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Full Paper

New 3-Substituted-2-(4-hydroxyanilino)pyridine Derivatives: Synthesis, Antitumor Activity, and Tubulin Polymerization Inhibition

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A series of new pyridine derivatives **4a–c**, **5a–d**, **6a–d**, **7a–f**, and **8a–f** structurally related to ABT-751 were synthesized and characterized by spectroscopic means and elemental analysis. All the synthesized compounds were tested for their cytotoxic activity *in vitro* against the HCT-116 and HepG-2 cancer cell lines using the MTT assay. The results showed that compound **8d** has higher cytotoxic activity than the reference antimitotic agent colchicine, against both tested cell lines, with $IC_{50} = 0.52$ and 1.40 μM, respectively. The three most active compounds, **5d**, **8b**, and **8d**, were further screened *in vitro* for inhibition of tubulin and showed remarkable results in comparison to colchicine.

Keywords: Cytotoxic activity / Inhibitors / Synthesis

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Introduction

Antimitotic drugs have been successfully used as chemotherapeutic agents in management of cancer via disruption of microtubule/tubulin dynamics [1–4]. Microtubules are important cytoskeletal structures formed by polymerization of α and β tubulin heterodimers, which play a crucial role in the formation of mitotic spindle required for proper chromosomal separation during cell division. Therefore, drugs that target microtubules or interfere with its dynamic stability cause mitotic arrest and consequently lead to apoptosis [5–11]. Over the past years, diverse classes of tubulin polymerization inhibitors of natural and synthetic origin have been exploited and investigated. Some of them were found to display toxicity toward tumor vasculature and are referred to as vascular disrupting agents (VDAs) [12–16]. ABT-751 (E7010) (Fig. 1) is a synthetic antimitotic agent that inhibits tubulin polymerization through reversible binding to β tubulin at the colchicine binding site, leading to a block in the cell cycle at the G2/M phase and cellular apoptosis. It was reported to exhibit potential anticancer activity against several human tumors especially non-small cell lung cancer and colon cancer [17–19]. Additionally, it showed good *in vivo* antitumor activity and reached clinical phase II trials [20–24]. Previously reported structure–activity relationship (SAR) studies on ABT-751 analogs demonstrated the importance of the hydroxyl group at position 4 of the anilino moiety on the *in vivo* activity [25]. It has been also reported that the 4-hydroxy function plays an important role in binding with βTyr202 amino residue in a similar manner to that observed by the co-crystallized ligand ABT-751 (PDB code: 3HKC) [26]. Herein, we report the synthesis and cytotoxic activity of certain new 2-(4-hydroxyanilino)pyridine derivatives **4a–c**, **5a–d**, **6a–d**, **7a–f**, and **8a–f** structurally related to ABT-751.

On the other hand, literature studies reported certain 1,3,4-oxadiazole derivatives as antimitotic agents targeting tubulin. IMC-038525 (Fig. 1) is one of the lead oxadiazoles that showed mitotic arrest at nanomolar concentrations in epidermoid and breast cancers [27–29]. Further, the oxadiazoline derivative A-105972 is a colchicine site binder that

