e)

2-Chloro nicotinoethylester (**2**, 10 mmol) and substituted anilines (**3a**–**d**, 10 mmol) were heated in ethylene glycol up to 160 °C with stirring for 6 h. Then the reaction mixture was cooled to room temperature and the compound was extracted in ethyl acetate from the aqueous layer and purified by column chromatography (silica gel 60–120).

6.2.1. Ethyl 2-(4-chloroanilino)nicotinate (4a). Compound **2** (185 mg, 1 mmol) and 4-chloro aniline (**3a**, 127 mg, 1 mmol) were taken in ethylene glycol and refluxed at 160 °C for 6 h. Then the reaction mixture was cooled and extracted in ethyl acetate (4 × 25 mL) from the aqueous layer and concentrated in vacuo. The compound was further purified by column chromatography using 60–120 silica gel (ethyl acetate/hexane, 1:9). Yield 75%; mp 98–100 °C; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 10.26 (br s, 1H), 8.34 (q, J = 2.66 Hz, 1H), 8.20 (dd, J = 8.30 Hz, 2.66, 1H), 7.67 (d, J = 9.065 Hz, 2H), 7.24 (d, J = 9.06 Hz, 2H), 6.70 (dd, J = 7.55, 4.52 Hz, 1H), 4.38 (q, J = 7.55 Hz, 2H), 1.42 (t, J = 7.55 Hz, 3H); EI MS *m*/*z* 276; IR (KBr) (ν_{max}/cm^{-1}): 1253, 1528, 1586, 1620 (C–N), 1685 (C=O), 2986, 3196, 3264 (NH). Anal. Calcd for $C_{14}H_{13}ClN_2O_2$: C, 60.77; H, 4.73; N, 10.12. Found: C, 60.81; H, 4.72; N, 10.09.

6.2.2. Ethyl 2-(4-methoxyanilino)nicotinate (4b). Yield 80%; mp 88–90 °C; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.00 (s, 1H), 8.32 (dd, J = 4.68,

2.34 Hz, 1H), 8.20 (dd, J = 7.81, 2.34 Hz, 1H), 7.52 (dd, J = 7.03, 2.34 Hz, 2H), 6.88 (dd, J = 7.03, 2.34 Hz, 2H), 6,64 (dd, J = 7.81, 4.68 Hz, 1H), 4.38 (q, J = 7.03 Hz, 2H), 3.82 (s, 3H), 1.43 (t, J = 7.03 Hz, 3H); EI MS m/z 272; IR (KBr) (ν_{max}/cm^{-1}): 1244, 1507, 1580, 1621 (C–N), 1686 (C═O), 2829, 2927, 2969, 3002, 3317 (NH). Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.11; H, 5.90; N, 10.32.

6.2.3. Ethyl 2-(4-fluoroanilino)nicotinate (4c). Yield 75%; mp 67–69 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.19 (br s, 1H), 8.32 (dd, J = 4.69, 2.01 Hz, 1H), 8.22 (dd, J = 8.04, 2.01 Hz, 1H), 7.65 (m, 2H), 7.00 (m, 2H), 6.70 (dd, J = 7.37, 4.69 Hz, 1H), 4.40 (q, J = 7.37 Hz, 2H), 1.44 (t, J = 7.37 Hz, 3H); EI MS m/z 260; Anal. Calcd for $C_{14}H_{13}FN_2O_2$: C, 64.61; H, 5.03; N, 10.76. Found: C, 64.56; H, 5.01; N, 10.72.

6.2.4. Ethyl 2-(2,4-dichloroanilino)nicotinate (4d). Yield 80%; mp 96–98 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.66 (br s, 1H), 8.38 (q, 2.35, 1H), 8.27 (dd, J = 7.86, 2.35 Hz, 1H), 7.40 (d, J = 2.35 Hz, 1H), 7.22 (d, J = 2.35 Hz, 1H), 7.18 (d, J = 2.35 Hz, 1H), 6.78 (dd, J = 7.07, 3.93 Hz, 1H), 4.42 (q, J = 7.07 Hz, 2H), 1.44 (t, J = 7.07 Hz, 3H); EI MS m/z 311; IR (KBr) (ν_{max}/cm^{-1}): 1253, 1292, 1514, 1592 (C–N), 1696 (C═O), 3310 (NH). Anal. Calcd for $C_{14}H_{12}Cl_2N_2O_2$: C, 54.04; H, 3.89; N, 9.00. Found: C, 54.01; H, 3.91; N, 9.05.

