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Full Paper

Indole-3-carbinol and 1,3,4-Oxadiazole Hybrids: Synthesis and Study of Anti-Proliferative and Anti-Microbial Activity

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In the present study, molecular hybrids of indole-3-carbinol and 1,3,4-oxadiazole-2-thiols have been designed and synthesized. The thiol analogues consisted of diversely substituted benzyl and alkyl groups with different electronic properties. The structures of all the newly synthesized scaffolds and target compounds were ascertained using ^{1}H NMR, ^{13}C NMR, mass spectrometry, and elemental analyses. All the final compounds were screened in vitro for their anti-proliferative and anti-microbial activity. Three compounds showed excellent anti-proliferative activity with more than 70 % cell growth inhibition against three cancer cell lines, HepG2 (human liver hepatocellular carcinoma), HeLa (human cervix carcinoma), and MCF-7 (human breast carcinoma). In the anti-microbial studies, compounds with electron-withdrawing fluoro or nitro substituent displayed appreciable activity similar to that of standard drugs. Also, the final compounds are non-toxic to non-cancerous Vero cell line.

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Introduction

Cancer continues to be one of the major causes of death in developing as well as in developed nations, with lung and breast cancer topping the list.[1] A variety of clinically approved drugs are on the market, and some of them are being applied for the treatment of neoplastic disorders such as leukaemia and testicular cancer. But, most of the drugs used for the treatment of cancer are either poorly specific or have toxic side effects, urging the need to develop novel chemotherapeutic agents through an efficient synthetic approach. One of the approaches in this direction is to explore the various heterocyclic motifs that are presently in use and modify their structure to enhance their efficacy. Indole, the most pharmacodynamic nucleus in nature, has been a major constituent of several bio-molecules such as indole-3-carbinol (I3C) and its related bioactive derivatives, which are anti-carcinogenic,[2] anti-oxidant,[3] and anti-atherogenic agents. I3C is the degradation product of glucobrassicin and it exhibits anti-proliferative activity in various types of human cancer cells[4] including oestrogen-responsive and oestrogen-independent breast cancer cells[5–7] and human prostate cancer cells.[8] Indole is a sub-structural element of many natural products such as alkaloids and is commonly used as a scaffold in medicinal chemistry research.[9] Likewise, oxadiazole-based heterocycles have attracted interest in medicinal chemistry as bioisosteres of amides and esters as they enhance the biological activity by participating in hydrogen bonding interactions with different receptors. They have acquired a great prominence due to their broad spectrum of metabolic profile.[10] Furthermore, the pharmacological activity of these heterocycles can also be attributed to the presence of toxophoric –N–C=O– linkage.[11] Among the four possible isomers, 1,3,4-oxadiazole and its derivatives are widely exploited due to their varied pharmacological activities, namely, anti-microbial,[12] anti-viral,[13] fungicidal,[14] anti-neoplastic,[15] anti-cancer,[16] anti-HIV,[17] and inhibition of tyrosinase.[18] Amongst the new drug moieties used in clinical medicine, Zibotentan, an anti-cancer agent, perfectly illustrates the cytotoxicity of the compounds containing the 1,3,4-oxadiazole unit.[19] It is this versatility that establishes this moiety as an important bioactive class of heterocyclic compounds. Also, amidst various types of oxadiazole-containing molecules, many 2,5-disubstituted-1,3,4-oxadiazoles have shown better biological activities. One such compound is A-204197, which is useful for the treatment of neoplastic diseases in in vivo studies as an anti-mitotic agent (Fig. 1).[20] On the other hand, 1,3,4-oxadiazole-2-thiols are also acquiring significance owing to the fact that the thiol group on the oxadiazole ring undergoes nucleophilic substitution reactions readily[21,22] that provides an efficient method for structural modification.

Moreover, in most drug molecules, it is observed that fluorine substitution has substantial influence on the pharmacological properties of a molecule.[23] The bioisosteric replacement of hydrogen by fluorine has long been known as a tool to

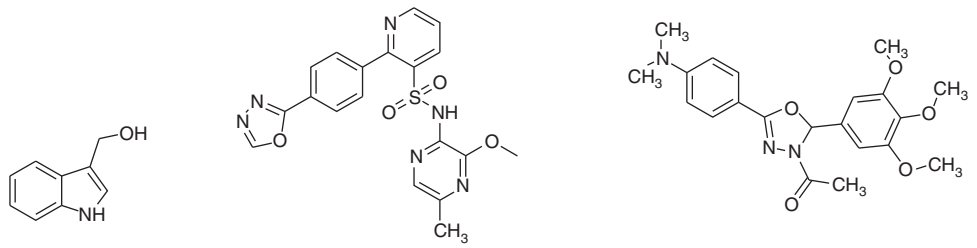
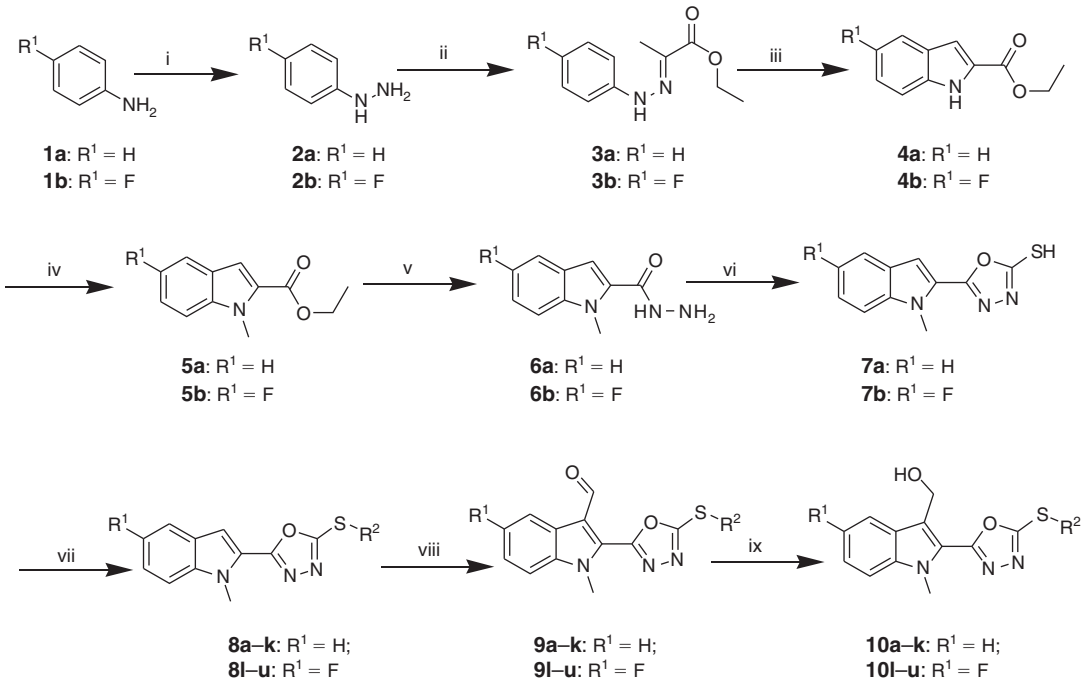


Fig. 1. Bioactive molecules based on indole and 1,3,4-oxadiazole moieties.



Scheme 1. Synthesis of indole–oxadiazole hybrids. Reagents and conditions: (i) HCl, $NaNO_2$, $SnCl_2$, −5°C to RT overnight; (ii) ethyl pyruvate, cat. HOAc, EtOH, 80°C, 1 h; (iii) polyphosphoric acid, toluene, 100°C, 5 h; (iv) K_2CO_3, *n*-TBAB, DMF, MeI, RT, 12 h; (v) $NH_2NH_2{\cdot}H_2O$, EtOH, 80°C, 2 h; (vi) CS_2, KOH, MeOH, 65°C, 24 h; (vii) K_2CO_3, DMF, *n*-TBAB, alkyl halide, 80°C, 90 min; (viii) DMF, $POCl_3$, −5°C to 80°C, 2 h, H_2O; and (ix) $NaBH_4$, THF, RT, 1 h.

enhance the metabolic stability, modify the chemical reactivity, and improve the transportation and absorption characteristics of pharmaceuticals.[24] The highly fluorinated systems have potential for the delivery of drugs, prodrugs, vaccines, genes, markers, contrast agents, and other materials.[25] Keeping all these points in view and as an extension of our comprehensive research aimed at developing novel bioactive pharmacophores, we have described here, our use of indole-3-carbinol as a lead structure in carrying out the synthesis of analogues having simple structures and significant biological activity. Reports on indole-2-oxadiazole acting as a bioactive molecule[26] also gave us an impetus to further explore the hybrid structure. For this purpose, we have joined two pharmacologically active moieties, indole and 1,3,4-oxadiazole, into a single framework to improve the potency of the molecules. The thiol functional group linked to the oxadiazole ring offered us an array of substitution patterns affording compounds **8a**–**u**. Subsequent reactions led to the formation of 21 new hybrids **10a**–**u** that were evaluated for their anti-proliferative activities against three human cancer cell lines, i.e. HeLa (human cervix carcinoma), HepG2 (human liver hepatocellular carcinoma), and MCF-7 (human breast carcinoma), by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In addition to this, we have screened the synthesized target molecules for their anti-microbial activity against few strains of bacteria and fungi. The compounds showing promising activity in each case were identified on the basis of which the structure–activity relationship (SAR) was established.