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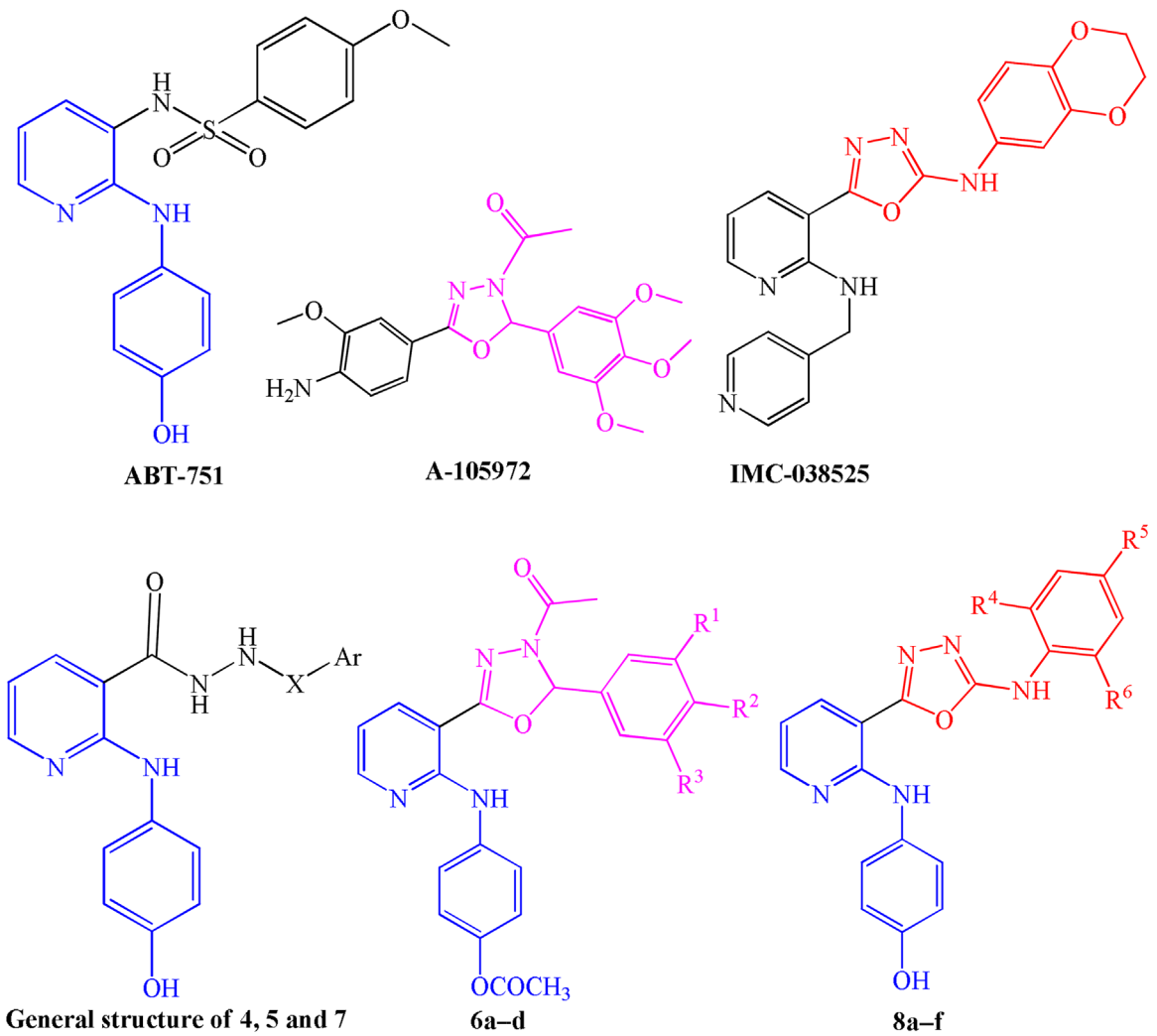

Figure 1. Structure of antimitotic agents acting on colchicine binding site ABT-751, A-105972, IMC-038525 and the general structures of the newly synthesized compounds **4a–c**, **5a–d**, **6a–d**, **7a–f**, and **8a–f**.

interacts with microtubules, inhibits tubulin polymerization, and induces apoptosis [30–32]. In this context, two series of 4-hydroxyanilinopyridine derivatives containing 1,3,4-oxadiazole pharmacophore (**6a–d** and **8a–f**) were synthesized. The design of the compounds **6a–d** was based upon the potent cytotoxic activity displayed by the oxadiazoline derivative A-105972. On the other hand, compounds **8a–f** included NH linking between the oxadiazole and the aromatic rings in a manner analogous to IMC-038525, which is significant for tubulin inhibitory activity [29]. All compounds were evaluated for potential cytotoxic activity against colon cancer cell line HCT-116 and liver cancer cell line HepG-2. Percentage of tubulin inhibition was recorded for the most active compounds at the IC_{50} concentration of each compound.

Results and discussion

Chemistry

The synthetic route to the key intermediate hydrazide **3** is outlined in Scheme 1 and the pathway to the target compounds **4a–c**, **5a–d**, **6a–d**, **7a–f**, and **8a–f** is presented in Scheme 2. The newly synthesized 2-[(4-hydroxyphenyl)-amino]nicotinic acid (**1**) was prepared via two synthetic approaches. The first pathway is a traditional procedure that involved heating of 2-chloronicotinic acid and 4-aminophenol in presence of *p*-toluenesulphonic acid and pyridine for 12 h to give **1** in 72% yield. In the second pathway, the same components were heated under microwave irradiation for a shorter period of time (2.5 h), with the aim to improve yield,

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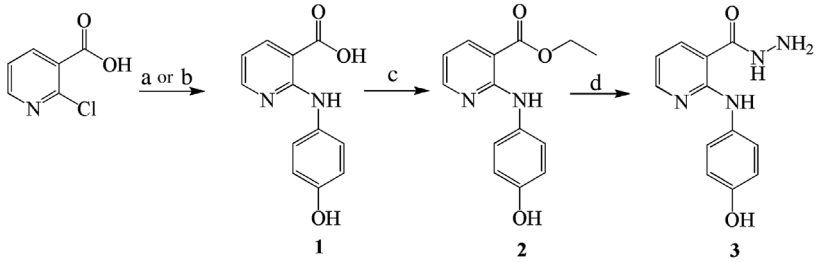

Scheme 1. General synthetic pathways of the key intermediate hydrazide **3**.