6.2.5. Ethyl 2-anilinonicotinate (4e). Yield 75%; mp 54–56 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.22 (br s, 1H), 8.34 (dd, J = 4.71, 1.57 Hz, 1H), 8.20 (dd, J = 7.86, 2.35 Hz, 1H), 7.68 (m, 2H), 7.30 (m, 2H), 7.00 (dt, J = 7.07, 1.57 Hz, 1H), 6.66 (dd, J = 7.86, 4.71 Hz, 1H), 4.38 (q, J = 7.07 Hz, 2H), 1.43 (t, J = 7.07 Hz, 3H); EI MS m/z 242; Anal. Calcd for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.37; H, 5.80; N, 11.52.

6.3. General procedure for the synthesis of substituted 2-anilino nicotino hydrazides (5a–e)

2-Anilinonicotinoethylesters (**4a**–**e**, 1 mmol) were refluxed with hydrazine hydrate (5 mmol) in ethanol for 2 h. The reaction mixture was cooled and left for overnight, crystals were obtained, filtered and washed with ethanol to give pure compounds (**5a**–**e**).

6.3.1. 2-(4-Chloroanilino)-3-pyridinecarbohydrazide (5a). The title compound was obtained from compound **4a** (276 mg, 1 mmol) and hydrazine hydrate (0.25 mL, 5 mmol) as described in Section 6.3 Yellow crystalline needles, yield 70%; mp 195–197 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.10 (s, 1H), 8.25 (dd, J = 4.46, 1.48 Hz, 1H), 7.79 (dd, J = 7.43 Hz, 1H), 7.69 (d, J = 8.92 Hz, 2H), 7.21 (d, J = 8.92, 2H), 6.71 (dd, J = 7.43, 5.20. 1H); EI MS m/z 262; IR (KBr) (ν_{max}/cm^{-1}): 1247, 1420, 1460, 1512 (C–N), 1606 (C═O),3024, 3196 (NH). Anal. Calcd for $C_{12}H_{11}ClN_4O_2$: C, 54.87; H, 4.22; N, 21.33. Found: C, 54.82; H, 4.24; N, 21.30.

6.3.2. 2-(4-Methoxyanilino)-3-pyridinecarbohydrazide (5b). Yield 78%; mp 150–152 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.20 (br s, 1H), 8.20 (dd, J = 4.58, 1.52 Hz, 1H), 7.90 (dd. J = 7.63, 1.52 Hz, 1H), 7.50 (d, J = 9.16 Hz 2H), 6.80 (d, J = 9.16 Hz, 2H), 6.60 (m, 1H); EI MS m/z 258; IR (KBr) (ν_{max}/cm^{-1}): 1247, 1420, 1460, 1508 (C–N), 1606 (C═O), 3024 (methyl), 3196, 3328 (NH). Anal. Calcd for $C_{13}H_{14}N_4O_2$: C, 60.46; H, 5.46; N, 21.69. Found: C, 60.50; H, 5.44; N, 21.65.

6.3.3. 2-(4-Fluoroanilino)-3-pyridinecarbohydrazide (5c). Yield 75%; mp 159–161 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.20 (br s, 1H), 8.22 (dd, J = 5.00, 1.43 Hz, 1H), 7.99 (dd, J = 7.86, 1.43 Hz, 1H), 7.66 (dd, J = 9.29, 5.00 Hz, 2H), 6.96 (m, 2H), 6.70 (dd, J = 7.86, 5.00 Hz, 1H); EI MS m/z 246; IR (KBr) (ν_{max}/cm^{-1}): 1253, 1410, 1504, 1527 (C–N), 1609 (C═O), 3071, 3128, 3314 (NH). Anal. Calcd for $C_{12}H_{11}FN_4O$: C, 58.53; H, 4.50; N, 22.75. Found: C, 58.59; H, 4.48; N, 22.72.

