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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of 2-(5-substituted-1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-*N*-substituted-hydrazinecarbothioamides

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Abstract Various 5-substituted-2-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)hydrazinecarbothioamide (**4a**, **b**) and 5-substituted-2-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-*N*-(phenyl-4-substituted)hydrazinecarbothioamide (**5a**–**h**) derivatives were synthesized. The compounds were screened for cytotoxicity against human HeLa and CEM T-lymphocytes as well as murine L1210 cells. The compounds were also screened for β-lactamase inhibitory activity, antiviral, antibacterial, and antifungal activity against various strains of microorganisms. Several of these compounds were endowed with low micromolar 50 %-cytostatic concentration (IC_{50}) values, and some were virtually equally potent as melphalan. The most potent inhibitors against the murine leukemia cells (L1210) were also the most inhibitory against human T-lymphocyte (CEM) tumor cells. Derivative 2-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-*N*-(4-methoxyphenyl)hydrazinecarbothioamide **5c** emerged as the most potent cytostatic compound among the tested compounds. Derivatives **4b**, **5a**, **5b**, and **5d** showed antiviral activity against HEL cell cultures (IC_{50} 11–20 μM). Moderate antimicrobial activity was observed for all derivatives. The encouraging cytostatic and antiviral activity data provide an adequate rationale for further modification of these molecular scaffolds.

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Introduction

Isatin has been known for about 150 years and has recently been found, like 2,3-dioxo-indoles and endogenous polyfunctional heterocyclic compounds, to exhibit biological activity in mammals (Somogyi, 2001). Schiff bases and Mannich bases of isatin are known to possess a wide range of pharmacological properties including antibacterial (Pandeya *et al.,* 1998; Sarangapani and Reddy, 1994; Varma and Nobles, 1975), anticonvulsant (Sridhar *et al.,* 2002; Varma *et al.,* 2004), anti-HIV (Pandeya *et al.,* 1998, 1999a, b, 2000; Sriram *et al.,* 2000), antifungal (Pandeya *et al.,* 1999a, b), antiviral (Singh *et al.,* 1983), and anticancer activity (Fig. 1) (Karki *et al.,* 2004, 2007, 2009). A variety of 3-substituted indolin-2-ones have been utilized as anticancer drugs or drug candidates (Mologni *et al.,* 2010, Beauchard *et al.,* 2009, Zhang and Go 2009, Andreani *et al.,* 2010). A representative member of this class is sunitinib [SU11248, Sutent™; Pfizer, Inc.] which is currently used in the clinics as a multi-targeting tyrosine kinase inhibitor with antiangiogenic activity (Fig. 1) (Sun *et al.,* 2003). 6-Methoxycarbonyl group-substituted indolin-2-ones [BIBF1000, BIBF1120] are potent inhibitors of VEGFR-1/2/3, PDGFRa, and FGFR-1, with low cross-reactivity against a panel of other kinases (Fig. 1) (Roth *et al.,* 2009). While BIBF1120 is currently being evaluated in phase III clinical trials in the treatment of non-small cell lung cancer and is in clinical development for other tumor types. Indirubin was identified as the active ingredient of a traditional Chinese recipe [Danggui Longhui Wan] that

was used for the treatment of chronic myelogenous leukemia (CML) (Fig. 1) (Xiao *et al.,* 2002).

Thiosemicarbazones of various aldehydes and ketones occupy a special place among organic ligands, since they contain various donor atoms and are able to change density depending on the starting reagents and their reaction conditions. Isatin-3-thiosemicarbazones (1*H*-indole-2,3-dioxo-3-thiosemicarbazones) have been studied extensively due to their important biological activities (Karki *et al.,* 2009), since 1-methylisatin-3-thiosemicarbazone (Marboran) was found to be active in the treatment of smallpox. Previous studies by our group have revealed the promising cytotoxic, and anticonvulsant properties of various 2,3-dioxo-2,3-dihydroindole thiosemicarbazones (Fig. 1) (Karki *et al.,* 2009). Therefore, we have performed the synthesis of new *N*-4-aryl thiosemicarbazone derivatives of substituted 2,3-dioxo-2,3-dihydroindoles and evaluated them for cytotoxic, antiviral, and antimicrobial activity.