Reagents and conditions: a) *p*-Aminophenol, *p*-toluenesulphonic acid, pyridine, reflux 12 h; b) *p*-aminophenol, *p*-toluenesulphonic acid, pyridine, MW 2.5 h; c) abs. ethanol, conc. H_2SO_4, reflux 8 h; d) $NH_2NH_2.H_2O$, stirr at RT 5 h.

reduce reaction time, and consequently reduce environmental hazards. By this approach, the product was obtained in higher yield (80%). Further, esterification of **1** with ethanol containing catalytic amount of conc. H_2SO_4 afforded the corresponding ethyl ester **2**. Hydrazinolysis of **2** accomplished the key intermediate hydrazide **3**. The synthesis of the desired targets **4a–c** was carried out by the reaction of **3** with a number of arylsulphonyl chlorides in pyridine as a solvent. On the other hand, Schiff bases **5a–d** were obtained from **3** and certain aromatic aldehydes. Subsequent cyclization of **5a–d** using acetic anhydride afforded *N*-acetyl-1,3,4-oxadiazoline derivatives **6a–d**. New thiosemicarbazide derivatives **7a–f** were obtained in 69–87% yield from the reaction of **3** with certain isothiocyanates. Further, *p*-toluenesulphonyl chloride/pyridine-mediated cyclization of **7a–f** assembled the target 1,3,4-oxadiazole derivatives **8a–f** in 74–92% yield.

The structures of the newly synthesized compounds were confirmed by elemental analyses and spectral data. ^{1}H NMR spectrum of **1** showed two additional D_2O exchangeable signals at 9.15 and 10.10 ppm corresponding to NH and phenolic OH. ^{1}H NMR spectrum of **2** revealed a triplet and a quartet at 1.32 and 4.32 ppm, respectively, assignable to ethyl group protons. In addition, the spectrum showed absence of carboxylic group hydrogen at the expected chemical shift. On the other hand, ^{1}H NMR spectrum of the corresponding hydrazide **3** displayed four D_2O exchangeable signals; NH_2 signal at 4.56 ppm integrating for two protons, NH and OH signals at 9.05, 9.93, and 10.41 ppm integrating for one proton. Additionally, its ^{1}H NMR and ^{13}C NMR spectra showed absence of ethyl group signals of the ester **2**. Moreover, the presence of amidic carbonyl function was substantiated by IR (1645 cm^{-1}) and ^{13}C NMR (167.47 ppm) data. Analyzing ^{1}H NMR spectra of **4a–c**, **5a–d**, and **7a–f** revealed absence of NH_2 protons. Compounds **4a–c** displayed an additional NH signal in the range of 10.84–10.87 ppm. IR spectra of **4a–c** showed two bands corresponding to SO_2 group at 1157 and 1338–1334 cm^{-1}. ^{13}C NMR spectrum of **4c** showed a signal at 21.43 ppm corresponding to CH_3 carbon and a signal at 167.19 ppm due to carbonyl carbon. On the other hand, ^{1}H NMR spectra of the hydrazone derivatives **5a–d** displayed a singlet signal of the imine hydrogen in the range of 8.35–8.44 ppm. ^{13}C NMR spectrum of **5d** showed two signals at 56.43 and 60.60 ppm corresponding to the three OCH3 ^{1}H NMR spectra of **7a–f** showed two additional signals assignable to two D_2O exchangeable NH hydrogens of thiosemicarbazide moiety carbons and a signal at 164.70 assignable to the carbonyl function. Furthermore, their IR spectra showed a band in the region of 1247–1234 cm^{-1} corresponding to C=S group.

In compounds **6a–d**, IR spectra revealed two carbonyl functions in the range of 1662–1672 and 1705–1751 cm^{-1}. In ^{1}H NMR, acetylation of OH group and ring closure was confirmed by the appearance of two signals corresponding to the two methyl group protons at 2.26–2.27 ppm and 2.32–2.37 ppm. ^{13}C NMR spectrum of **6b** showed three signals at 21.30, 22.05, and 55.75 ppm assignable to two CH_3 carbons and OCH_3 carbon, respectively. A signal corresponding to CH of the 1,3,4-oxadiazoline ring system was observed at 91.57 ppm. Additionally, two signals at 167.24 and 169.90 ppm confirmed the existence of two carbonyl carbons. The structures of 1,3,4-oxadiazole derivatives **8a–f** were confirmed by disappearance of C=O and C=S stretching bands in their IR spectra. Furthermore, in ^{1}H NMR of **8a–f** the D_2O exchangeable signal of NH linked to oxadiazole ring was observed at 10.04–11.08 ppm. Also, their spectra lacked two NH signals of the thiosemicarbazide intermediate as a result of ring closure. ^{13}C NMR spectrum of **8e** showed a signal at 20.80 ppm corresponding to CH_3 carbon with absence of C=O and C=S signals. The protons belonging to the aromatic system and phenyl substituents were observed at the expected chemical shifts and integral values.