6.3.4. 2-(2,4-Dichloroanilino)-3-pyridinecarbohydrazide (5d). Yield 80%; mp 197–199 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 11.20 (s, 1H), 8.72 (d, J = 7.92 Hz, 1H), 8.30 (d, J = 5.28 Hz, 1H), 8.10 (d, J = 7.92 Hz, 1H), 7.36 (s, 1H), 7.18 (dd, J = 9.24, 2.64 Hz, 1H), 6.80 (dd, J = 7.92, 5.28 Hz, 1H); EI MS m/z 297; IR (KBr) (ν_{max}/cm^{-1}): 1257, 1464, 1513 (C–N), 1591 (C═O), 3312 (NH). Anal. Calcd for $C_{12}H_{10}ClN_4O$: C, 48.51; H, 3.39; N, 18.86. Found: C, 48.47; H, 3.37; N, 18.89.

6.3.5. 2-Anilino-3-pyridinecarbohydrazide (5e). Yield 75%; mp 125–127 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.75 (s, 1H), 9.80 (br s, 1H), 8.24 (dd, J = 5.25, 1.50 Hz, 1H), 7.96 (dd, J = 7.50, 1.50 Hz, 1H), 7.68 (d, J = 8.25 Hz, 2H), 7.24 (t, J = 8.25 Hz, 2H), 6.90 (t, J = 7.50 Hz, 1H), 6.68 (dd, J = 7.50, 5.25 Hz, 1H); EI MS m/z 228; IR (KBr) (ν_{max}/cm^{-1}): 1256, 1441, 1581 (C–N), 1644 (C═O), 3021, 3308 (NH). Anal. Calcd for $C_{12}H_{12}lN_4O$: C, 63.15; H, 5.30; N, 24.55. Found: C, 63.11; H, 5.32; N, 24.51.

6.4. General procedure for the synthesis of compounds 7a–i

To a stirred solution of substituted 2-anilino nicotino hydrazides (**5a**–**e**) (1 mmol) in pyridine (10 mL), substituted aryl sulfonylchlorides (1.2 mmol) were added at 0 °C. Then the resulting mixture was stirred at room temperature for 4–5 h and then the reaction mixture was diluted with 1 M HCl and extracted in ethyl acetate (4× 25 mL) from the aqueous layer. The combined layer was washed with sodium bicarbonate solution and dried over anhydrous Na_2SO_4. The resulting products were purified by column chromatography employing EtOA/Hexane as an eluent.

6.4.1. *N*′-1-[2-(4-Chloroanilino)-3-pyridyl]carbonyl-4-methyl-1-benzenesulfonohydrazide (7a). The title compound

was obtained from 2-(4-chloroanilino)-3-pyridinecarbohydrazide (**5a**, 263 mg, 1 mmol) and 4-methyl benzenesulfonyl chloride (**6a**, 228 mg, 1.2 mmol) as described in Section 6.4.

Yield 68%; mp 241–243 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.96 (1H, d, J = 4.46 Hz), 9.79 (br s, 1H), 9.36 (d, J = 4.46 Hz, 1H), 8.23 (dd, J = 5.20, 1.48 Hz, 1H), 8.02 (dd, J = 7.43, 1.48 Hz, 1H), 7.75 (d, J = 8.17 Hz, 2H), 7.50 (d, J = 8.176 Hz, 2H), 7.20 (dd, J = 15.61, 8.92 Hz, 4H), 6.71 (dd, J = 8.17, 5.20 Hz, 1H), 2.30 (s, 3H); FABMS m/z 417 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 1150, 1328 (SO_2), 1248 (C–N), 1521, 1602, 1648 (C=O), 1721, 3036 (methyl), 3323 (NH). Anal. Calcd for $C_{19}H_{17}ClN_4O_3S$: C, 54.74; H, 4.11; N, 13.44. Found: C, 54.79; H, 4.21; N, 13.35.

6.4.2. *N*′-1-[2-(4-Methoxyanilino)-3-pyridyl]carbonyl-4-methyl-1-benzenesulfonohydrazide (7b). The title compound was obtained from 2-(4-methoxyanilino)-3-pyridinecarbohydrazide (**5b**, 258 mg, 1 mmol) and 4-methyl benzenesulfonyl chloride (**6a**, 228 mg, 1.2 mmol) as described in Section 6.4.