Results and discussion

Chemistry

The compounds in series **4** and **5** were prepared by the methodologies outlined in Scheme 1. The synthesis of 1-[(diethylamino)methyl]-1*H*-indole-2,3-dione derivatives (**3**) was carried out by mannich base reaction of 2,3-dioxy-2,3-dihydroindoles or 5-chloro-2,3-dioxy-2,3-dihydroindoles (**2**) with diethylamine in the presence of formaldehyde. The synthesis of *N*-diethylaminomethyl-2-oxo-1,2-dihydroindole hydrazinecarbothioamides (**4a**, **b**, **5a**–**h**) was carried out by reacting 1-[(diethylamino)methyl]-1*H*-indole-2,3-dione derivatives (**3**) with thiosemicarbazide and N^4-aryl thiosemicarbazides under reflux in ethanol in the presence of catalytic amounts of glacial acetic acid. ^{1}H-NMR spectroscopy indicated that the compounds exist as single isomers in solution.

Biological activity

All of the compounds were assayed for cytotoxicity against human HeLa and CEM T-lymphocytes as well as murine L1210 cells. These data are summarized in Table 1. Compound **5c** was the most cytostatic among all compounds evaluated against the tumor cell lines (IC_{50} in the low micromolar range (1.9–4.4 μM)).

The most potent inhibitor of murine L1210 cell proliferation (**5c**) was also the most inhibitory to human T-lymphocyte (CEM) and HeLa cell proliferation. The introduction of R_2-benzyl substitution in the thiosemicarbazone derivative of series **5** often led to more potent inhibitors of tumor cell proliferation (**5a**–**h**). Replacement of the hydrogen by chlorine atom did not improve the potentiation of the cytostatic activity (compare **5a** with **5b**) (Table 1). Whereas, replacement of the chlorine atom at R_2 by a methoxy group often resulted in a marked (equal) potentiation of cytostatic activity (**5b** with **5c**).

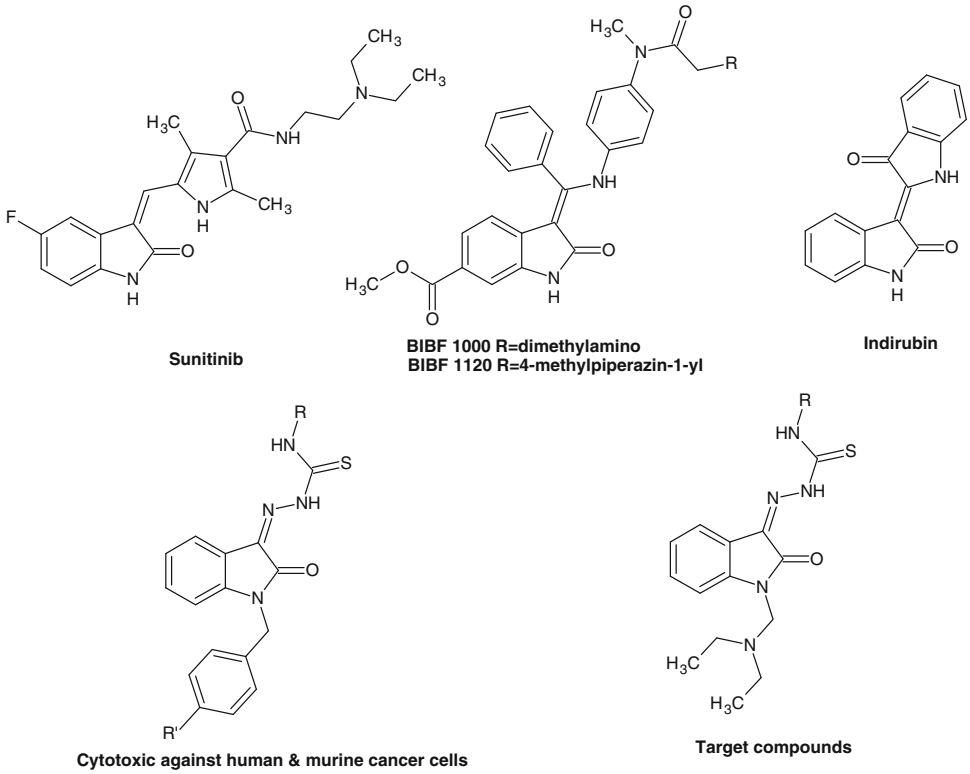
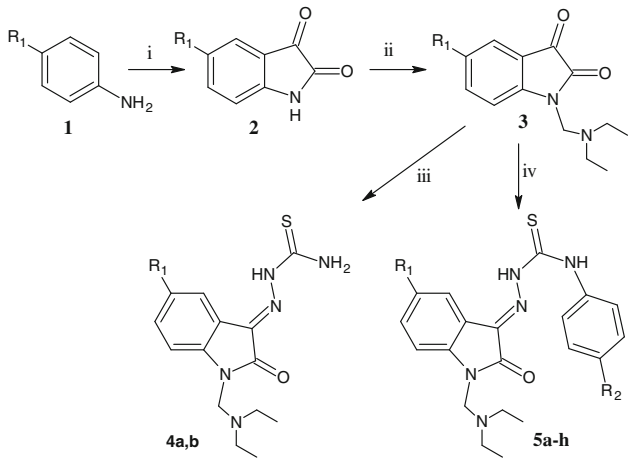