Mass spectra of all compounds showed the molecular ion peak. In addition, compounds **4b**, **7b**, **7c**, **7f**, **8b**, **8c**, and **8f** showed the characteristic M+2 peaks confirming presence of Cl or Br. Moreover, mass spectra of the target compounds showed a characteristic fragmentation peak at *m/z* 79.95 corresponding to pyridinium cation. Elemental analyses were within ±0.4% of the theoretical values. The synthetic pathways for the preparation of the new compounds **1–3**,

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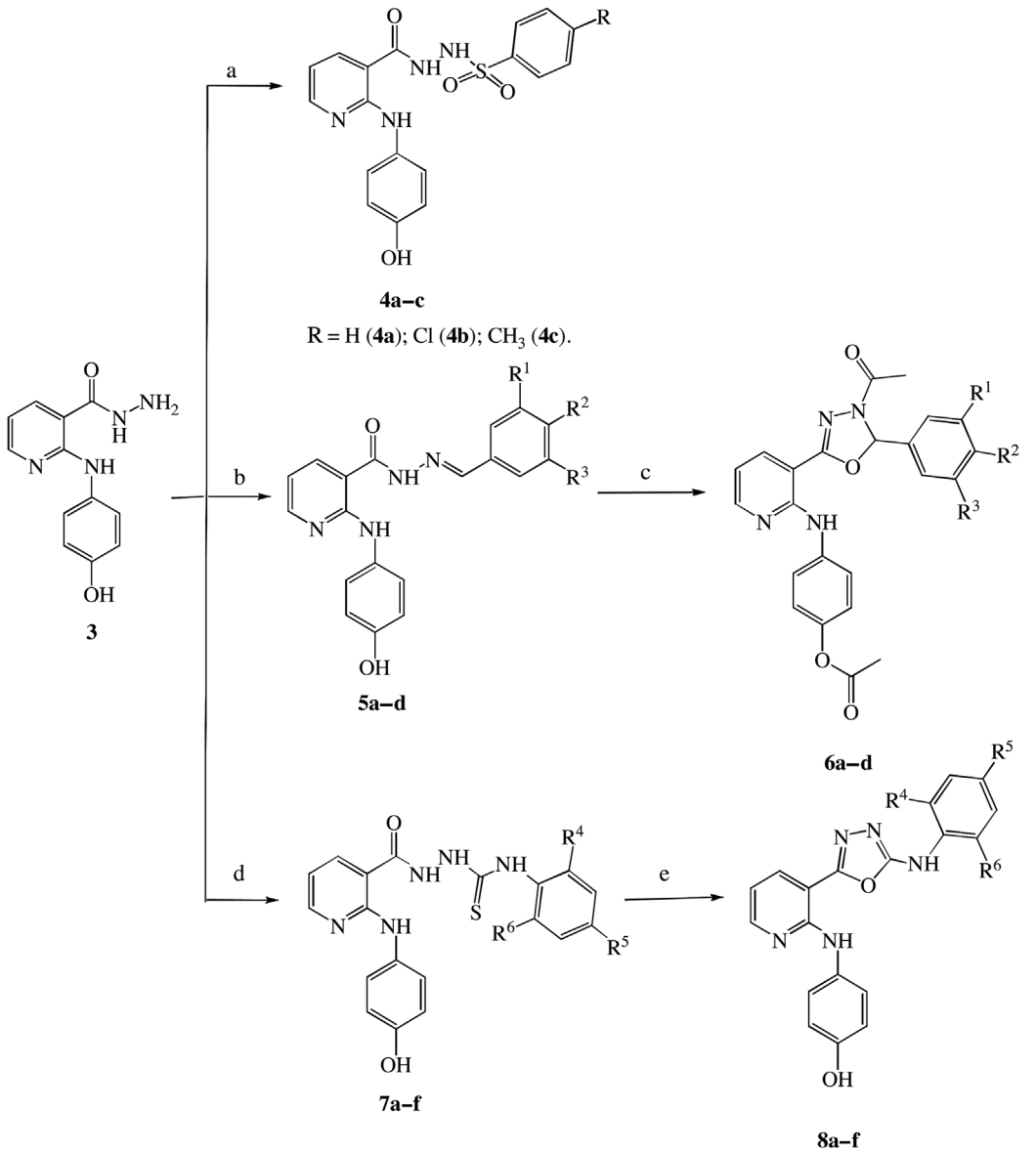

	R^1	R^2	R^3
5a, 6a:	H	H	H
5b, 6b:	H	OCH_3	H
5c, 6c:	OCH_3	OCH_3	H
5d, 6d:	OCH_3	OCH_3	OCH_3

	R^4	R^5	R^6
7a, 8a:	H	H	H
7b, 8b:	H	Br	H
7c, 8c:	H	Cl	H

	R^4	R^5	R^6
7d, 8d:	H	OCH_3	H
7e, 8e:	H	CH_3	H
7f, 8f:	Cl	H	CH_3

Reagents and conditions: a) Arylsulphonyl chloride, pyridine, stirr at RT 5 h; b) aromatic aldehyde, abs. ethanol, reflux 6 h; c) acetic anhydride, reflux 6–8 h; d) substituted phenyl isothiocyanate, abs. ethanol, reflux 6 h; e) *p*-toluenesulphonyl chloride, pyridine, dry THF, reflux 6 h.

Scheme 2. General synthetic pathways of the target compounds **4a–c**, **5a–d**, **6a–d**, **7a–f**, and **8a–f**.