Yield 75%; mp 159–161 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.80 (1H, d, J = 4.29 Hz), 9.49 (d, J = 4.29 Hz, 1H), 9.45 (br s, 1H), 8.11 (dd, J = 3.57 Hz, 1.43, 1H), 7.92 (1H, dd, J = 7.15, 1.43, 1H), 7.70 (dd, J = 7.86, 0.71 Hz, 2H), 7.30 (d, J = 8.58 Hz, 2,), 7.18 (d, J = 7.86 Hz, 2H), 6.72 (d, J = 8.58 Hz, 2H), 6.58 (dd, J = 5.00, 7.86 Hz, 1H), 3.70 (s, 3H), 2.25 (s, 3H); FABMS m/z 413 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 1163, 1337 (SO_2), 1245 (C–N), 1517, 1603, 1659 (C=O), 1740, 2854, 2924 (methyl), 3213, 3325 (NH). Anal. Calcd for $C_{20}H_{20}N_4O_4S$: C, 58.24; H, 4.89; N, 13.58. Found: C, 58.31; H, 4.84; N, 13.62.

6.4.3. *N*′-1-[2-(4-Fluoroanilino)-3-pyridyl]carbonyl-4-fluoro-1-benzenesulfonohydrazide (7c). The title compound was obtained from 2-(4-fluoroanilino)-3-pyridinecarbohydrazide (**5c**, 246 mg, 1 mmol) and 4-fluoro benzenesulfonyl chloride (**6b**, 232 mg, 1.2 mmol) as described in Section 6.4.

Yield 70%; mp 225–227 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.92 (d, J = 3.18 Hz, 1H), 9.78 (br s, 1H), 9.73 (d, J = 3.18 Hz, 1H), 8.22 (dd, J = 4.77, 1.59 Hz, 1H), 8.02 (dd, J = 7.95, 1.59 Hz, 1H), 7.94 (dd, J = 8.76, 4.77 Hz, 2H), 7.48 (dd, J = 8.76, 4.77 Hz, 1H), 7.15 (t, J = 8.76 Hz, 2H), 6.94 (t, J = 8.76 Hz, 2H), 6.70 (dd, J = 7.96, 4.77 Hz, 1H); FABMS m/z 405 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 1154, 1344 (SO_2), 1256 (C–N), 1506, 1585, 1691 (C=O), 3030 (Ar–H), 3232, 3355 (NH). Anal. Calcd for $C_{18}H_{14}F_2N_4O_3S$: C, 53.46; H, 3.49; N, 13.85. Found: C, 53.51; H, 3.46; N, 13.76.

6.4.4. *N*′-1-[2-(2,4-Dichloroanilino)-3-pyridyl]carbonyl-4-fluoro-1-benzenesulfono-hydrazide (7d). The title compound was obtained from 2-(2,4-dichloroanilino)-3-pyridinecarbohydrazide (**5d**, 297 mg, 1 mmol) and 4-fluoro benzenesulfonyl chloride (**6b**, 232 mg, 1.2 mmol) as described in Section 6.4.

Yield 75%; mp 237–239 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.96 (d, J = 5.00, 1H), 10.29 (br s, 1H), 9.42 (d, J = 5.00, 1H), 8.52 (d, J = 9.29. 1H), 8.22 (dd, J = 5.00, 1.43, 1H), 8.08 (dd, J = 7.86, 1.43 Hz, 1H), 7.88 (dd, J = 8.58,5.00 Hz, 2H), 7.28 (d, J = 2.14 Hz, 1H), 7.12 (d, J = 2.14 Hz, 1H), 7.04 (t, J = 8.58 Hz, 1H), 6.74(dd, J = 7.86, 4.29 Hz, 1H); LC–MS m/z 454.9 (M^+), 476.9 (M^++Na); IR (KBr) (ν_{max}/cm^{-1}): 1164, 1326 (SO_2), 1247 (C–N), 1500, 1604, 1660 (C=O), 3103 (Ar–H), 3268, 3355 (NH). Anal. Calcd for $C_{18}H_{13}Cl_2FN_4O_3S$: C, 47.49; H, 2.88; N, 12.31. Found: C, 47.52; H, 2.86; N, 12.28.

6.4.5. *N*′-1-[2-(4-Fluoroanilino)-3-pyridyl]carbonyl-4-chloro-1-benzenesulfonohydrazide (7e). The title compound was obtained from 2-(4-fluoroanilino)-3-pyridinecarbohydrazide (**5c**, 246 mg, 1 mmol) and 4-chloro benzenesulfony lchloride (**6c**, 252 mg, 1.2 mmol) as described in Section 6.4.