Results and Discussion

Chemistry

The synthetic route for the target molecules is outlined in Scheme 1. The core indole-2-ester **4** was conveniently prepared using the Fischer indole protocol.[27] The indole-NH was then methylated using potassium carbonate (K_2CO_3) as a base and tetra-*n*-butyl ammonium bromide (*n*-TBAB) as a phase transfer catalyst to give intermediates **5a** and **5b**. The ethyl ester functional group present in **5a** and **5b** was then converted into a hydrazide group using hydrazine hydrate yielding **6a** and **6b**, respectively. Intermediates **6a** and **6b** upon reaction with carbon disulfide (CS_2) in methanolic potassium hydroxide (KOH)

Table 1. Physical data of the final compounds 10a–u[A]

Code	R^1	R^2	Molecular formula	Molecular weight	Nature of compound	Melting point [°C]	Yield [%]
10a	H	4F–C_6H_4–CH_2–*	$C_{19}H_{16}FN_3O_2S$	369.41	White solid	156–157	91
10b	H	4OMe–C_6H_4–CH_2–*	$C_{20}H_{19}N_3O_3S$	381.45	Pale yellow solid	112–113	88
10c	H	4CN–C_6H_4–CH_2–*	$C_{20}H_{16}N_4O_2S$	376.43	Off-White solid	175–176	92
10d	H	2F–C_6H_4–CH_2–*	$C_{19}H_{16}FN_3O_2S$	369.41	White solid	130–131	94
10e	H	4NO_2–C_6H_4–CH_2–*	$C_{19}H_{16}N_4O_4S$	396.42	Pale yellow solid	163–164	78
10f	H	CH_3–CH*–CH_3	$C_{15}H_{17}N_3O_2S$	303.38	White solid	110–111	80
10g	H	C_5H_9–*	$C_{17}H_{19}N_3O_2S$	329.42	White solid	122–123	65
10h	H	4CF_3O–C_6H–CH_2–*	$C_{20}H_{16}F_3N_3O_3S$	435.42	Pale yellow solid	128–129	90
10i	H	4CF_3–C_6H_4–CH_2–*	$C_{20}H_{16}F_3N_3O_2S$	419.42	White solid	141–142	91
10j	H	C_6H_4–CH_2–*	$C_{19}H_{17}N_3O_2S$	351.42	Pale yellow solid	114–115	85
10k	H	CH_3–CH_2–*	$C_{14}H_{15}N_3O_2S$	289.35	Beige solid	98–99	75
10l	F	4F–C_6H_4–CH_2–*	$C_{19}H_{15}F_2N_3O_2S$	387.40	White solid	161–162	90
10m	F	4CN–C_6H_4–CH_2–*	$C_{20}H_{15}FN_4O_2S$	394.42	Pale yellow solid	168–169	94
10n	F	2F–C_6H_4–CH_2–*	$C_{19}H_{15}F_2N_3O_2S$	387.40	White solid	148–149	90
10o	F	4NO_2–C_6H_4–CH_2–*	$C_{19}H_{15}FN_4O_4S$	414.41	Pale brown solid	80–81	75
10p	F	CH_3–CH*–CH_3	$C_{15}H_{16}FN_3O_2S$	321.37	White solid	109–110	78
10q	F	C_5H_9–*	$C_{17}H_{18}FN_3O_2S$	347.41	White solid	123–124	71
10r	F	4CF_3O–C_6H_4–CH_2–*	$C_{20}H_{15}F_4N_3O_3S$	453.41	Off-White solid	154–155	87
10s	F	4CF_3–C_6H_4–CH_2–*	$C_{20}H_{16}F_3N_3O_2S$	437.41	White solid	143–144	90
10t	F	C_6H_4–CH_2–*	$C_{19}H_{16}FN_3O_2S$	369.41	White solid	141–142	86
10u	F	CH_3–CH_2–*	$C_{14}H_{14}FN_3O_2S$	307.34	Off-White solid	112–113	79

[A]Symbol * denotes the point of attachment.

under reflux condition yielded **7a** and **7b**, respectively. These were then treated with different substituted alkyl and aryl halides using K_2CO_3 as a base in anhydrous DMF to give S-alkylated intermediates **8a**–**u**. Each of these intermediates, **8a**–**u**, was subjected to formylation at position-3 via the Vilsmeier–Haack procedure[28] to obtain **9a**–**u**, correspondingly. Finally, the –CHO group in all the intermediates (**9a**–**u**) was reduced to a $–CH_2OH$ group using sodium borohydride ($NaBH_4$) in anhydrous THF to afford the target compounds **10a**–**u**, respectively. All the target compounds were purified by column chromatography on silica gel using *n*-hexane and ethyl acetate solvent systems. All the intermediates and target compounds were characterized using ^{1}H NMR, ^{13}C NMR, and electrospray ionization mass spectrometry (ESI–MS) followed by elemental analyses. The hydrazide intermediates **6a** and **6b** showed distinct downfield signals at δ 9.75 and 9.80 ppm corresponding to –CONH proton whereas the $–NH_2$ peak resonated at δ 4.48 and 4.50 ppm. These peaks were absent in the ^{1}H NMR spectra of intermediates **7a** and **7b**, whereas a new singlet peak for –SH at δ 3.97 and 3.98 ppm appeared for **7a** and **7b**, respectively. The intermediate **8j** showed five new aromatic peaks in the ^{1}H NMR spectrum confirming the S-alkylation. The singlet at δ 4.60 ppm indicates the presence of benzyl $–CH_2$ group. In its ^{13}C NMR spectrum, peaks at δ 36.42 and 32.48 ppm are attributed to $S–CH_2$ and $N–CH_3$ carbons, respectively. Furthermore, the presence of 1,3,4-oxadiazole unit in the molecule was supported by the appearance of signals (C2, C5) in the range of δ 160.5–166.5 ppm. The formation of intermediate **9j** is clearly evidenced by the appearance of a singlet at δ 10.28 ppm in the ^{1}H NMR spectrum that occurs due to –CHO proton, whereas in the ^{13}C NMR spectrum, the –CHO carbon resonates at δ 186.7 ppm. The –CHO peak disappeared in the ^{1}H NMR spectrum of target compound **10j**, whereas new singlet peaks appeared at δ 4.97 and 4.88 ppm due to –OH and $O–CH_2$ protons, respectively. The signal at δ 54.26 ppm for $–CH_2–OH$ carbon in its ^{13}C NMR spectrum further justifies the structure of the molecule. The ESI–MS pattern of compound **10j** shows $[M-17]^+$ and/or $[M-18]^+$ peak owing to the cleavage of aliphatic –OH group. This is primarily because of the impact of high-energy electrons used in the mass spectrometry analysis that often break a molecule into a cation and a radical. As cations are detected in the spectra, a prominent $[M-17]^+$ and/or $[M-18]^+$ peak is observed due to the loss of a hydroxyl radical or a neutral molecule such as H_2O.[29] A similar pattern of peaks was observed for other compounds of the series. The detailed spectral data of all the title compounds are presented in the experimental section and their physical data are tabulated in Table 1.

*X-Ray Crystallography Study of **10n***

One of the final compounds, **10n**, was subjected to single-crystal X-ray diffraction studies (SCXRD), and the 3-dimentional structure of the molecule was established. The crystal structure solution was worked out using *SHELXL-2014/6* software package. All the atoms were located in different Fourier maps and refined isotropically, using a riding model, and all the projections were generated using *ORTEP*. The single crystal suitable for the X-ray diffraction analysis was grown by slow evaporation of compound **10n** in dichloromethane solution at room temperature. The *ORTEP* diagram (50 % probability) of **10n** is depicted in Fig. 2, whereas Table 2 presents the crystal refinement details.

Anti-Proliferative Studies

The cytotoxicity of the synthesized indole–oxadiazole hybrids was ascertained using MTT assay[30] over a panel of three human cancer cell lines. The data in Table 3 present the anti-tumour activity in terms of percentage growth inhibition (%GI) values caused by the test compounds at 10 μM concentration. An assessment of the anti-proliferative profile of the synthesized compounds helped us to select the most potent compounds. HeLa, HepG2, and MCF-7 cells were chosen to evaluate the efficacy of the target compounds. MCF-7 appears to be the

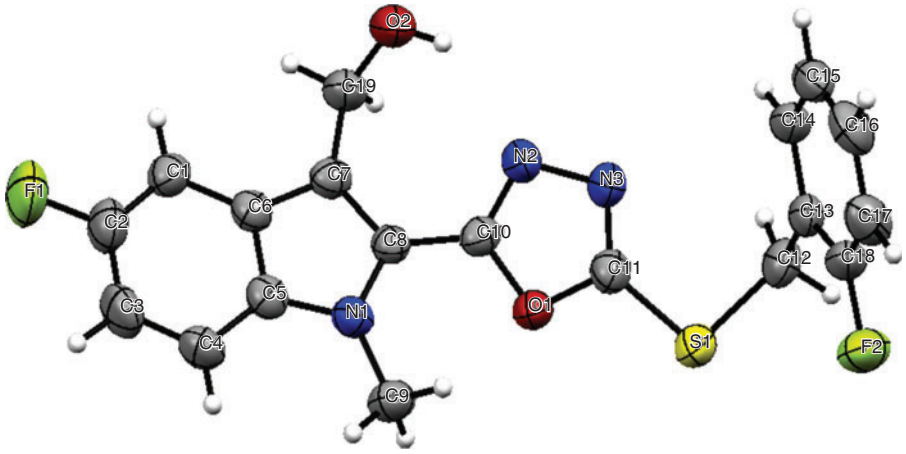


Fig. 2. *ORTEP* diagram (50 % probability) of compound **10n**.