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4a–c, **5a–d**, **6a–d**, **7a–f**, and **8a–f** are outlined in Schemes 1 and 2.

Biological studies

Cytotoxic activity

All the synthesized compounds **4a–c**, **5a–d**, **6a–d**, **7a–f**, and **8a–f** were evaluated for cytotoxic activity against colon cancer cell line HCT-116 and liver cancer cell line HepG-2 using MTT assay. The results were summarized and represented graphically (Table 1 and Fig. 2). Compounds **5d**, **8b**, and **8d** displayed the highest inhibitory activity on both tested cell lines in comparison to the positive control colchicine and doxorubicin.

Analysing data in Table 1 revealed that existence of an additional amide function between the two aromatic rings in ABT-751 analogs **4a–c** resulted in significant decline of cytotoxic activity in both tested cell lines. Interestingly, replacement of the sulphonamide group in **4a–c** with azomethine in compounds **5a–d** significantly improved the activity especially in **5d**. Further, cyclization of **5a–d** to the 1,3,4-oxadiazoline derivatives **6a–d** led to a noteworthy poor activity with the exception of **6d**, which showed moderate cytotoxic city against HCT-116 cell line and good activity against HepG-2 cell line. The improved cytotoxicity in the compounds **5d** and **6d** may be referred to H-bond interaction of the 4-methoxy group of trimethoxyphenyl moiety with βCys241 (Fig. 6), which is an important key amino acid residue at the colchicine binding site [33]. Among the thiosemicarbazide derivatives **7a–f**, which showed moderate cytotoxic activity, compound **7f** was found to be the most potent with IC_{50} of 4.40 and 11.0 μM against HCT-116 and HepG-2 cell lines, respectively. This relatively good activity may be attributed to better hydrophobic interaction of the chloro and methyl substitution on phenyl ring. Cyclization of **7a–f** to the corresponding 1,3,4-oxadiazole derivatives **8a–f** significantly enhanced cytotoxicity especially against HepG-2 cell line. Compound **8d** with 4-methoxy substitution on the phenyl ring showed better activity than colchicine against both cancer cell lines HCT-116 and HepG-2 with $IC_{50} = 0.52$ and 1.40 μM, respectively. Also, cyclization of **7b** including 4-bromo substitution on the phenyl ring to the corresponding oxadiazole **8b** resulted in threefold increase in cytotoxicity with $IC_{50} = 5.53$ μM against HCT-116 cell line and about eightfold increase in cytotoxicity with $IC_{50} = 1.49$ μM against HepG-2 cell line. Similarly, the oxadiazole derivative **8c** was found to be about fourfold more active than the corresponding thiosemicarbazide **7c** with $IC_{50} = 7.70$ μM against HCT-116 cell line, also it showed about eightfold increase in cytotoxicity against HepG-2 cell line with $IC_{50} = 2.17$ μM.

Table 1. **IC_{50} values in μM of compounds 4a–c, 5a–d, 6a–d, 7a–f, and 8a–f against HCT-116 and HepG-2 cell lines using MTT assay.**

Compound number	IC_{50} (μM) ± SE	
	HCT-116	HepG-2
4a	>100	>100
4b	86.90 ± 4.50	62.11 ± 2.80
4c	>100	90.55 ± 8.30
5a	>100	NT
5b	7.85 ± 0.75	20.8 ± 1.27
5c	92 ± 4.20	43 ± 7.30
5d	5.80 ± 0.35	2.22 ± 0.04
6a	>100	NT
6b	>100	>100
6c	>100	>100
6d	20.41 ± 2.20	3.90 ± 0.27
7a	8.90 ± 0.36	24.75 ± 1.01
7b	15.14 ± 1.20	11.48 ± 0.65
7c	33.88 ± 0.45	17.37 ± 1.16
7d	5.34 ± 0.23	19.76 ± 1.50
7e	9.50 ± 0.33	24.76 ± 2.54
7f	4.40 ± 0.85	11 ± 0.30
8a	12.60 ± 0.41	11.65 ± 0.60
8b	5.53 ± 0.088	1.49 ± 0.10
8c	7.70 ± 0.63	2.17 ± 0.065
8d	0.52 ± 0.008	1.40 ± 0.004
8e	26.41 ± 1.07	1.58 ± 0.12
8f	13.90 ± 0.35	9.80 ± 0.66
Colchicine	3.40 ± 0.001	5.10 ± 0.001
Doxorubicin	0.26 ± 0.004	1.14 ± 0.115

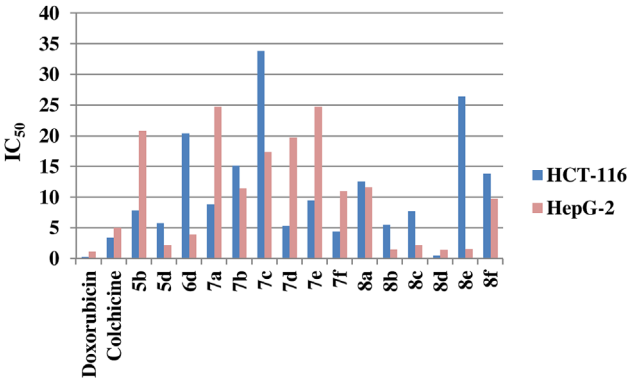

Figure 2. Cytotoxic activity of the target compounds **5b**, **5d**, **6d**, **7a–f**, **8a–f**, doxorubicin, and colchicine against HCT-116 and HepG-2 cell lines.