Fig. 1 Structures of indolin-2-ones



Scheme 1 The reagents used were as follows: *i* $CCl_3CH(OH)_2$/H_2SO_4/Na_2SO_4; R_1=H, Cl, *ii* $(C_2H_5)NH/HCHO$, *iii* $NH_2C(S)NHNH_2$, *iv* $R_2C_6H_4NHC(S)NHNH_2$. The nature of the R_1 and R_2 substituent are presented in Table 1

Replacement of hydrogen atom at R_1 by chlorine atom did not yield any improvement in potency (compare **5a**, **5b** and **5d** with **5e**–**h**). Replacing hydrogen atom at R_1 and R_2 by chlorine resulted in a complete loss of activity (**5f**). By keeping chlorine atom at R_1 and CH_3 and OCH_3 at R_2, resulted in slight improvement of cytostatic activity (**5g** and **5h**).

The antiviral screening of **4a**, **b** and **5a**–**h** was performed using an MTS-based CPE reduction assay (Kumar *et al.*, 2010) against feline corona virus (FIPV) and feline herpes virus in CRFK cell culture; herpes simplex virus-1 (KOS) (HSV-1 KOS), herpes simplex virus-2 (G) (HSV-2G), vaccinia virus (VV), vesicular stomatitis virus (VSV), and herpes simplex virus-1 TK KOS ACV^r (HSV-1 TK KOS ACV^r) in HEL cell cultures; VSV, Coxsackie virus B4 (CV-B4), and respiratory syncytial virus (RSV) in HeLa cell cultures; influenza A virus H1N1 subtype, influenza A virus H3N2 subtype, and influenza B virus in MDCK cell cultures; parainfluenza-3 virus (PI-3V), reovirus-1 (RV-1), Sindbis virus (SV), CV-B4, and Punta Toro virus (PTV) in Vero cell cultures.

Compounds **4b**, **5a**, **5b**, and **5d** exhibited moderate antiviral activity in HEL cells in comparison to standard compounds. No specific antiviral effects were noted for any of the compounds in CRFK, MDCK, or Vero cell cultures (Tables 2, 3, 4, 5, 6).

All the synthesized compounds were screened for β-lactamase inhibitory activity and results are shown in Table 1. Compounds namely **5a** and **5g** were capable of inactivating β-lactamase activity, and for other compounds activity was moderate in comparing to standard potassium clavulanate. Titled compounds were also screened for antibacterial activity against *S. aureus, B. subtilis, K. pneumoniae, E. coli, P. vulgaris, and S. typhi* and antifungal activity against *A. niger and C. albicans* and exhibited moderate antimicrobial activity (Table 7).

Experimental

Chemistry

All reagents were obtained from Sigma-Aldrich, Mumbai, and Loba Chemie, Mumbai. All the solvents used in these studies were dried and distilled before use. Melting points (Mp): Veego VMP-PM digital melting point apparatus, and are uncorrected. TLC: solvent benzene:ethanol (8:2). UV spectra: Shimadzu Pharmspec 1700, UV–Vis spectrophotometer. IR

Table 1 Results of cytotoxicity in murine L1210 cells, human HeLa, and CEM T-lymphocytes, and β-lactamase inhibitory activity