Table 2. Crystal data and measurement details of compound 10n

Parameters	Crystal data
CCDC No.	1060838
Chemical formula	$C_{19}H_{15}F_2N_3O_2S$
Formula weight	387.40
Crystal system	triclinic
Space group	*P*-1
a [Å]	7.6017(9)
b [Å]	9.5136(11)
c [Å]	12.9546(14)
Volume [Å^3]	863.52(18)
Angle α, β, γ [°]	105.453(7), 105.744(7), 91.883(8)
Z	2
F_{000}	400
μ [mm^{-1}]	0.229
Temperature [K]	296
Radiation wavelength [Å]	0.71073
Radiation type	Mo Kα
Radiation source	Fine-focus sealed tube
Radiation monochromator	Graphite
R-Factor [%]	11.10

most sensitive cell line with several compounds showing substantial growth inhibitory activity against it. Most of the compounds showed moderate-to-good inhibition. All the tested compounds have different patterns of substitution, with a benzyl ring having either an electron-donating or an electron-withdrawing group or alkyl chain attached to the thiol group (R^2) of the oxadiazole ring. Compounds **10c**, **10f**, and **10o** displayed remarkable cytotoxic activity against all three cell lines with growth inhibition ranging from 71.8 % to 94.3 %. These compounds are nearly twice as potent as 5-fluorouracil and of equal potency with respect to doxorubicin in inhibiting the growth of cells (Table 3). Compound **10c** contains an aryl ring with an electron-withdrawing functional group i.e. cyano, which is in turn attached to the oxadiazole–thiol unit (R^2), whereas compound **10f** has an electron-donating isopropyl group attached at R^2. However, it can be noted that the fluoro analogues (at R^1) of **10c** and **10f**, namely **10m** and **10p** respectively, showed only moderate inhibition. Compound **10o** exhibited excellent inhibition, whereas almost comparable inhibition was shown by compound **10e** against all three cell lines that may be due to the electron-withdrawing nitro group (R^2). Selective inhibition

Table 3. Cytotoxic activity (% cell growth inhibition) of compounds 10a–u against three human cancer cell lines (at 10 µM) and against non-cancerous Vero cell line (at 50 µM)

The percentage inhibition values above 70 % are presented in bold.

Compound code	Growth inhibitory activity[A]			
	HeLa	HepG2	MCF-7	Vero
10a	**73.2 ± 0.9**	62.8 ± 0.5	**71.1 ± 0.4**	10.4 ± 3.7
10b	56.2 ± 0.2	**78.2 ± 0.4**	**75.1 ± 0.4**	9.23 ± 1.5
10c	**77.9 ± 2.7**	**90.4 ± 0.3**	**88.2 ± 0.6**	7.00 ± 3.4
10d	57.4 ± 1.4	67.1 ± 0.7	66.8 ± 0.3	–
10e	**75.8 ± 1.8**	**71.1 ± 0.5**	**78.8 ± 0.7**	8.5 ± 1.3
10f	**71.8 ± 2.2**	**84.5 ± 0.2**	**91.5 ± 0.6**	10.93 ± 1.0
10g	24.9 ± 0.5	64.6 ± 0.4	67 ± 0.2	–
10h	41.8 ± 0.8	61.8 ± 0.4	56.1 ± 0.6	–
10i	38.1 ± 1.2	55.8 ± 0.5	41.6 ± 0.2	–
10j	27.7 ± 1.8	**70.1 ± 0.7**	**76.1 ± 0.3**	14.2 ± 2.3
10k	31.8 ± 1.7	46.3 ± 0.4	37.7 ± 0.5	–
10l	29.9 ± 1.1	51.9 ± 0.7	41.2 ± 0.4	–
10m	66.8 ± 1.1	45.6 ± 0.4	48.2 ± 0.4	–
10n	36.5 ± 0.7	68.7 ± 0.7	58.3 ± 0.5	–
10o	**94.3 ± 1.0**	**73.2 ± 0.7**	**73.5 ± 0.3**	10.8 ± 1.2
10p	43.3 ± 2.5	47.9 ± 0.5	47.2 ± 0.5	–
10q	47.6 ± 0.9	55.6 ± 0.5	53.3 ± 0.4	–
10r	39 ± 0.4	62.8 ± 0.4	35.2 ± 0.2	–
10s	29.76 ± 0.8	64.2 ± 0.6	62.6 ± 0.2	–
10t	26.5 ± 1.3	**78.9 ± 1.8**	**71.7 ± 0.5**	10.28 ± 1.2
10u	21.8 ± 0.5	50.5 ± 0.5	61.7 ± 0.6	–
Doxorubicin	77 ± 2.4	65 ± 0.8	88 ± 0.3	46 ± 0.1
5-Fluorouracil	45 ± 3.3	42 ± 0.8	35 ± 2.2	22 ± 3.0

[A]Experiment was performed in triplicate. All values are expressed as mean ± standard error of the mean ($n = 3$).
– Not determined.

towards HepG2 and MCF-7 cells was demonstrated by compounds **10j** and **10t**, both bearing a simple unsubstituted benzyl ring. Similarly, compounds **10h** and **10i**, and their respective fluoro equivalents, **10r** and **10s**, showed relatively similar inhibition on HepG2 and MCF-7 cell growth. At the same time, it can also be perceived that a functional group may provide different effects on the inhibition activity depending on its position in the molecule. For instance, compounds **10d** and **10n** in which fluorine is present at *ortho* position (2-F) on the benzyl ring showed lower inhibition activity than compound **10a** which has a *para*-fluoro group (4-F). However, it is to be noted that

Table 4. Anti-Microbial activity of compounds 10a–u (10 μg per disc)
The zone of inhibition values of the active compounds are presented in bold.

Compound code	Zone of inhibition [mm]					
	Anti-Bacterial activity				Anti-Fungal activity	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
10a	**27**	**26**	**28**	**26**	**26**	**25**
10b	09	08	08	10	11	13
10c	14	16	16	13	10	08
10d	**27**	**26**	**28**	**26**	**25**	**24**
10e	**29**	**27**	**25**	**25**	**27**	**23**
10f	09	08	08	09	11	10
10g	10	08	10	09	08	09
10h	**28**	**30**	**25**	**24**	**27**	**25**
10i	**25**	**26**	**25**	**23**	**23**	**24**
10j	10	11	09	10	10	11
10k	07	10	09	08	08	10
10l	**25**	**28**	**25**	**24**	**26**	**26**
10m	22	23	25	24	20	21
10n	**28**	**24**	**26**	**22**	**24**	**23**
10o	**27**	**28**	**27**	**25**	**25**	**24**
10p	16	18	15	14	12	14
10q	14	10	13	16	19	16
10r	**28**	**30**	**29**	**25**	**27**	**25**
10s	**23**	**26**	**25**	**24**	**24**	**23**
10t	15	13	15	16	14	12
10u	13	15	18	13	15	14
Ciprofloxacin	27	26	28	25	–	–
Fluconazole	–	–	–	–	27	26

compounds **10d**, **10n**, **10h**, **10i**, **10r**, and **10s** exhibit comparable inhibition values pertaining to 5-fluorouracil on all the three cells. Furthermore, their inhibition activity on HepG2 cells is comparable with that of doxorubicin. Likewise, the electron-donating ethyl group (R^2) in compounds **10k** and **10u**, and lipophilic cyclopentyl group (R^2) in compounds **10g** and **10q** had very little impact on the cytotoxic activity. In contrast, compound **10b**, with a methoxy group on the benzyl ring (R^2), displayed fairly good inhibition activity. This testifies that there is very little variation in the cytotoxicity of the compounds regardless of the electron donor or electron acceptor property of the attached substituents at R^2 position. In addition, none of the active compounds were toxic to the benign Vero cells, with a cell growth inhibition of less than 15 % at a concentration of 50 μM, thereby boosting further work in future in developing them into effective leads.

Anti-Microbial Studies

The anti-microbial activity was determined using disc diffusion method[31] by measuring the zone of inhibition in millimetre. All the compounds (**10a**–**u**) were screened in vitro at a concentration of 10 μg per disc for their anti-bacterial activity against two Gram-positive strains (*Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*)) and two Gram-negative strains (*Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)). The anti-fungal evaluation of the compounds was carried out against *Candida albicans* (*C. albicans*) and *Aspergillus niger* (*A. niger*) at a concentration of 10 μg per disc. The standard anti-bacterial drug, ciprofloxacin (10 μg per disc), and the anti-fungal drug, fluconazole (10 μg per disc), were tested under similar conditions against these organisms. All synthesized compounds exhibited significant anti-bacterial and anti-fungal activities as demonstrated in Table 4. Each experiment was performed in triplicate and the average reading was taken. The activity was classified as highly active (≥26 mm), moderately active (11–25 mm), and least active (<11 mm). Accordingly, compounds **10a**, **10d**, **10e**, **10h**, **10i**, **10l**, **10n**, **10o**, **10r**, and **10s** have shown excellent activity compared with other compounds of the series. Notably, all these compounds comprise electron-withdrawing groups, namely, nitro (**10e** and **10o**), fluoro (**10a**, **10d**, **10l**, and **10n**), trifluoromethoxy (**10h** and **10r**), and trifluoromethyl (**10i** and **10s**), attached to the thiol group of the oxadiazole ring (R^2 position) that may have contributed to an increase in the activity. These results also indicate a marked preference for the presence of fluorine atoms, which are present either at R^1 or at R^2 position. Moreover, it is interesting to note that the majority of the molecules is almost as potent as standard drugs (ciprofloxacin and fluconazole), with some of them showing superior activity than the standards.