Tubulin polymerization assay

The inhibition of tubulin polymerization is one of the goals for chemotherapy. In this context, *in vitro* tubulin inhibitory activity was evaluated for the compounds that showed the highest cytotoxic activity against HCT-116 and HepG-2 cell lines (**5d**, **8b**, and **8d**). The inhibitory activity of tubulin was given as the percentage inhibition at IC_{50} concentration of each compound and the results were presented in Table 2. It was obvious that all tested compounds showed substantial activity in suppressing

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Table 2. Percentage inhibition of tubulin polymerization at IC_{50} for compounds 5d, 8b, and 8d.

Compound no.	IC_{50} (μM) HCT-116	% Inhibition of tubulin polymerization (HCT-116)	IC_{50} (μM) HepG-2	% Inhibition of tubulin polymerization (HepG-2)
5d	5.80 ± 0.35	86.10 ± 0.002	2.22 ± 0.04	93.30 ± 0.002
8b	5.53 ± 0.088	86.40 ± 0.004	1.49 ± 0.10	91.70 ± 0.002
8d	0.52 ± 0.008	92.60 ± 0.002	1.40 ± 0.004	93.20 ± 0.002
Colchicine	3.40 ± 0.001	82.70 ± 0.002	5.10 ± 0.001	85.70 ± 0.003

tubulin polymerization with percentage inhibition ranging from (86.10–92.60%) and (91.70–93.30%) against HCT-116 and HepG-2 cells, respectively, in comparison with the reference antimitotic agent colchicine (Table 2). The results indicated that the mechanism of the most active compounds, **5d**, **8b**, and **8d**, might be correlated to their tubulin inhibitory activity.

Effect of representative target compounds on normal human colon cells and liver cells

One of the main problems of cancer chemotherapy is the unwanted damage to normal cells caused by the high toxicities of anticancer drugs. Two of the most active target compounds against HCT-116 and HepG-2 cancer cell lines that caused the highest percentage inhibition of tubulin polymerization **8b** and **8d** were selected to be tested for cytotoxicity against normal colon cell line (CCD-33Co) and normal liver cell line (THLE-2) and their IC_{50} values were determined via MTT assay. The IC_{50} values of the representative target compounds against normal colon and liver cells were very high in comparison to their IC_{50} doses against the cancer cell lines (Table 3).

Molecular modeling

Docking simulation was performed to predict the probable binding mode of the most active compounds **8b**, **8d**, and **5d** with the crystal structure of tubulin at the colchicine binding site (PDB ID: 3HKC) using Discovery Studio Software version 2.55. The co-crystallized ligand revealed H-bond interaction of OH group of the 4-hydroxyaniline moiety with βTyr202. Additionally, the pyridine ring nitrogen showed binding with βCys241. The 2D interactions of ABT-751 with tubulin at the colchicine binding site is illustrated in Fig. 3.

Compound **8d** was capable of occupying the colchicine binding site while maintaining the essential key interaction of the 4-hydroxyl group of the anilino moiety with the amino acid residue βTyr202 (distance = 2.57 Å), and a H-bond interaction between pyridine ring nitrogen and SH of βCys241 amino residue (distance = 2.25 Å) as well, being comparable to ABT-751 binding mode. An additional H-bond interaction of OH group with NH_2 of βAsn167 was observed (distance = 2.74 Å) with an estimated binding energy score of −11.34 kcal/mol. The 2D interactions of **8d** with tubulin at the colchicine binding site was illustrated in Fig. 4. Similarly, compound **8b** showed binding mode analogous to **8d** with the same H-bond interactions and an estimated binding energy score = −11.28 kcal/mol (Fig. 5).

On the other hand, compound **5d** showed a disparate orientation within the colchicine binding site, thereby producing different H-bond interactions compared to ABT-751 (Fig. 6). The 4-methoxy group of trimethoxyphenyl moiety showed binding to SH of βCys241, an important key amino acid residue in the colchicine binding site in a manner similar to colchicine binding mode (distance = 2.74 Å). Also, OH group of the 4-hydroxyanilino moiety displayed strong H-bond with the C=O of βAsn350 (distance = 1.94 Å). In addition, the anilino moiety was involved in π–cation interaction with βLys352, with an estimated binding energy score of −9.53 kcal/mol.

The most active compounds in the cytotoxicity screen, **5d**, **8b**, and **8d** showed good fitting in the colchicine binding site, producing good interactions with H-bond length of less than 3 Å and binding free energy scores of −9.53 to −11.34 kcal/mol in comparison to ABT-751 (−13.72 kcal/mol).