Compound	R_1	R_2	IC_{50}[a] (μM)			Time for decolorization of I_2 (s)	Activity (u ml^{-1})	Inactivation (%)
			L1210	CEM	HeLa			
4a	H	–	121 ± 35	164 ± 21	123 ± 85	128.6	46.7	38.2
4b	Cl	–	148 ± 15	71 ± 7	44 ± 22	121.5	49.4	34.6
5a	H	H	13 ± 3	11 ± 0	8.3 ± 0.0	159.5	37.6	50.2
5b	H	Cl	11 ± 1	10 ± 1	7.6 ± 0.9	129.7	46.3	38.7
5c	H	OCH_3	2.4 ± 0.0	1.9 ± 0.9	4.4 ± 2.4	123.6	48.5	35.7
5d	H	CH_3	29 ± 3	12 ± 0	12 ± 0	145.8	41.2	45.5
5e	Cl	H	49 ± 2	40 ± 3	34 ± 0	156.3	38.4	49.2
5f	Cl	Cl	>125	>125	>125	142.3	42.2	44.2
5g	Cl	OCH_3	11 ± 2	6.9 ± 4.3	9.2 ± 0.9	167.9	35.7	52.6
5h	Cl	CH_3	9.5 ± 0.5	4.6 ± 4.0	8.6 ± 0.3	120.6	49.8	34.1
Melphalan	–	–	3.2 ± 0.6	2.2 ± 0.2	2.1 ± 0.02	–	–	–
Control	–	–	–	–	–	79.5	75.5	–
Potassium clavulanate	–	–	–	–	–	240.50	25.0	67.0

[a] IC_{50} concentrations of compounds required to inhibit the growth of the tumor cells by 50 %

Table 2 Results of anti-FIPV and anti-feline herpes virus activity and cytotoxicity in CRFK cell cultures

Compound	CC_{50}[a] (μM)	EC_{50}[b] (μM)	
		FIPV	Feline herpes virus
4a	73.8	>20	>20
4b	>100	>100	>100
5a	32.3	>20	>20
5b	4.0	>0.8	>0.8
5c	3.5	>0.8	>0.8
5d	3.8	>0.8	>0.8
5e	6.9	>4	>4
5f	9.1	>4	>4
5g	3.6	>0.8	>0.8
5h	17.5	>4	>4
HHA (μg ml^{-1})	>100	19.5	1.8
UDA (μg ml^{-1})	>100	9.1	2.4
Ganciclovir	>100	>100	7.3

[a] 50 % cytotoxic concentration

[b] 50 % effective concentration, determined by colorimetric formazan-based MTS assay

spectra: Shimadzu 8400 S, FT-IR. ^{1}H-NMR spectra: 300 MHz JEOL NMR Spectrophotometer in $CDCl_3$ and DMSO-d_6. Mass spectra: GCMS QP 5050 Shimadzu. All spectra were obtained from Pune University, Maharashtra, India.

Syntheses of the intermediate 2,3-dioxy-2,3-dihydroindoles

The synthesis of the intermediate 2,3-dioxo-2,3-dihydroindoles was accomplished using a literature methodology (Marvel and Heirs, 1941) and a previously reported procedure was used to convert these compounds to the corresponding 1-[(diethylamino)methyl]-1*H*-indole-2,3-dione. The N^4-arylthiosemicarbazides required for the preparation of **4a**, **b** and **5a**–**h** was prepared by a literature methodology (Sen and Sengupta, 1962; Lieber *et al.,* 1957).

*General procedure for syntheses of **4a**, **b***

A mixture of the 1-[(diethylamino)methyl]-1*H*-indole-2,3-dione or 5-chloro-1-[(diethylamino)methyl]-1*H*-indole-2,3-dione (0.005 mol), thiosemicarbazides (0.005 mol), acetic acid (0.5–1.0 ml), and ethanol (100 ml) was heated under reflux until the reaction was completed (~4 h). Approximately half of the ethanol was removed in vacuo and the solution was left overnight at room temperature. The precipitated solid was collected, washed with cold ethanol, and recrystallized from ethanol:chloroform (9:1) to give the following compounds.

*2-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)hydrazinecarbothioamide (**4a**)* % Yield: 83, m.p.: 134–136 °C; IR (KBr) (cm^{-1}): 1131 (C=S), 1307 (C–N), 1696 (C=O), 3005 (C–H), 3143 (NH), 3238, 3256 (NH_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.4 (q, 4H, $2CH_2$), 4.03 (s, 2H, N–CH_2), 7.0–7.7 (m, 4H, Ar–H), 9.53 (s, 2H,