Conclusion

The present study describes the successful synthesis of 21 new compounds with indole-3-carbinol as a base structure and 1,3,4-oxadiazole-2-thiol analogues attached at position-2 of the indole ring. The formation of intermediates and target compounds was verified by spectroscopic techniques, and the single crystal structure of one of the target compounds (**10n**) was determined. All the target compounds were screened against three cancerous human cell lines, amidst which, compounds **10c**, **10f**, and **10o** (with cyano, isopropyl, and nitro groups at R^2 position, respectively) exhibited more potent and a broad spectrum of anti-tumour activity. Fluorine substitution at position-5 of the indole nucleus did not contribute significantly to the cytotoxicity. However, in case of anti-microbial profile,

compounds containing fluorine atoms namely, **10a**, **10d**, **10e**, **10h**, **10i**, **10l**, **10n**, **10o**, **10r**, and **10s** offered excellent activity against all the bacterial and fungal strains, thereby providing promising leads for future structural optimization.

Experimental

Materials and Methods

The chemicals used in the present work were procured from Sigma-Aldrich (Germany) or Spectrochem Chemicals Pvt. Ltd. All the solvents used are of analytical grade. They were purchased, distilled, and dried before use. The progress of the reaction was monitored by thin layer chromatography, performed on a Silica gel 60 F254-coated aluminium sheet. Melting points were determined using open capillaries on a Stuart SMP3 (BIBBY STERLIN Ltd UK) apparatus and were uncorrected. ^{1}H NMR analysis of the intermediates and final compounds was performed on a Bruker 400 MHz NMR spectrometer using TMS as internal reference and [D6]DMSO as solvent. ^{13}C NMR spectra of the compounds were recorded using a Bruker 100 MHz NMR spectrometer. Elemental analyses were performed on a Flash EA-1112 CHNS analyzer (Thermo Electron Corporation). Mass spectra were recorded on an Agilent Triple quad LC-MS model: 0430 using methanol as a solvent. The SCXRD analysis was performed on a Bruker SMART APEXII DUO CCD diffractometer using MoKα as radiation source. All reactions were performed under inert conditions.

Synthesis

*Procedure for the Synthesis of 1-Methyl-1H-indole-2-carbohydrazide (**6a**)*

A round-bottom flask containing methylated indole-2-ester **5a** (5 g, 24.60 mmol) was charged with hydrazine hydrate (2.98 mL, 61.50 mmol) at room temperature (RT) under argon atmosphere. Contents were diluted with sufficient quantity of dry ethanol until a clear solution was obtained and then refluxed at 80°C for ~2 h. Following completion of the reaction, excess ethanol was distilled off and the residue obtained was filtered, washed with ice-cold water, dried, and recrystallized from ethanol to afford a white crystalline solid (4.7 g, 94 %), mp 150–151°C. δ_H ([D6]DMSO, 400 MHz) 9.75 (1H, s, NHCO), 7.61–6.99 (5H, m, Ar–H), 4.48 (2H, s, NH_2), 3.97 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 162.25, 138.74, 137.48, 131.57, 130.88, 121.91, 120.01, 119.61, 111.27, 31.69. *m*/*z* (ESI) 190.2 ([M + 1]$^+$). Anal. Calc. for $C_{10}H_{11}N_3O$: C 63.48, H 5.86, N 22.21. Found: C 63.44, H 5.83, N 22.18 %.

*Procedure for the Synthesis of 5-Fluoro-1-methyl-1H-indole-2-carbohydrazide (**6b**)*

The above procedure was followed for the synthesis of **6b** using **5b** (5.5 g, 24.86 mmol) and hydrazine hydrate (3.02 mL, 62.15 mmol) in ethanol solvent to afford a white solid (5.1 g, 93 %), mp 180–181°C. δ_H ([D6]DMSO, 400 MHz) 9.80 (1H, s, NHCO), 7.56–6.97 (4H, m, Ar–H), 4.50 (2H, s, NH_2), 3.97 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 162.56, 159.72, 139.11, 131.25, 122.79, 121.65, 120.09, 119.63, 114.33, 31.52. *m*/*z* (ESI) 208.2 ([M + 1]$^+$). Anal. Calc. for $C_{10}H_{10}FN_3O$: C 57.97, H 4.86, N 20.28. Found: C 57.92, H 4.83, N 20.23 %.

*Procedure for the Synthesis of 5-(1-Methyl-1H-indol-2-yl)-1,3,4-oxadiazol-2-thiol (**7a**)*

To a stirred solution of hydrazide **6a** (4.5 g, 23.78 mmol) in anhydrous methanol (45 mL), potassium hydroxide (2.00 g, 35.67 mmol) was added in portions followed by dropwise addition of carbon disulfide (2.26 mL, 35.67 mmol) at 0°C under argon atmosphere. This reaction mixture was heated at 65°C for 24 h. Following completion of the reaction as monitored by TLC, the solvent was removed under vacuum. The residue obtained was diluted with water and later extracted with ethyl acetate. The organic phase obtained was washed with saturated brine solution and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue obtained was recrystallized from ethanol to obtain an olive green solid (3.9 g, 87 %), mp 131–133°C. δ_H ([D6]DMSO, 400 MHz): 7.62–6.87 (5H, m, Ar–H), 4.08 (3H, s, N–CH_3), 3.97 (1H, s, –SH). δ_C ([D6]DMSO, 100 MHz): 163.51, 162.34, 139.29, 131.50, 129.45, 122.30, 120.58, 119.47, 113.22, 111.08, 31.54. *m*/*z* (ESI) 232.1 ([M + 1]$^+$). Anal. Calc. for $C_{11}H_9N_3OS$: C 57.13, H 3.92, N 18.17, S 13.86. Found: C 57.10, H 3.88, N 18.14, S 13.84 %.

*Procedure for the Synthesis of 5-(5-Fluoro-1-methyl-1H-indol-2-yl)-1,3,4-oxadiazol-2-thiol (**7b**)*

Intermediate **7b** was prepared by treating compound **6b** (5 g, 24.13 mmol) with potassium hydroxide pellets (2.03 g, 36.19 mmol) and carbon disulfide (2.29 mL, 36.19 mmol) in anhydrous methanol (50 mL) by following the above-mentioned procedure to obtain a buff-coloured solid (4.3 g, 86 %), mp 126–127°C. δ_H ([D6]DMSO, 400 MHz) 7.62–6.85 (4H, m, Ar–H), 4.04 (3H, s, N–CH_3), 3.99 (1H, s, –SH). δ_C ([D6]DMSO, 100 MHz) 163.55, 162.29, 159.62, 132.81, 130.37, 129.56, 115.06, 114.43, 113.22, 111.08, 31.54. *m*/*z* (ESI) 250.2 ([M + 1]$^+$). Anal. Calc. for $C_{11}H_8FN_3OS$: C 53.00, H 3.23, N 16.86, S 12.86. Found: C 52.95, H 3.17, N 16.83, S 12.81 %.

*General Procedure for the Synthesis of Alkylated Intermediates **8a**–**u***

The thione intermediate **7a** (0.3 g) was suspended in anhydrous DMF (3 mL), to which anhydrous K_2CO_3 (2 equiv.) was added at RT and stirred for about 0.5 h. *n*-TBAB was added in catalytic amount and substituted aryl halides (1.5 equiv.) were added. The reaction refluxed at 80°C for 90 min. The progress of the reaction was monitored by TLC. Following completion of the reaction, the entire mass was poured into ice-cold water and stirred properly to yield crystals of the product. These were then filtered, washed with cold water, dried, and recrystallized from methanol.

The structural characterization data of intermediates **8a**–**u** are presented as follows.

2-(5-(4-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8a**): White solid (0.285 g, 95 %), mp 118–119°C. δ_H ([D6]DMSO, 400 MHz) 7.70–7.14 (9H, m, Ar–H), 4.60 (2H, s, S–CH_2), 4.10 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.33, 163.00, 160.90, 139.57, 133.41, 131.70, 131.62, 126.77, 124.96, 122.78, 122.18, 121.12, 115.99, 115.78, 111.24, 106.78, 35.59, 32.48. *m*/*z* (ESI) 340.1 ([M + 1]$^+$). Anal. Calc. for $C_{18}H_{14}FN_3OS$: C 63.70, H 4.16, N 12.38, S 9.45. Found: C 63.67, H 4.11, N 12.36, S 9.40 %.

2-(5-(4-Methoxybenzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8b**): Pale yellow solid (0.25 g, 83 %), mp 109–110°C. δ_H ([D6]DMSO, 400 MHz) 7.71–7.14 (9H, m, Ar–H), 4.61 (2H, s, S–CH_2), 4.11 (3H, s, N–CH_3), 3.69 (3H, s, O–CH_3). δ_C ([D6] DMSO, 100 MHz) 162.76, 161.55, 160.93, 133.09, 131.70, 130.62, 127.98, 124.96, 123.06, 122.80, 121.12, 118.53, 115.99, 115.78, 111.24, 106.70, 54.41, 35.59, 32.48. *m*/*z* (ESI)

352.4 ([M + 1]$^+$). Anal. Calc. for $C_{19}H_{17}N_3O_2S$: C 64.94, H 4.88, N 11.96, S 9.12. Found: C 64.90, H 4.85, N 11.91, S 9.08 %.