Yield 70%; mp 232–235 °C (charred); ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ (ppm): 10.85 (br s, 1H), 9.66 (br s, 2H), 8.14 (dd, J = 4.45, 1.48, 1H), 7.95 (dd, J = 8.17, 1.48, 1H), 7.80 (d, J = 8.17, 2H), 7.38 (m, 4H), 6.68 (t, J = 8.91, 2H), 6.60 (dd, J = 8.17, 4.45, 1H); MS m/z 420.9 (M^+), 443 (M^++Na); IR (KBr) (ν_{max}/cm^{-1}): 1157, 1337 (SO_2), 1253 (C–N), 1506, 1588, 1613, 1735 (C=O), 3021 (Ar–H), 3330 (NH). Anal. Calcd for $C_{18}H_{14}ClFN_4O_3S$: C, 51.37; H, 3.35; N, 13.31. Found: C, 51.32; H, 3.37; N, 13.28.

6.4.6. *N*′-1-[2-(4-Chloroanilino)-3-pyridyl]carbonyl-2,4-dichloro-1-benzenesulfono-hydrazide (7f). The title compound was obtained from 2-(4-chloroanilino)-3-pyridinecarbohydrazide (5a, 263 mg, 1 mmol) and 2,4-dichloro benzenesulfonyl chloride (**6d**, 293 mg, 1.2 mmol) as described in Section 6.4.

Yield 70%; mp 215–216 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.85 (br s, 1H), 9.86 (br s, 1H), 9.62 (br s, 1H), 8.18 (dd, J = 4.21, 1.68 Hz, 1H), 7.95 (d, J = 1.68 Hz, 1H), 7.89 (d, J = 8.43 Hz, 1H), 7.51 (d, J = 1.68 Hz, 1H), 7.46 (d, J = 8.43 Hz, 2H), 7.25 (dd, J = 8.43, 1.68 Hz, 1H), 7.12 (d, J = 8.43 Hz, 2H), 6.64 (dd, J = 7.59,4.21 Hz, 1H); FABMS m/z 473 $(M+2)^+$; IR (KBr) (ν_{max}/cm^{-1}): 1162, 1339 (SO_2), 1251 (C–N), 1518, 1607, 1644 (C=O), 1738, 3020 (Ar–H), 3339 (NH). Anal. Calcd for $C_{18}H_{13}Cl_3N_4O_3S$: C, 45.83; H, 2.78; N, 11.88. Found: C, 45.78; H, 2.76; N, 11.81.

6.4.7. *N*-1-4-[(2-[2-(4-Chloroanilino)-3-pyridyl]carbonylhydrazino)sulfonyl]phenyl-acetamide (7g). The title compound was obtained from 2-(4-chloroanilino)-3-pyridinecarbohydrazide (**5a**, 263 mg, 1 mmol) and 4-acetylamino benzenesulfonyl chloride (**6e**, 280 mg, 1.2 mmol) as described in Section 6.4.

Yield 65%; mp 245–247 °C (charred); ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 11.02 (d, J = 3.37, 1H), 10.26 (br s, 1H), 10.04 (d, J = 3.37 Hz, 1H), 9.72 (br s, 1H), 8.32 (dd, J = 5.06 Hz, 1H), 8.03 (dd, J = 7.59, 1.68 Hz, 1H), 7.74 (m, 4H), 7.54 (d,

J = 8.43 Hz, 2H), 7.30 (d, J = 8.43 Hz, 2H), 6.88 (dd, J = 5.06, 7.59 Hz, 1H), 2.03 (s, 3H); FABMS m/z 460 (M+1); IR (KBr) (ν_{max}/cm^{-1}): 1171, 1328 (SO_2), 1261 (C–N), 1517, 1590, 1671 (C=O), 2854, 2923 (methyl), 3101 (Ar–H), 3220, 3306, 3384 (NH). Anal. Calcd for $C_{20}H_{18}ClN_5O_4S$: C, 52.23; H, 3.94; N, 15.23. Found: C, 52.28; H, 3.92; N, 15.19.

6.4.8. *N*-1-4-[(2-[2-(4-Fluoroanilino)-3-pyridyl]carbonylhydrazino)sulfonyl]phenyl-acetamide (7h). The title compound was obtained from 2-(4-fluoroanilino)-3-pyridinecarbohydrazide (**5c**, 246 mg, 1 mmol) and 4-acetylamino benzenesulfonyl chloride (**6e**, 280 mg, 1.2 mmol) as described in Section 6.4.