Table 3 Results of cytotoxicity and antiviral activity of compounds in HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50}[b] (μM)				
		HSV-1 (KOS)	HSV-2 (G)	VV	VSV	HSV-1 TK^- KOS ACV^r
4a	>100	>100	>100	>100	>100	>100
4b	>100	>100	>100	20	>100	>100
5a	>100	20	15	20	>100	>100
5b	100	15	15	≥20	>20	≥20
5c	100	>20	>20	>20	>20	>20
5d	100	14	12	11	>20	≥20
5e	100	>20	>20	>20	>20	>20
5f	100	>20	>20	>20	>20	>20
5g	100	>20	>20	>20	>20	>20
5h	100	>20	>20	>20	>20	>20
Brivudin	>250	0.08	150	29	>250	>250
Cidofovir	>250	5	1.5	10	>250	6
Acyclovir	>250	1.0	0.7	>250	>250	>250
Ganciclovir	>100	0.09	0.07	>100	>100	≥20

[a] Required to cause a microscopically detectable alteration of normal cell morphology

[b] Required to reduce virus-induced cytopathogenicity by 50 %

Table 4 Results of cytotoxicity and antiviral activity of compounds in HeLa cell cultures

Compound	Cytotoxicity (μM)		EC_{50}[b] (μM)					
	CC_{50}[a]	Minimum cytotoxic concentration[b]	VSV		CV-B4		RSV	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
4a	>100	>100	>100	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100	>100	>100	>100
5a	10.8	≥20	>20	>20	>20	>20	>20	>20
5b	9.8	≥4	>4	>4	>4	>4	>4	>4
5c	>100	≥4	>4	>4	>4	>4	>4	>4
5d	10.7	≥20	>20	>20	>20	>20	>20	>20
5e	13.5	≥20	>20	>20	>20	>20	>20	>20
5f	9.4	100	>20	>20	>20	>20	>20	>20
5g	13.2	4	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
5h	>100	≥4	>4	>4	>4	>4	>4	>4
DS-5000[c]	>100	>100	20	14.8	>100	>100	4	2.8
(S)-DHPA	>250	>250	>250	>250	>250	>250	>250	>250
Ribavirin	>250	>250	50	12.1	50	28.5	10	4.6

[a] 50 % cytotoxic concentration

[b] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology

[c] 50 % effective concentration, as determined by a colorimetric formazan-based MTS assay. Data in μg ml^{-1}

Table 5 Results of cytotoxicity and antiviral activity of compounds in Vero cell cultures

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50}[b] (μM)				
		PI-3V	RV-1	SV	CV-B4	PTV
4a	>100	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100	>100
5a	100	>20	>20	>20	>20	>20
5b	20	>4	>4	>4	>4	>4
5c	4	>0.8	>0.8	>0.8	>0.8	>0.8
5d	100	>20	>20	>20	>20	>20
5e	100	>20	>20	>20	>20	>20
5f	100	>20	>20	>20	>20	>20
5g	4	>0.8	>0.8	>0.8	>0.8	>0.8
5h	≥4	>4	>4	>4	>4	>4
DS-5000[c]	>100	>100	>100	20	100	100
(S)-DHPA	>250	250	250	>250	>250	>250
Ribavirin	>250	50	>250	>250	>250	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology

[b] Required to reduce virus-induced cytopathogenicity by 50 %

[c] Data in μg ml^{-1}

NH_2), 11.18 (s, 1H, NH); calc. for $C_{14}H_{19}N_5OS$: C-55.06, H-6.27 and N-22.93, found C-55.18, H-6.15 and N-22.69.

*2-(5-chloro-1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)hydrazinecarbothioamide (**4b**)* % Yield: 64, m.p.: 214–216 °C; IR (KBr) (cm^{-1}): 1127 (C=S), 1305 (C–N), 1698 (C=O), 3008 (C–H), 3136 (NH), 3227, 3246 (NH_2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.4 (q, 4H, $2CH_2$), 4.03 (s, 2H, N–CH_2), 7.0–7.7 (m, 3H, Ar–H), 9.53 (s, 2H, NH_2), 11.18 (s, 1H, NH); calc. for $C_{14}H_{18}ClN_5OS$: C-49.48, H-5.34 and N-20.61, found C-49.34, H-5.21 and N-20.69.