Table 3. Effect of selected target compounds 8b and 8d on normal human colon cells (CCD-33Co) and liver cells (THLE-2).

Compound no.	IC_{50} (μM) HCT-116	IC_{50} (μM) CCD-33Co	IC_{50} (μM) HepG-2	IC_{50} (μM) THLE-2
8b	5.53 ± 0.088	>100	1.49 ± 0.10	>100
8d	0.52 ± 0.008	52.66 ± 0.001	1.40 ± 0.004	>100

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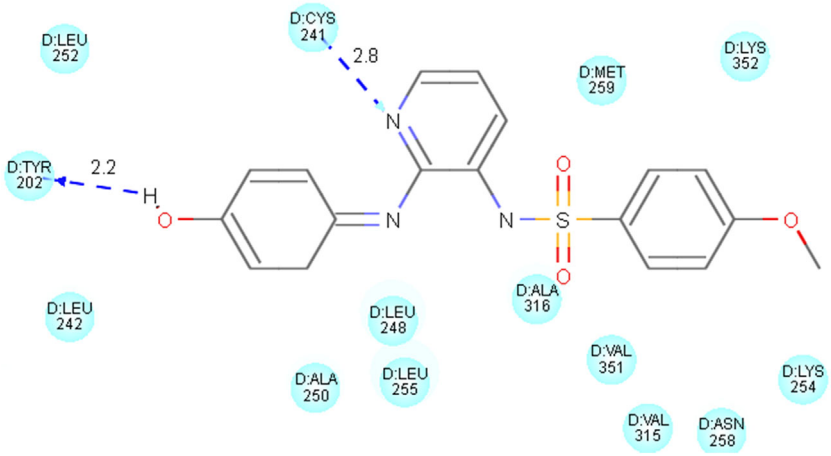

Figure 3. 2D interaction of ABT-751 with tubulin at the colchicine binding site (3HKC).

Conclusions

Modification of prior leads ABT-751, A-105972, and IMC-038525 resulted in the synthesis and biological evaluation of 23 new compounds (series **4–8**). During this process, the active compound **8d** showed high cytotoxic activity against HCT-116 and HepG-2 cell lines with IC_{50} = 0.52 and 1.40 μM, respectively, more potent than colchicine in the same assay. Compound **8d** also displayed significant tubulin inhibitory activity; 92.60% at 0.52 μM against HCT-116 cells and 93.20% at 1.40 μM against HepG-2 cells. With the aim to rationalize the biological results, molecular docking was further performed. After analysis of the binding modes of the most active compounds **5d**, **8b**, and **8d** with the colchicine site of tubulin and comparing the results with that of the co-crystallized ligand ABT-751, it was obvious that these compounds could effectively bind to the colchicine binding site of α- and β-tubulin through H-bond interactions with βTyr202 and/or βCys241, which may play a crucial role in its antitubulin polymerization and cytotoxic activities.

Experimental

Chemistry

General

All chemicals and reagents were obtained from Aldrich and were used without further purification. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254. The solvent system was benzene, chloroform, and methanol (5:9:1) and spots were visualized using UV lamp. IR spectra were determined on Shimadzu IR 8400s spectrophotometer (KBr, cm^{-1}). ^{1}H NMR spectra were carried out using a Mercury 300-BB 300 MHz and Bruker 400 MHz usig tetramethylsilane (TMS) as internal standard. ^{13}C NMR spectra were carried out using a Mercury 300-BB 75 MHz and Bruker 100 MHz using TMS as internal standard. Chemical shifts (δ) are recorded in ppm on δ scale, Microanalytical Unit, Faculty of Pharmacy, Cairo University, Egypt. Mass spectra were performed on Shimadzu QP-2010 plus mass spectrophotometer at 70 eV. Elemental analyses were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt. Melting points

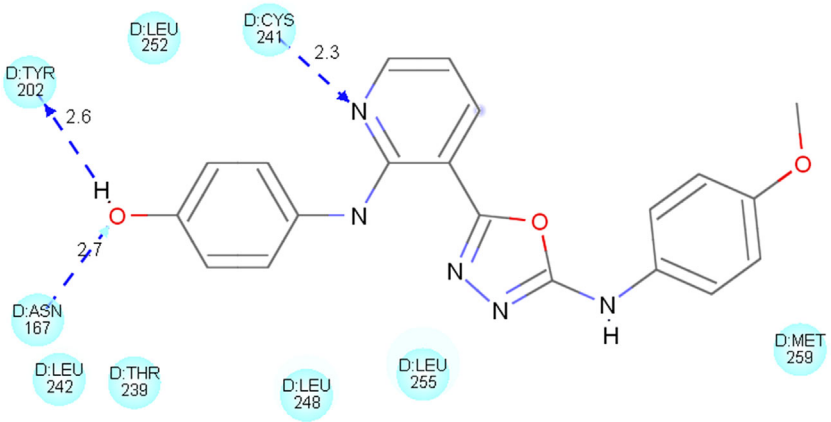

Figure 4. 2D interaction of **8d** with tubulin at the colchicine binding site (3HKC).