Yield 60%; mp 215–216 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.88 (d, J = 3.71 Hz, 1H) 10.04 (br s, 1H), 9.70 (d, J = 3.17 Hz, 1H), 9.62 (br s, 1H), 8.16 (dd, J = 4.45 Hz, 1H), 7.96 (dd, J = 7.43, 1.48 Hz, 1H), 7.68 (dd, J = 8.91, 14.12 Hz, 4H), 7.42 (q, J = 4.45 Hz, 2H), 6.90 (t, J = 8.17, 2H), 6.66 (dd, J = 5.20, 7.43 Hz, 1H), 1.99 (s, 3H); LC–MS m/z 444.0 (M^+), 466.9 (M^++Na); Anal. Calcd for $C_{20}H_{18}FN_5O_4S$: C, 54.17; H, 4.09; N, 15.79. Found: C, 54.13; H, 4.10; N, 15.75.

6.4.9. *N*′-1-[(2-Anilino-3-pyridyl)carbonyl]-1-benzenesulfonohydrazide (7i). The title compound was obtained from 2-anilino-3-pyridinecarbohydrazide (**5e**, 228 mg, 1 mmol) and benzenesulfonyl chloride (**6f**, 0.15 ml, 1.2 mmol) as described in Section 6.4.

Yield 78%; mp 172–174 °C; ^{1}H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ 10.86 (br s, 1H), 9.64 (br s,1H), 9.34 (br s, 1H), 8.18 (dd, J = 4.29, 1.43 Hz, 1H), 7.96 (dd. J = 7.86, 1.43 Hz, 1H), 7.84 (dd, J = 7.86, 1.43 Hz, 2H), 7.40 (t, J = 7.86 Hz 5H), 7.16 (t, J = 7.86 Hz, 2H), 6.86 (t, J = 7.86 Hz, 1H), 6.62 (dd, J = 5.00, 7.86 Hz, 1H); FABMS m/z 369 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 1157, 1335 (SO_2), 1253 (C–N), 1521, 1602, 1645 (C=O), 3048 (Ar–H), 3325 (NH). Anal. Calcd for $C_{18}H_{16}N_4O_3S$: C, 58.68; H, 4.38; N, 15.21. Found: C, 58.61; H, 4.40; N, 15.19.

7. Biological activity

7.1. In vitro anticancer screening

In vitro anticancer screening assays were performed according to NCI procedures.[24,26–28]

7.2. Antibacterial activity

All synthesized compounds were screened for their antibacterial activity against *E. coli* MTCC 448, *P. aeruginosa* MTCC 424, *S. epidermidis* MTCC 435, *B. subtilis* MTCC 441 and *Vibrio species* using Mueller-Hinton agar medium (Hi-Media Laboratories, India). Minimum inhibitory concentrations (MIC) were determined using Kirby–Bauer method.[32] The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate. The test compounds were prepared with different concentrations using *N*,*N*-dimethylformamide (DMF). Discs of each concentrations were placed in triplicate in the medium inoculated with fresh bacteria separately ($1–5 \times 10^4$ cfu mL^{-1}). The incubation was carried out at 30 °C for 18 h. Sulfanilamide and sulfadiazine were used as positive controls and solvent did not show inhibition.