Table 6 Results of anti-influenza virus activity and cytotoxicity in MDCK cell cultures

Compound	Cytotoxicity (μM)		Antiviral EC_{50}[c] (μM)					
	CC_{50}[a]	Minimum cytotoxic concentration[b]	Influenza A H1N1 subtype		Influenza A H3N2 subtype		Influenza B	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
4a	85.8	100	>20	>20	>20	>20	>20	>20
4b	78.3	100	>20	>20	>20	>20	>20	>20
5a	1.6	0.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
5b	0.4	0.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
5c	9.2	4	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
5d	0.2	0.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
5e	3.0	≥0.8	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
5f	2.4	≥0.8	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
5g	11.1	≥0.8	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
5h	11.3	4	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
Oseltamivir carboxylate	>100	>100	45	39.1	4	5.7	45	21.8
Ribavirin	>100	≥100	9	11.5	9	6.8	9	8.4
Amantadine	>500	>500	45	65.5	2	1.5	>500	>500
Rimantadine	258.9	500	9	24.2	0.1	0.08	>100	>100

[a] 50 % cytotoxic concentration, as determined by colorimetric formazan-based MTS assay

[b] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology

[c] 50 % effective concentration, as determined by colorimetric formazan-based MTS assay

Table 7 Zone of Inhibition in mm (using 50 μg ml^{-1} as test solution)

Compound	Antibacterial activity						Antifungal activity	
	S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*	*P. vulgaris*	*S. typhi*	*A. niger*	*C. albicans*
4a	++	++	++	++	++	++	++	++
4b	+++	++	++	++	++	++	++	++
5a	++	++	++	++	+++	+++	+++	+++
5b	++	+++	++	+++	++	++	++	++
5c	++	++	++	++	+++	+++	++	++
5d	+++	+++	++	+++	++	+++	++	++
5e	++	++	++	++	+++	++	++	+++
5f	+++	+++	++	+++	+++	+++	+++	+++
5g	++	++	++	++	+++	+++	++	++
5h	++	++	++	+++	++	++	++	++
Ofloxacin	++++	++++	++++	++++	++++	++++	–	–
Fluconazole	–	–	–	–	–	–	++++	++++
Control	+	+	+	+	+	+	+	+

++++ (31–36), +++ (21–30), ++ (11–20), + (8–10) in mm

General procedure for syntheses of ***5a–h***

A mixture of the 1-[(diethylamino)methyl]-1*H*-indole-2, 3-dione or 5-chloro-1-[(diethylamino)methyl]-1*H*-indole-2,3-dione (0.005 mol), N^4-aryl thiosemicarbazides (0.005 mol), acetic acid (0.5–1.0 ml), and ethanol (100 ml) was heated under reflux until the reaction was completed (approximately 4 h). Approximately half of the ethanol was removed in vacuo and the solution was left overnight at room temperature. The precipitated solid was collected, washed with cold ethanol, and recrystallized from ethanol:chloroform (9:1) to give the following compounds.

*2-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-N-phenylhydrazinecarbothioamide (**5a**)* % Yield: 69, m.p.: 230–232 °C; IR (KBr) (cm^{-1}): 1127 (C=S), 1304 (C–N), 1713 (C=O), 3008 (C–H), 3143, 3217 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.4 (q, 4H, $2CH_2$), 4.03 (s, 2H, N–CH_2), 6.9–7.7 (m, 9H, Ar–H), 11.29 (s, 1H, N–NH), 11.52 (s, 1H, NH); calc. for $C_{20}H_{23}N_5OS$: C-62.97, H-6.08 and N-18.36, found C-62.53, H-5.87 and N-18.15.

*N-(4-chlorophenyl)-2-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)hydrazine carbothioamide (**5b**)* % Yield: 74, m.p.: 151–153 °C; IR (KBr) (cm^{-1}): 1129 (C=S), 1317 (C–N), 1716 (C=O), 3006 (C–H), 3128, 3209 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.4 (q, 4H, $2CH_2$), 4.03 (s, 2H, N–CH_2), 6.4–7.7 (m, 8H, Ar–H), 11.29 (s, 1H, N–NH), 11.52 (s, 1H, NH); calc. for $C_{20}H_{22}ClN_5OS$: C-57.75, H-5.33 and N-16.84, found C-57.39, H-5.26 and N-16.53.

*2-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-N-(4-methoxyphenyl)hydrazinecarbothioamide (**5c**)* % Yield: 77, m.p.: 177–179 °C; IR (KBr) (cm^{-1}): 1119 (C=S), 1325 (C–N), 1706 (C=O), 3008 (C–H), 3120, 3202 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.4 (q, 4H, $2CH_2$), 3.75 (s, 3H, O–CH_3), 4.03 (s, 2H, N–CH_2), 6.3–7.7 (m, 8H, Ar–H), 11.29 (s, 1H, N–NH), 11.52 (s, 1H, NH); calc. for $C_{21}H_{25}N_5O_2S$: C-61.29, H-6.12 and N-17.02, found C-61.09, H-6.09 and N-16.80.