4-((5-(1-Methyl-1H-indol-2-yl)-1,3,4-oxadiazol-2-ylthio) methyl)benzonitrile (**8c**): Off-white solid (0.275 g, 92 %), mp 149–150°C. δ_H ([D6]DMSO, 400 MHz) 7.71–7.10 (9H, m, Ar–H), 4.60 (2H, s, S–CH_2), 4.09 (3H, s, N–CH_3). δ_C ([D6] DMSO, 100 MHz) 160.98, 158.95, 143.10, 136.65, 134.78, 134.71, 130.54, 129.98, 129.81, 127.69, 124.30, 122.80, 121.12, 118.53, 115.78, 111.21, 106.70, 35.43, 32.49. *m/z* (ESI) 347.4 ([M + 1]$^+$). Anal. Calc. for $C_{19}H_{14}N_4OS$: C 65.88, H 4.07, N 16.17, S 9.26. Found: C 65.84, H 4.06, N 16.14, S 9.22 %.

2-(5-(2-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8d**): White solid (0.26 g, 87 %), mp 109–110°C. δ_H ([D6] DMSO, 400 MHz) 7.74–7.16 (9H, m, Ar–H), 4.58 (2H, s, S–CH_2), 4.09 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.00, 162.13, 160.90, 139.57, 131.19, 130.31, 129.47, 128.30, 126.77, 124.96, 124.10, 122.18, 121.12, 115.99, 111.24, 106.48, 32.48, 29.49. *m/z* (ESI) 340.1 ([M + 1]$^+$). Anal. Calc. for $C_{18}H_{14}FN_3OS$: C 63.70, H 4.16, N 12.38, S 9.45. Found: C 63.66, H 4.10, N 12.36, S 9.40 %.

2-(5-(4-Nitrobenzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8e**): Pale brown solid (0.27 g, 90 %), mp 111–112°C. δ_H ([D6]DMSO, 400 MHz): 7.65–7.13 (9H, m, Ar–H), 4.59 (2H, s, S–CH_2), 4.10 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 160.91, 158.87, 147.33, 146.71, 138.64, 131.21, 129.66, 129.49, 128.15, 122.63, 122.48, 122.04, 120.79, 115.93, 111.18, 106.71, 35.36, 32.41. *m/z* (ESI) 367.2 ([M + 1]$^+$). Anal. Calc. for $C_{18}H_{14}N_4O_3S$: C 59.01, H 3.85, N 15.29, S 8.75. Found: C 58.99, H 3.83, N 15.27, S 8.72 %.

2-(5-(Isopropylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8f**): White solid (0.265 g, 88 %), mp 84–85°C. δ_H ([D6]DMSO, 400 MHz) 7.70–7.14 (5H, m, Ar–H), 4.13 (3H, s, N–CH_3), 3.94 (1H, sept, *J* 6.8, S–CH), 1.48 (6H, d, *J* 6.8, –$(CH_3)_2$). δ_C ([D6] DMSO, 100 MHz) 162.78, 160.66, 139.56, 126.78, 126.62, 121.96, 121.24, 121.10, 111.23, 106.72, 32.49, 32.38, 23.55, 20.41. *m/z* (ESI) 274.2 ([M + 1]$^+$). Anal. Calc. for $C_{14}H_{15}N_3OS$: C 61.51, H 5.53, N 15.37, S 11.73. Found: C 61.47, H 5.49, N 15.36, S 11.70 %.

2-(5-(Cyclopentylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8g**): White solid (0.255 g, 85 %), mp 93–94°C. δ_H ([D6] DMSO, 400 MHz) 7.79–7.12 (5H, m, Ar–H), 4.10 (3H, s, N–CH_3), 4.08–4.00 (1H, m, S–CH), 2.22–1.47 (8H, m, aliphatic). δ_C ([D6]DMSO, 100 MHz) 162.77, 160.58, 139.71, 129.59, 126.78, 121.92, 121.24, 121.10, 111.23, 106.72, 44.19, 35.28, 35.31, 32.49, 26.35, 26.18. *m/z* (ESI) 300.1 ([M + 1]$^+$). Anal. Calc. for $C_{16}H_{17}N_3OS$: C 64.19, H 5.72, N 14.04, S 10.71. Found: C 64.17, H 5.69, N 14.01, S 10.68 %.

2-(5-(4-(Trifluoromethoxy)benzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8h**): Off-white solid (0.28 g, 93 %), mp 98–99°C. δ_H ([D6]DMSO, 400 MHz) 7.71–7.14 (9H, m, Ar–H), 4.65 (2H, s, S–CH_2), 4.09 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 161.71, 160.57, 159.63, 139.62, 133.30, 129.97, 129.89, 128.47, 128.16, 126.73, 121.24, 121.10, 120.28, 115.68, 115.38, 111.23, 106.72, 35.58, 32.49. *m/z* (ESI) 406.2 ([M + 1]$^+$). Anal. Calc. for $C_{19}H_{14}F_3N_3O_2S$: C 56.29, H 3.48, N 10.37, S 7.91. Found: C 56.25, H 3.46, N 10.35, S 7.89 %.

2-(5-(4-(Trifluoromethyl)benzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8i**): White solid (0.271 g, 90 %), mp 108–109°C. δ_H ([D6]DMSO, 400 MHz) 7.77–7.14 (9H, m, Ar–H), 4.65 (2H, s, S–CH_2), 4.11 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 160.57, 159.63, 146.19, 139.62, 129.89, 129.40, 128.47, 128.16, 126.73, 125.64, 125.37, 125.11, 122.18, 121.12, 115.91, 111.23, 106.72, 35.57, 32.49. *m/z* (ESI) 390.3 ([M + 1]$^+$). Anal. Calc. for $C_{19}H_{14}F_3N_3OS$: C 58.60, H 3.63, N 10.79, S 8.23. Found: C 58.58, H 3.59, N 10.76, S 8.21 %.

2-(5-(Benzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8j**): White solid (0.282 g, 94 %), mp 84–85°C. δ_H ([D6]DMSO, 400 MHz) 7.70–7.14 (10H, m, Ar–H), 4.60 (2H, s, S–CH_2), 4.10 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.07, 160.76, 139.57, 138.50, 129.08, 128.99, 128.87, 128.48, 128.28, 127.48, 126.77, 122.18, 121.81, 121.12, 111.24, 106.76, 36.42, 32.48. *m/z* (ESI) 322.2 ([M + 1]$^+$). Anal. Calc. for $C_{18}H_{15}N_3OS$: C 67.27, H 4.70, N 13.07, S 9.98. Found: C 67.24, H 4.67, N 13.04, S 9.94 %.

2-(5-(Ethylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8k**): White solid (0.266 g, 89 %), mp 95–96°C. δ_H ([D6]DMSO, 400 MHz) 7.69–7.12 (5H, m, Ar–H), 4.06 (3H, s, N–CH_3), 3.35 (2H, q, *J* 7.2, S–CH_2), 1.44 (3H, t, *J* 7.2, –CH_3). δ_C ([D6]DMSO, 100 MHz) 163.10, 160.17, 139.55, 131.28, 126.59, 122.18, 121.73, 121.19, 111.23, 106.72, 32.48, 30.06, 15.86. *m/z* (ESI) 260.3 ([M + 1]$^+$). Anal. Calc. for $C_{13}H_{13}N_3OS$: C 60.21, H 5.05, N 16.20, S 12.36. Found: C 60.19, H 5.01, N 16.18, S 12.33 %.

2-(5-(4-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indole (**8l**): Off-white solid (0.281 g, 93.6 %), mp 119–120°C. δ_H ([D6]DMSO, 400 MHz) 7.71–7.14 (8H, m, Ar–H), 4.60 (2H, s, S–CH_2), 4.11 (3H, s, N–CH_3). δ_C ([D6] DMSO, 100 MHz) 160.80, 160.29, 158.95, 156.63, 135.56, 133.32, 131.67, 131.59, 131.53, 126.95, 116.01, 115.80, 113.53, 112.65, 112.55, 106.33, 35.70, 32.76. *m/z* (ESI) 358.2 ([M + 1]$^+$). Anal. Calc. for $C_{18}H_{13}F_2N_3OS$: C 60.49, H 3.67, N 11.76, S 8.97. Found: C 60.46, H 3.66, N 11.73, S 8.93 %.

4-((5-(5-Fluoro-1-methyl-1H-indol-2-yl)-1,3,4-oxadiazol-2-ylthio)methyl)benzonitrile (**8m**): Off-white solid (0.262 g, 87 %), mp 151–152°C. δ_H ([D6]DMSO, 400 MHz) 7.76–7.13 (8H, m, Ar–H), 4.59 (2H, s, S–CH_2), 4.10 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 162.42, 161.53, 159.87, 145.41, 132.26, 132.23, 131.91, 130.50, 129.61, 129.57, 128.82, 120.54, 115.61, 113.88, 112.84, 112.57, 111.04, 34.59, 32.73. *m/z* (ESI) 365.4 ([M + 1]$^+$). Anal. Calc. for $C_{19}H_{13}FN_4OS$: C 62.63, H 3.60, N 15.38, S 8.80. Found: C 62.60, H 3.57, N 15.36, S 8.77 %.

2-(5-(2-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indole (**8n**): White solid (0.272 g, 91 %), mp 109–110°C. δ_H ([D6]DMSO, 400 MHz) 7.68–7.14 (8H, m, Ar–H), 4.62 (2H, s, S–CH_2), 4.11 (3H, s, N–CH_3). δ_C ([D6] DMSO, 100 MHz) 162.38, 160.92; 159.87, 158.53, 131.89, 130.31, 129.47, 128.30, 127.39, 126.77, 124.96, 121.12, 113.98, 112.61, 111.24, 106.48, 34.68, 32.74. *m/z* (ESI) 358.2 ([M + 1]$^+$). Anal. Calc. for $C_{18}H_{13}F_2N_3OS$: C 60.49, H 3.67, N 11.76, S 8.97. Found: C 60.46, H 3.63, N 11.75, S 8.95 %.