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References and notes

1. Buolamwini, J. K. *Curr. Opin. Chem. Biol.* **1999**, *3*, 500.
2. Jones, D. A.; Fitzpatrick, F. A. *Curr. Opin. Chem. Biol.* **1999**, *3*, 71.
3. Drew, J. *Science* **2000**, *287*, 1960.
4. Supuran, C. T.; Casini, A.; Scozzafava, A.; Mastrolorenzo, A. *Curr. Cancer Drug Targets* **2002**, *2*, 55.
5. Owa, T.; Nagasu, T. *Exp. Opin. Ther. Patents* **2000**, *10*, 1725.
6. Supuran, C. T.; Scozzafava, A. *Exp. Opin. Ther. Patents* **2000**, *10*, 575.
7. Supuran, C. T.; Scozzafava, A. *Curr. Med. Chem.—Immunol., Endocrinol. Metab. Agents* **2001**, *1*, 61.
8. Lobb, K. L.; Hipskind, P. A.; Aikins, J. A.; Alvarez, E.; Cheung, Y.; Considine, E. L.; De Dios, A.; Durst, G. L.; Ferritto, R.; Grossman, C. S.; Giera, D. D.; Hollister, B. A.; Huang, Z.; Iversen, P. W.; Law, K. L.; Tiechao, L. i.; Ho-Shen Lin Lopez, B.; Lopez, J. E.; Cabrejas, L. M. M.; Denis J McCann, D. J.; Molero, V.; Reilly, J. E.; Richett, M. E.; Shih, C.; Teicher, B.; Wikel, J. H.; White, W. T.; Mader, M. M. *J. Med. Chem* **2004**, *47*, 5367.
9. 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.
10. Maren, T. H. *Annu. Rev. Pharmacol. Toxicol.* **1976**, *16*, 309.
11. Boyd, A. E. *Diabetes* **1988**, *37*, 847.
12. Koyanagi, N.; Nagasu, T.; Fujita, F.; Watanabe, T.; Tsukahara, K.; Funahashi, Y.; Fujita, M.; Taguchi, T.; Yoshino, H.; Kitoh, K. *Cancer Res.* **1994**, *54*, 1702.
13. Yokoi, A.; Kuromitsu, J.; Kawai, T.; Nagasu, T.; Sugi, N. H.; Yoshimatsu, K.; Yoshino, H.; Owa, T. *Mol. Cancer Ther.* **2002**, *1*, 275.
14. Yoshimatsu, K.; Yamaguchi, A.; Yoshino, H.; Koyanagi, N.; Kitoh, K. *Cancer Res.* **1997**, *57*, 3208.
15. Nakatsu, N.; Yoshida, Y.; Yamazaki, K.; Nakamura, T.; Dan, S.; Fukui, Y.; Yamori, T. *Mol. Cancer Ther.* **2005**, *4*, 399.
16. Mokotoff, M.; Brynes, S.; Burckart, G. J. *J. Med. Chem.* **1978**, *21*, 45.
17. Supuran, C. T.; Winum, J. Y.; Dogne, J. M.; Casini, A.; de Leval, X.; Montero, J. L.; Scozzafava, A.; Vullo, D.; Innocenti, A. *J. Med. Chem.* **2005**, *48*, 2121.
18. Ariesan, V.; Marie, Mrs.; Cuparencu, B.; Safta, L. *Therapie* **1972**, *27*, 309 (Fr).
19. Jouhari, R.; Quinn, P. *Heterocycles* **1994**, *38*, 2243.

20. Fand, T. I.; Spoerri, P. E. *J. Am. Chem. Soc.* **1952**, *74*, 1345.
21. Huntress, E. H.; Carten, F. H. *J. Am. Chem. Soc.* **1940**, *62*, 511.
22. Halperin, J. A.; Natarajan, A.; Guo, Y.; Harbinski, F.; Fan, Y. H.; Chen, H.; Luus, L.; Diercks, J.; Aktas, H.; Chorev, M. *J. Med. Chem.* **2004**, *47*, 4979.
23. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbot, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
24. Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622.
25. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
26. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
27. Weinstein, J. N.; Myers, T. G.; Connor, P. M.; S. H.; Friend, Fornace, A. J., Jr.; Kohn, K. W.; Fojo, T.; Bates, S. E.; Rubinstein, L. V.; Anderson, N. L.; Buolamwini, J. K.; van Osdol, W. W.; Monks, A. P.; Scudiero, D. A.; Sausville, E. A.; Zaharevitz, D. W.; Bunow, B.; Viswanadhan, V. N.; Johnson, G. S.; Wittes, R. E.; Paull, K. D. *Science* **1997**, *275*, 343.
28. Data concerning the NCI screening methods in detail are accessible from the NCI via the Internet from the following address: http://dtp.nci.nih.gov/branches/btb/ivclsp.html.
29. Yoshino, H.; Ueda, N.; Niijima, J.; Sugumi, H.; Kotake, Y.; Yoshimatsu, K.; Asada, M.; Watanabe, T.; Nagasu, T.; Tsukahara, K.; Iijima, A.; Kitoh, K. *J. Med. Chem.* **1992**, *35*, 2496.
30. ALOGPS 2.1 software available at http://www.vcclab.org/lab/alogps.
31. Tetko, I. V.; Tanchuk, V. Y. J. *Chem. Inf. Comput. Sci.* **2002**, *42*, 1136.
32. Prescott, L. M.; Harley, J. P.; Klein, D. A. *Microbiology*, 5th ed.; McGraw-Hill: New York, 2002, Chapter 35, pp 805–825.