*2-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-N-p-tolylhydrazinecarbothioamide (**5d**)* % Yield: 67, m.p.: 191–193 °C; IR (KBr) (cm^{-1}): 1125 (C=S), 1297 (C–N), 1706 (C=O), 3008 (C–H), 3135, 3219 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.32 (s, 3H, CH_3), 2.4 (q, 4H, $2CH_2$), 4.03 (s, 2H, N–CH_2), 6.3–7.7 (m, 9H, Ar–H), 11.29 (s, 1H, N–NH), 11.52 (s, 1H, NH); calc. for $C_{21}H_{25}N_5OS$: C-63.77, H-6.37 and N-17.71, found C-63.42, H-6.14 and N-17.39.

*2-(5-chloro-1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-N-phenylhydrazinecarbothioamide (**5e**)* % Yield: 72, m.p.: 181–183 °C; IR (KBr) (cm^{-1}): 1125 (C=S), 1315 (C–N), 1718 (C=O), 3008 (C–H), 3123, 3219 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.4 (q, 4H, $2CH_2$), 4.03 (s, 2H, N–CH_2), 6.9–7.7 (m, 8H, Ar–H), 11.29 (s, 1H, N–NH), 11.52 (s, 1H, NH); calc. for $C_{20}H_{22}ClN_5OS$: C-57.75, H-5.33 and N-16.84, found C-57.51, H-5.37 and N-16.27.

*2-(5-chloro-1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-N-(4-chlorophenyl)hydrazinecarbothioamide (**5f**)* % Yield: 79, m.p.: 219–221 °C; IR (KBr) (cm^{-1}): 1114 (C=S), 1310 (C–N), 1713 (C=O), 3007 (C–H), 3123, 3216 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.4 (q, 4H, $2CH_2$), 4.03 (s, 2H, N–CH_2), 6.4–7.6 (m, 8H, Ar–H), 11.29 (s, 1H, N–NH), 11.52 (s, 1H, NH); calc. for $C_{20}H_{21}Cl_2N_5OS$: C-53.34, H-4.70 and N-15.55, found C-53.29, H-4.54 and N-15.28.

*2-(5-chloro-1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-N-(4-methoxyphenyl)hydrazinecarbothioamide (**5g**)* % Yield: 73, m.p.: 165–167 °C; IR (KBr) (cm^{-1}): 1123 (C=S), 1329 (C–N), 1716 (C=O), 3008 (C–H), 3127, 3224 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.4 (q, 4H, $2CH_2$), 3.75 (s, 3H, O–CH_3), 4.03 (s, 2H, N–CH_2), 6.3–7.7 (m, 7H, Ar–H), 11.29 (s, 1H, N–NH), 11.52 (s, 1H, NH); calc. for $C_{21}H_{24}ClN_5O_2S$: C-56.56, H-5.42 and N-15.70, found C-56.26, H-5.32 and N-15.47.

*2-(5-chloro-1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-N-p-tolylhydrazinecarbothioamide (**5h**)* % Yield: 72, m.p.: 178–180 °C; IR (KBr) (cm^{-1}): 1128 (C=S), 1309 (C–N), 1711 (C=O), 3008 (C–H), 3125, 3228 (NH); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.32 (s, 3H, CH_3), 2.4 (q, 4H, $2CH_2$), 4.03 (s, 2H, N–CH_2), 6.3–7.7 (m, 8H, Ar–H), 11.29 (s, 1H, N–NH), 11.52 (s, 1H, NH); calc. for $C_{21}H_{24}ClN_5OS$: C-58.66, H-5.63 and N-16.29, found C-58.43, H-5.38 and N-16.31.

Cytotoxicity assays

The methodology for undertaking the HeLa, CEM, and L1210 assays has been published previously (Baraldi *et al.,* 2004). In brief, varying concentrations of the compounds were incubated at 37 °C for 72 h (HeLa and CEM T-lymphocytes) or 48 h (L1210 cells) in 200-µl 96-well-microtiter plates, and the viable tumor cell number was counted at the end of the incubation period using a Coulter Counter (Coulter Electronics, Harpenden Hertz, U.K.).