2-(5-(4-Nitrobenzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indole (**8o**): Yellow solid (0.276 g, 92 %), mp 123–124°C. δ_H ([D6]DMSO, 400 MHz) 7.69–7.10 (8H, m, Ar–H), 4.64 (2H, s, S–CH_2), 4.09 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 160.91, 158.87, 159.87, 147.66, 146.46, 132.20, 131.09, 129.66, 129.49, 128.15, 122.63, 122.48, 113.98, 112.64, 111.18, 106.71, 35.36, 32.41. *m/z* (ESI) 385.3 (([M + 1]$^+$). Anal. Calc. for $C_{18}H_{13}FN_4O_3S$: C 56.24, H 3.41, N 14.58, S 8.34. Found: C 56.20, H 3.38, N 14.55, S 8.32 %.

5-Fluoro-2-(5-(isopropylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indole (**8p**): White solid (0.26 g, 86 %), mp 116–117°C. δ_H ([D6]DMSO, 400 MHz) 7.67–7.19 (4H, m, Ar–H), 4.12 (3H, s, N–CH_3), 3.94 (1H, sept, *J* 6.8, S–CH), 1.48 (6H, d, *J* 6.8, –$(CH_3)_2$). δ_C ([D6]DMSO, 100 MHz) 162.78, 160.66, 159.86, 132.23, 131.91, 127.37, 113.98, 112.64, 111.23, 106.72, 35.89, 33.48,

23.55, 20.45. *m/z* (ESI) 292.2 ($[M+1]^+$). Anal. Calc. for $C_{14}H_{14}FN_3OS$: C 57.72, H 4.84, N 14.42, S 11.01. Found: C 57.69, H 4.81, N 14.39, S 10.99 %.

2-(5-(Cyclopentylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indole (**8q**): Pale yellow solid (0.268 g, 89 %), mp 110–111°C. δ_H ([D6]DMSO, 400 MHz) 7.67–7.19 (4H, m, Ar–H), 4.12 (3H, s, N–CH_3), 4.09–4.02 (1H, m, S–CH), 2.25–1.65 (8H, m, aliphatic). δ_C ([D6]DMSO, 100 MHz) 162.77, 160.58, 159.79, 133.33, 129.59, 127.32, 113.70, 112.62, 111.22, 106.72, 44.23, 35.28, 35.31, 32.49, 26.35, 26.29. *m/z* (ESI) 318.3 ($[M+1]^+$). Anal. Calc. for $C_{16}H_{16}FN_3OS$: C 60.55, H 5.08, N 13.24, S 10.10. Found: C 60.52, H 5.05, N 13.21, S 10.06 %.

2-(5-(4-(Trifluoromethoxy)benzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indole (**8r**): White solid (0.29 g, 96 %), mp 118–119°C. δ_H ([D6]DMSO, 400 MHz) 7.67–7.20 (8H, m, Ar–H), 4.63 (2H, s, S–CH_2), 4.10 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.17, 160.85, 160.56, 159.23, 135.59, 131.51, 131.43, 126.89, 126.79, 121.58, 121.36, 119.24, 113.86, 113.71, 113.44, 112.79, 106.51, 35.45, 32.77. *m/z* (ESI) 424.2 ($[M+1]^+$). Anal. Calc. for $C_{19}H_{13}F_4N_3O_2S$: C 53.90, H 3.09, N 9.92, S 7.57. Found: C 53.87, H 3.06, N 9.90, S 7.54.

2-(5-(4-(Trifluoromethyl)benzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indole (**8s**): White solid (0.279 g, 93 %), mp 108–109°C. δ_H ([D6]DMSO, 400 MHz) 7.74–7.20 (8H, m, Ar–H), 4.69 (2H, s, S–CH_2), 4.10 (3H, s, N–CH_3). δ_C ([D6] DMSO, 100 MHz) 160.57, 159.63, 159.17, 144.67, 133.32, 130.09, 129.89, 128.47, 128.16, 127.43, 125.64, 125.37, 125.21, 113.98, 112.73, 112.61, 106.70, 35.55, 32.48. *m/z* (ESI) 408.3 ($[M+1]^+$). Anal. Calc. for $C_{19}H_{13}F_4N_3OS$: C 56.02, H 3.22, N 10.31, S 7.87. Found: C 55.99, H 3.19, N 10.28, S 7.84 %.

2-(5-(Benzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indole (**8t**): Pale brown solid (0.274 g, 91 %), mp 115–116°C. δ_H ([D6]DMSO, 400 MHz) 7.87–7.16 (9H, m, Ar–H), 4.59 (2H, s, S–CH_2), 4.11 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.07, 160.76, 159.76, 140.03, 133.30, 129.08, 128.99, 128.87, 128.48, 128.69, 127.48, 127.17, 113.41, 112.12, 111.24, 106.76, 35.61, 32.48. *m/z* (ESI) 340.2 ($[M+1]^+$). Anal. Calc. for $C_{18}H_{14}FN_3OS$: C 63.70, H 4.16, N 12.38, S 9.45. Found: C 63.66, H 4.14, N 12.35, S 9.42 %.

2-(5-(Ethylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indole (**8u**): Off-white solid (0.254 g, 85 %), mp 98–99°C. δ_H ([D6]DMSO, 400 MHz) 7.67–7.19 (4H, m, Ar–H), 4.18 (3H, s, N–CH_3), 3.34 (2H, q, *J* 7.6, S–CH_2), 1.44 (3H, t, *J* 7.6, –CH_3). δ_C ([D6]DMSO, 100 MHz) 163.10, 160.17, 159.71, 133.35, 131.28, 129.59, 113.34, 112.19, 111.23, 106.72, 32.48, 30.06, 15.86. *m/z* (ESI) 278.2 ($[M+1]^+$). Anal. Calc. for $C_{13}H_{12}FN_3OS$: C 56.30, H 4.36, N 15.15, S 11.56. Found: C 56.27, H 4.33, N 15.12, S 11.55.

General Procedure for the Synthesis of Formylated Intermediates **9a**–**u**

To a dried two-neck round-bottom flask containing DMF (1.5 equiv.), phosphorus oxychloride ($POCl_3$; 1.5 equiv.) was added dropwise using a syringe under argon at a temperature below 0°C. After the formation of the iminium cation, a solution of intermediate **8a**–**u** (0.23 g) in DMF was added and the entire reaction mixture was heated at 80°C for 2 h. The reaction was monitored by TLC and after complete consumption of the starting material, the reaction mixture was poured into crushed ice and stirred well. The formed solid was collected by filtration, washed with cold water, dried, and used as such for the next step. The structural characterization data of intermediates **9a**–**u** are given in the Supplementary Material.

Procedure for the Synthesis of the Title Compounds **10a**–**u**

The final compounds were prepared by dissolving the formylated indole **9a**–**u** (0.12 g) in THF (1.2 mL) to which $NaBH_4$ (3 equiv.) was added at 0°C. It was then kept for stirring at RT for 1 h. Completion of the reaction was determined by TLC, after which ice-cold water was poured into the reaction mixture. The precipitated solid was filtered off, washed with ice-cold water, and dried well. The obtained crude product was then purified by column chromatography using ethyl acetate/petroleum ether (6 : 4) system to afford the pure title compounds.

The structural characterization of the final compounds is presented as follows.

(2-(5-(4-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10a**): δ_H ([D6]DMSO, 400 MHz) 7.85–7.14 (8H, m, Ar–H), 4.94 (1H, s, –OH), 4.87 (2H, s, O–CH_2), 4.60 (2H, s, S–CH_2), 3.99 (3H, s, N–CH_3). δ_C ([D6] DMSO, 100 MHz) 163.42, 163.35, 160.92, 138.80, 133.36, 133.33, 131.67, 131.59, 126.79, 125.08, 121.22, 120.64, 119.90, 116.01, 115.80, 111.04, 54.24, 35.69, 32.39. *m/z* (ESI) 352.9 ($[M-17]^+$). Anal. Calc. for $C_{19}H_{16}FN_3O_2S$: C 61.77, H 4.37, N 11.37, S 8.68. Found: C 61.75, H 4.35, N 11.34, S 8.66 %.

(2-(5-(4-Methoxybenzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10b**): δ_H ([D6]DMSO, 400 MHz) 7.70–6.90 (8H, m, Ar–H), 4.55 (2H, s, O–CH_2), 4.08 (3H, s, O–CH_3), 3.99 (1H, s br, –OH), 3.73 (3H, s, N–CH_3), 3.17 (2H, s, S–CH_2). δ_C ([D6]DMSO, 100 MHz) 164.22, 162.35, 159.92, 159.06, 133.36, 132.03, 131.67, 130.95, 125.78, 124.08, 121.87, 120.04, 117.90, 116.31, 115.30, 110.08, 55.89, 53.91, 34.69, 33.29. *m/z* (ESI) 364.6 ($[M-17]^+$). Anal. Calc. for $C_{20}H_{19}N_3O_3S$: C 62.97, H 5.02, N 11.02, S 8.41. Found: C 62.95, H 4.99, N 10.98, S 8.38 %.