Antiviral assay

The antiviral screening of **4a**, **b** and **5a**–**h** was performed against FIPV and feline herpes virus in CRFK cell culture; HSV-1 KOS, HSV-2G, VV, VSV, and HSV-1 TK KOS ACVr in HEL cell cultures; VSV, CVB4, and RSV in HeLa cell cultures; PI-3V, RV-1, SV, CV-B4, and PTV in Vero cell cultures; influenza A virus H1N1 subtype, influenza A virus H3N2 subtype, and influenza B virus in MDCK cell cultures and the results are expressed as the 50 % effective concentration (EC_{50}). Cells, grown to confluency in 96-well plates, were infected with 100 $CCID_{50}$ of virus, one $CCID_{50}$ being the 50 % cell culture infective dose. At the time of virus infection, serial dilutions of the compounds were added. The cultures were incubated at 37 °C

for 3 days, until complete CPE was observed in the infected and untreated virus control.

Cytotoxicity assay

The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity in uninfected cells, and is expressed as the minimum cytotoxic concentration to cause a microscopically detectable alteration of normal cell morphology (HEL, HeLa, CRFK, MDCK, and Vero cells).

Antiproliferative assays

The cytostatic effects of the test compounds on murine leukemia cells (L1210), human T-lymphocyte cells (CEM), and human cervix carcinoma cells (HeLa) were evaluated as follows: an appropriate number of cells suspended in growth medium were allowed to proliferate in 200-µl wells of 96-well-microtiter plates in the presence of variable amounts of test compounds at 37 °C in a humidified CO_2-controlled atmosphere. After 48 h (L1210), 72 h (CEM), or 96 h (HeLa), the number of cells was counted in a Coulter counter. The IC_{50} value was defined as the compound concentration required to inhibit cell proliferation by 50 %.

β-lactamase inhibitory assay

All reagents were equilibrated to 30 °C in a water bath before adding them to the reaction tubes (20 × 150 mm. Pyrex test tubes) in the following order: first, 1 ml of gelatin solution (1 % in 0.1 M phosphate buffer, pH 7.0), 50 µl of enzyme, one drop of starch solution (1 % soluble starch), 1 ml of Penicillin solution (Crystalline Sodium Penicillin G (Benzyl penicillin) IP, Alembic Ltd.) 1,660 µg mg^{-1}, dissolved in 0.1 M phosphate buffer, pH 7.0, to contain not less than 5,000 µg ml^{-1}), 3 ml of sample solution and finally, 2 ml of iodine (0.01 N iodine in 0.1 M potassium iodide) was added. Then the time of decolorization of iodine was recorded with a stopwatch; after addition of substrate (synthesized compounds), standard (Potassium Clavulanate) and blank was determined using water in place of sample solution.

Unit Penicillinase activity is expressed in Pollock and Torriani unit. One unit is the amount of enzyme which will hydrolyze 1 µM Sodium Penicillin G in 1 h at pH 7.0 at 30 °C.

Antimicrobial screening

The antimicrobial assays were performed for synthesized compounds by cup-plate method of all the synthesized compounds, as antibacterial activity against *S. aureus, B. subtilis, K. pneumoniae, E. coli, P. vulgaris, and S. typhi* and antifungal activity against *A. niger and C. albicans.* This activity was expressed in terms of diameters of zone of inhibition in mm.

Conclusion

In summary, a series of indolin-2-ones with *N*-diethyl amino and various thiosemicarbazide moiety were designed and synthesized. The cytotoxicity of all synthesized compounds was evaluated against two human cancer cell lines (CEM and HeLa) and murine leukemia cell line (L1210). Compound **5c** displayed an excellent cytotoxicity against all three cell lines tested; in particular, it showed potent cytotoxicity against CEM and L1210 cancer cell lines. The preliminary structure–activity relationship (SAR) studies revealed that combination of indolin-2-one core structure and 4-methoxy phenyl thiosemicarbazide moiety at the 3-position was more favorable. All the synthesized compounds were screened for cytotoxicity, β-lactamase inhibitory activity, antiviral, antibacterial, and antifungal activity against various strains of microorganisms. Derivative **4b**, **5a**, **5b**, and **5d** showed antiviral activity against HEL cell cultures in the range of 11–20 µM, in comparison with IC_{50} values of 29, 10, >250, and >100 µM for standard brivudin, cidofovir, acyclovir, and ganciclovir, respectively. Mild to moderate antimicrobial activity was observed. The encouraging biological data provide an adequate rationale for further modification of these molecular scaffolds.

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