4-((5-(3-(Hydroxymethyl)-1-methyl-1H-indol-2-yl)-1,3,4-oxadiazol-2-ylthio)methyl)benzonitrile (**10c**): δ_H ([D6] DMSO, 400 MHz) 7.85–7.14 (8H, m, Ar–H), 4.95 (1H, s br, –OH), 4.85 (2H, s, O–CH_2), 4.67 (2H, s, S–CH_2), 3.98 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.16, 160.71, 143.22, 138.80, 132.97, 132.79, 132.59, 130.81, 130.49, 130.17, 121.27, 121.22, 120.66, 119.85, 119.12, 111.05, 110.94, 54.21, 35.89, 32.39. *m/z* (ESI) 359.6 ($[M-17]^+$). Anal. Calc. for $C_{20}H_{16}N_4O_2S$: C 63.81, H 4.28, N 14.88, S 8.52. Found: C 63.78, H 4.26, N 14.85, S 8.49 %.

(2-(5-(2-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10d**): δ_H ([D6]DMSO, 400 MHz) 7.86–7.14 (8H, m, Ar–H), 4.97 (1H, s br, –OH), 4.87 (2H, s, O–CH_2), 4.63 (2H, s, S–CH_2), 3.99 (3H, s, N–CH_3). δ_C ([D6] DMSO, 100 MHz) 163.09, 162.35, 160.92, 137.36, 133.35, 133.21, 131.76, 130.59, 126.71, 123.88, 121.12, 121.04, 120.18, 115.91, 114.80, 110.91, 54.23, 35.69, 32.31. *m/z* (ESI) 351.6 ($[M-17]^+$). Anal. Calc. for $C_{19}H_{16}FN_3O_2S$: C 61.77, H 4.37, N 11.37, S 8.68. Found: C 61.75, H 4.35, N 11.36, S 8.66 %.

(2-(5-(4-Nitrobenzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10e**): δ_H ([D6]DMSO, 400 MHz) 8.24–7.14 (8H, m, Ar–H), 4.96 (1H, s br, –OH), 4.84 (2H, s, O–CH_2), 4.73 (2H, s, S–CH_2), 3.98 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 160.09, 159.35, 148.71, 145.61, 132.39, 132.21, 131.71, 129.59, 128.65, 123.08, 120.98, 120.74, 119.18, 115.10, 113.80, 108.56, 55.33, 34.09, 32.33. *m/z* (ESI) 379.9 ($[M-17]^+$). Anal. Calc. for

$C_{19}H_{16}N_4O_4S$: C 57.57, H 4.07, N 14.13, S 8.09. Found: C 57.55, H 4.05, N 14.10, S 8.07 %.

(2-(5-(Isopropylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10f**): δ_H ([D6]DMSO, 400 MHz) 7.86–7.14 (4H, m, Ar–H), 4.95 (1H, s, –OH), 4.89 (2H, s, O–CH_2), 4.03 (3H, s, N–CH_3), 3.94 (1H, sept, *J* 6.8, –CH), 1.49 (6H, d, *J* 7.2, –$(CH_3)_2$). δ_C ([D6]DMSO, 100 MHz) 163.15, 160.50, 138.80, 127.48, 126.80, 121.21, 121.16, 120.03, 119.92, 111.02, 110.94, 54.28, 32.41, 23.64, 20.46. *m/z* (ESI) 285.6 ($[M-17]^+$). Anal. Calc. for $C_{15}H_{17}N_3O_2S$: C 59.38, H 5.65, N 13.85, S 10.57. Found: C 59.37, H 5.62, N 13.82, S 10.55 %.

(2-(5-(Cyclopentylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10g**): δ_H ([D6]DMSO, 400 MHz) 7.85–7.14 (4H, m, Ar–H), 4.90 (3H, s br, –CH_2–OH), 4.07 (1H, m, S–CH), 4.02 (3H, s, N–CH_3), 2.26–1.65 (8H, m, aliphatic). δ_C ([D6]DMSO, 100 MHz) 163.09, 160.59, 139.71, 129.59, 127.75, 121.92, 121.24, 121.10, 111.23, 109.92, 44.19, 35.28, 35.34, 32.40, 26.35, 26.18. *m/z* (ESI) 312.9 ($[M-17]^+$). Anal. Calc. for $C_{17}H_{19}N_3O_2S$: C 61.98, H 5.81, N 12.76, S 9.73. Found: C 61.96, H 5.78, N 12.73, S 9.70 %.

(2-(5-(4-(Trifluoromethoxy)benzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10h**): δ_H ([D6]DMSO, 400 MHz) 7.85–7.12 (8H, m, Ar–H), 4.93 (1H, s br, –OH), 4.86 (2H, s, O–CH_2), 4.63 (2H, s, S–CH_2), 3.98 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 161.70, 160.58, 159.63, 139.62, 133.30, 129.97, 129.89, 128.47, 128.16, 126.98, 121.24, 121.10, 120.28, 115.68, 115.38, 111.23, 110.67, 55.02, 35.58, 32.41. *m/z* (ESI) 417.9 ($[M-17]^+$). Anal. Calc. for $C_{20}H_{16}F_3N_3O_3S$: C 55.17, H 3.70, N 9.65, S 7.36. Found: C 55.14, H 3.67, N 9.63, S 7.33 %.

(2-(5-(4-(Trifluoromethyl)benzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10i**): δ_H ([D6]DMSO, 400 MHz) 7.85–7.14 (8H, m, Ar–H), 4.96 (1H, s br, –OH), 4.85 (2H, s, O–CH_2), 4.69 (2H, s, S–CH_2), 3.98 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 160.43, 158.62, 139.75, 135.21, 129.49, 129.18, 128.51, 126.55, 125.54, 122.43, 122.12, 122.01, 120.94, 120.11, 119.91, 113.04, 110.45, 54.35, 35.29, 32.69. *m/z* (ESI) 401.9 ($[M-17]^+$). Anal. Calc. for $C_{20}H_{16}F_3N_3O_2S$: C 57.27, H 3.85, N 10.02, S 7.65. Found: C 57.25, H 3.81, N 9.98, S 7.62 %.

(2-(5-(Benzylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10j**): δ_H ([D6]DMSO, 400 MHz) 7.85–7.14 (9H, m, Ar–H), 4.97 (1H, s br, –OH), 4.88 (2H, s, O–CH_2), 4.61 (2H, s, S–CH_2), 3.99 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.50, 160.56, 138.80, 136.94, 129.51, 129.45, 129.11, 128.29, 126.79, 125.19, 121.22, 120.64, 120.44, 119.91, 111.03, 110.94, 54.26, 36.52, 32.39. *m/z* (ESI) 333.9 ($[M-17]^+$). Anal. Calc. for $C_{19}H_{17}N_3O_2S$: C 64.94, H 4.88, N 11.96, S 9.12. Found: C 64.91, H 4.86, N 11.94, S 9.09 %.

(2-(5-(Ethylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10k**): δ_H ([D6]DMSO, 400 MHz) 7.85–7.01 (4H, m, Ar–H), 4.97 (1H, s br, –OH), 4.87 (2H, s, O–CH_2), 4.02 (3H, s, N–CH_3), 3.34 (2H, q, *J* 7.2, S–CH_2), 1.46 (3H, t *J* 7.2, –CH_3). δ_C ([D6]DMSO, 100 MHz) 163.10, 160.17, 139.55, 131.28, 126.59, 122.20, 121.73, 121.19, 111.17, 110.72, 54.44, 33.18, 30.05, 15.86. *m/z* (ESI) 271.6 ($[M-17]^+$). Anal. Calc. for $C_{14}H_{15}N_3O_2S$: C 58.11, H 5.23, N 14.52, S 11.08. Found: C 58.09, H 5.21, N 14.48, S 11.06 %.

(2-(5-(4-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indol-3-yl)methanol (**10l**): δ_H ([D6]DMSO, 400 MHz) 7.65–7.17 (7H, m, Ar–H), 5.00 (1H, s, –OH), 4.83 (2H, s, O–CH_2), 4.60 (2H, s, S–CH_2), 3.99 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 160.80, 160.29, 158.95, 156.63, 135.56, 133.32, 131.67, 131.59, 131.53, 126.95, 116.01, 115.80, 113.80, 113.53, 112.65, 112.55, 54.34, 35.70, 32.76. *m/z* (ESI) 369.9 ($[M-17]^+$). Anal. Calc. for $C_{19}H_{15}F_2N_3O_2S$: C 58.91, H 3.90, N 10.85, S 8.28. Found: C 58.89, H 3.87 N 10.82, S 8.27 %.

4-((5-(5-Fluoro-3-(hydroxymethyl)-1-methyl-1H-indol-2-yl)-1,3,4-oxadiazo-2-ylthio)methyl)benzonitrile (**10m**): δ_H ([D6]DMSO, 400 MHz) 7.85–7.10 (7H, m, Ar–H), 4.91 (1H, s, *J* 5.2, –OH), 4.85 (2H, d, *J* 5.2, O–CH_2), 4.34 (2H, s, S–CH_2), 3.98 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 162.42, 161.53, 159.87, 145.41, 132.26, 132.23, 131.91, 131.50, 129.61, 129.59, 128.82, 120.54, 115.61, 113.89, 112.84, 112.57, 111.04, 55.53, 34.59, 32.73. *m/z* (ESI) 376.6 ($[M-17]^+$). Anal. Calc. for $C_{20}H_{15}FN_4O_2S$: C 60.90, H 3.83, N 14.20, S 8.13. Found: C 60.87, H 3.81, N 14.18, S 8.10 %.

(2-(5-(2-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indol-3-yl)methanol (**10n**): δ_H ([D6]DMSO, 400 MHz) 7.66–7.18 (7H, m, Ar–H), 5.01 (1H, s, –OH), 4.84 (2H, s, O–CH_2), 4.63 (2H, s, S–CH_2), 3.99 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 162.38, 160.92; 159.87, 158.53, 132.54, 130.31, 129.47, 128.30, 127.39, 126.77, 124.96, 115.39, 113.98, 113.54, 112.99, 111.24, 55.45, 34.68, 33.51. *m/z* (ESI) 369.9 ($[M-17]^+$). Anal. Calc. for $C_{19}H_{15}F_2N_3O_2S$: C 58.91, H 3.90, N 10.85, S 8.28. Found: C 58.89, H 3.87, N 10.82, S 8.27 %.

(2-(5-(4-Nitrobenzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indol-3-yl)methanol (**10o**): δ_H ([D6]DMSO, 400 MHz) 8.24–7.14 (7H, m, Ar–H), 4.96 (1H, s br, –OH), 4.84 (2H, s, O–CH_2), 4.71 (2H, s, S–CH_2), 3.99 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 160.91, 158.87, 159.87, 147.66, 146.46, 132.20, 131.09, 129.66, 129.49, 128.15, 122.63, 122.09, 113.98, 113.54, 112.64, 111.18, 54.67, 35.36, 33.41. *m/z* (ESI) 397.9 ($[M-17]^+$). Anal. Calc. for $C_{19}H_{15}FN_4O_4S$: C 55.07, H 3.65, N 13.52, S 7.74. Found: C 55.04, H 3.61, N 13.50, S 7.71 %.

(5-Fluoro-2-(5-(isopropylthio)-1,3,4-oxadiazol-2-yl)-1-methyl-1H-indol-3-yl)methanol (**10p**): δ_H ([D6]DMSO, 400 MHz) 7.66–7.20 (3H, m, Ar–H), 5.00 (1H, s, –OH), 4.86 (2H, s, O–CH_2), 4.03 (3H, s, N–CH_3), 3.94 (1H, sept, *J* 7.2, –CH), 1.49 (6H, d, *J* 7.2, aliphatic). δ_C ([D6]DMSO, 100 MHz) 162.78, 160.66, 159.86, 132.23, 131.91, 127.37, 113.98, 113.56, 112.64, 111.23, 55.32, 35.89, 33.48, 23.55, 20.45. *m/z* (ESI) 303.6 ($[M-17]^+$). Anal. Calc. for $C_{15}H_{16}FN_3O_2S$: C 56.06, H 5.02, N 13.08, S 9.98. Found: C 56.04, H 4.99, N 13.05, S 9.96 %.

(2-(5-(Cyclopentylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indol-3-yl)methanol (**10q**): δ_H ([D6]DMSO, 400 MHz) 7.66–7.58 (2H, m, Ar–H), 7.24–7.20 (1H, m, Ar–H), 4.99 (1H, s, –OH), 4.86 (2H, s, O–CH_2), 4.06 (1H, m, S–CH), 4.02 (3H, s, N–CH_3), 2.23–1.65 (8H, m, aliphatic). δ_C ([D6]DMSO, 100 MHz) 162.77, 160.58, 159.78, 133.33, 129.59, 127.32, 113.70, 113.51, 112.62, 111.20, 55.78, 44.23, 35.28, 35.31, 32.49, 26.35, 26.29. *m/z* (ESI) 329.9 ($[M-17]^+$). Anal. Calc. for $C_{17}H_{18}FN_3O_2S$: C 58.77, H 5.22, N 12.10, S 9.23. Found: C 58.75, H 5.19, N 12.08, S 9.20 %.

(2-(5-(4-(Trifluoromethoxy)benzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indol-3-yl)methanol (**10r**): δ_H ([D6]DMSO, 400 MHz) 7.64–7.22 (7H, m, Ar–H), 5.00 (1H, s br, –OH), 4.83 (2H, s, O–CH_2), 4.64 (2H, s, S–CH_2), 3.99 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.58, 160.34, 158.95, 156.62, 135.55, 131.49, 126.93, 126.83, 121.79, 121.60, 121.37, 121.09, 113.80, 113.54, 112.64, 112.55, 105.71, 54.31, 35.52, 32.69. *m/z* (ESI) 435.9 ($[M-17]^+$). Anal. Calc.

for $C_{20}H_{15}F_4N_3O_3S$: C 52.98, H 3.33, N 9.27, S 7.07. Found: C 52.95, H 3.30, N 9.25, S 7.04 %.

(2-(5-(4-(Trifluoromethyl)benzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indol-3-yl)methanol (**10s**): δ_H ([D6]DMSO, 400 MHz) 7.74–7.20 (7H, m, Ar–H), 5.01 (1H, s br, –OH), 4.83 (2H, s, O–CH_2), 4.69 (2H, s, S–CH_2), 3.98 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 160.57, 159.63, 159.17, 144.67, 133.32, 129.79, 129.71, 128.47, 128.16, 127.43, 125.64, 125.37, 125.21, 113.98, 113.49, 112.71, 112.61, 54.98, 35.55, 32.69. *m/z* (ESI) 419.9 ($[M-17]^+$). Anal. Calc. for $C_{20}H_{15}F_4N_3O_2S$: C 54.92, H 3.46, N 9.61, S 7.33. Found: C 54.90, H 3.43, N 9.59, S 7.29 %.

(2-(5-(Benzylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indol-3-yl)methanol (**10t**): δ_H ([D6]DMSO, 400 MHz) 7.65–7.20 (8H, m, Ar–H), 5.01 (1H, s, –OH), 4.84 (2H, s, O–CH_2), 4.61 (2H, s, S–CH_2), 3.99 (3H, s, N–CH_3). δ_C ([D6]DMSO, 100 MHz) 163.07, 160.76, 159.72, 140.06, 133.31, 129.62, 128.99, 128.84, 128.48, 128.69, 127.48, 127.19, 113.91, 113.41, 112.12, 111.24, 55.43, 35.61, 32.48. *m/z* (ESI) 351.5 ($[M-17]^+$). Anal. Calc. for $C_{19}H_{16}FN_3O_2S$: C 61.7, H 4.37, N 11.37, S 8.68. Found: C 61.75, H 4.33, N 11.35, S 8.64 %.

(2-(5-(Ethylthio)-1,3,4-oxadiazol-2-yl)-5-fluoro-1-methyl-1H-indol-3-yl)methanol (**10u**): δ_H ([D6]DMSO, 400 MHz) 7.65–7.19 (3H, m, Ar–H), 4.97 (1H, s br, –OH), 4.87 (2H, s, O–CH_2), 4.02 (3H, s, N–CH_3), 3.34 (2H, q, *J* 7.2, S–CH_2), 1.46 (3H, t, *J* 7.2, –CH_3). δ_C ([D6]DMSO, 100 MHz) 163.10, 160.17, 159.71, 133.09, 130.68, 129.58, 113.86, 113.34, 112.19, 112.23, 54.99, 32.48, 30.06, 15.86. *m/z* (ESI) 289.9 ($[M-17]^+$). Anal. Calc. for $C_{14}H_{14}FN_3O_2S$: C 54.71, H 4.59, N 13.67, S 10.43. Found: C 54.68, H 4.56, N 13.63, S 10.41 %.

Pharmacology

Cell Lines, Chemicals, and Culture Medium

HeLa, HepG2, MCF-7, and Vero (African green monkey kidney) cell lines were procured from the National Centre for Cell Sciences (NCCS; Pune, India). MTT, fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), and trypsin were obtained from Sigma-Aldrich (St Louis, USA). Ethylenediaminetetraacetic acid (EDTA), glucose, and antibiotics were obtained from Hi-Media Laboratories Ltd (Mumbai, India), and DMSO and propanol were from Merck Ltd (Mumbai, India). Stock cells were cultured in DMEM supplemented with 10 % inactivated FBS, penicillin (100 IU mL^{-1}), streptomycin (100 µg mL^{-1}), and amphotericin B (5 µg mL^{-1}) in a humidified atmosphere of 5 % CO_2 at 37°C until confluent. The cells were dissociated with TPVG (trypsin phosphate versene glucose) solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The stock cultures were grown in 25 cm^3 culture flasks and all experiments were carried out in 96 microtiter plates (Tarsons India Pvt. Ltd, Kolkata, India).

In Vitro Cytotoxic Activity

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells mL^{-1} using DMEM containing 10 % FBS. To each well of the 96-well microtiter plate, 0.1 mL of the diluted cell suspension (~10000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off. The monolayer was washed once with medium and 100 µL of different test concentrations of test drugs was added on to the partial monolayer in the microtiter plates. The plates were then incubated at 37°C for 3 days in 5 % CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µL of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5 % CO_2 atmosphere. The supernatant was removed and 100 µL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula where OD is optical density.

$$\%\ \text{Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100\,\%$$

Anti-Microbial Studies

The disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method.[31] Nutrient agar (20 mL) was poured into each sterile petri dish after injecting cultures (100 µL) of microorganisms, and medium was distributed in the petri dish homogeneously. Compounds were filtered using a membrane with a pore size of 0.45 µm for sterilization. The compounds were dissolved in DMSO to achieve a concentration of 5 mg mL^{-1}. Empty sterilized discs of 6 mm diameter (Schleicher and Schuell, No. 2668, Germany) were impregnated with 10 µg of compounds. The discs were placed on agar plates, and the plates were incubated at 37°C for 24 h. The culture suspensions were prepared and adjusted by comparing against 0.3 McFarland turbidity standard tubes. Inhibition zones formed on the medium were evaluated in millimetre (mm). The negative solvent control (DMSO) did not show any anti-microbial activity. Studies were performed in triplicate and the average reading was taken. Inhibition zones were compared with those of the reference disc.

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