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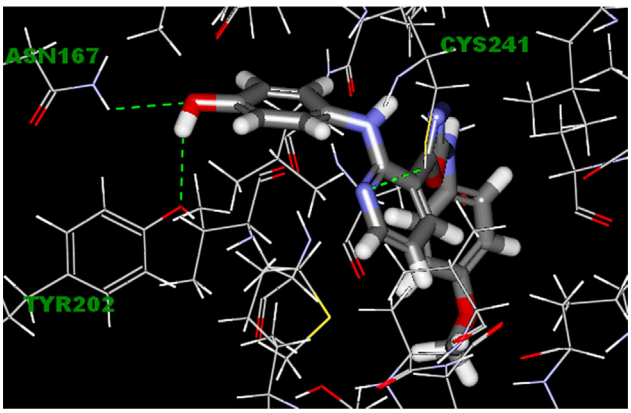

Figure 5. 3D interaction of **8d** with tubulin at the colchicine binding site (3HKC). H-bond interactions are represented as green-dashed lines.

were determined on Stuart apparatus and the values given are uncorrected. The microwave synthesis of **I** was performed using Sineo Microwave Chemistry Technology Co., Ltd. MAS-II plus apparatus.

The NMR spectra as well as the InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

*2-[(4-Hydroxyphenyl)amino]nicotinic acid (**1**)*

Route 1: A mixture of 2-chloronicotinic acid (3.83 g, 24.3 mmol), *p*-aminophenol (2.65 g, 24.3 mmol), pyridine (1.92 g, 24.3 mmol) and *p*-toluenesulphonic acid (1.0 g, 5.8 mmol) in H_2O (40 mL) was heated under reflux for 12 h. After cooling, the precipitated solid product was filtered and crystallized from ethanol. Yield: 72%; m.p.: 245–246°C; IR (KBr) cm^{-1}: 3446–2503 (OH and NH), 1654 (C=O), 1616 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 6.72–6.77 (m, 3H, 2ArH and H_5 pyridine), 7.41 (d, 2H, J = 8.76 Hz, ArH), 8.17 (dd, 1H, J_{H4H5} = 7.68 Hz, J_{H4H6} = 1.76 Hz, H_4 pyridine), 8.28 (dd, 1H, J_{H5H6} = 4.60 Hz, J_{H4H6} = 1.76 Hz, H_6 pyridine), 9.15, 10.10 (2s, 2H, NH and OH, D_2O exchangeable), 13.39 (s, 1H, OH acidic, D_2O exchangeable); EIMS *m/z* (% rel. int.): 231.05 (M+H, 14.43), 230.05 ($M^{\neg\dagger}$, 100), 229.05 (M−H, 37.03), 79.95 ($C_5H_6N^{\neg+}$, 21.80). Anal. calcd. for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17. Found: C, 62.84; H, 4.46; N, 12.39.

Route 2: The same procedure was applied under microwave irradiation for 2.5 h at 100°C and the power was adjusted at 600 W. Reaction completion was monitored by TLC. The reaction mixture was cooled and the precipitated solid was filtered and crystallized from ethanol. Yield: 80%; m.p.: 245–246°C.

*Ethyl 2-[(4-hydroxyphenyl)amino]nicotinate (**2**)*

To a solution of **1** (2 g, 8.69 mmol) in absolute ethanol (30 mL), conc. H_2SO_4 (2 mL) was added, and the reaction mixture was heated under reflux for 8 h. The solvent was distilled off under reduced pressure, the residue was cooled and washed with an aqueous sodium carbonate solution (20 mL, 10% w/v). The separated solid was filtered, dried, and crystallized from ethanol/H_2O. Yield: 80%; m.p.: 160–161°C; IR (KBr) cm^{-1}: 3398, 3323 (NH and OH), 3084 (CH aromatic), 2978, 2924 (CH aliphatic), 1666 (C=O), 1608 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.32 (t, 3H, J = 7.08 Hz, CH_3), 4.32 (q, 2H, J = 7.08 Hz, CH_2), 6.72 (d, 2H, J = 8.80 Hz, ArH), 6.77 (dd, 1H, J_{H4H5} = 7.76 Hz, J_{H5H6} = 4.72 Hz, H_5 pyridine), 7.40 (d, 2H, J = 8.80 Hz, ArH), 8.19 (dd, 1H, J_{H4H5} = 7.76 Hz, J_{H4H6} = 1.80 Hz, H_4 pyridine), 8.31 (dd, 1H, J_{H5H6} = 4.72 Hz, J_{H4H6} = 1.80 Hz, H_6 pyridine), 9.24, 9.82 (2s, 2H, NH and OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) ppm: 14.52 (CH_3), 61.55 (OCH_2), 106.50, 113.47, 115.63, 123.22, 131.42, 140.49, 153.60, 153.73, 156.24 (Ar C′s), 167.41 (C=O); EIMS *m/z* (% rel. int.): 258.05 ($M^{\neg\dagger}$, 6.47), 79.90 ($C_5H_6N^{\neg+}$,

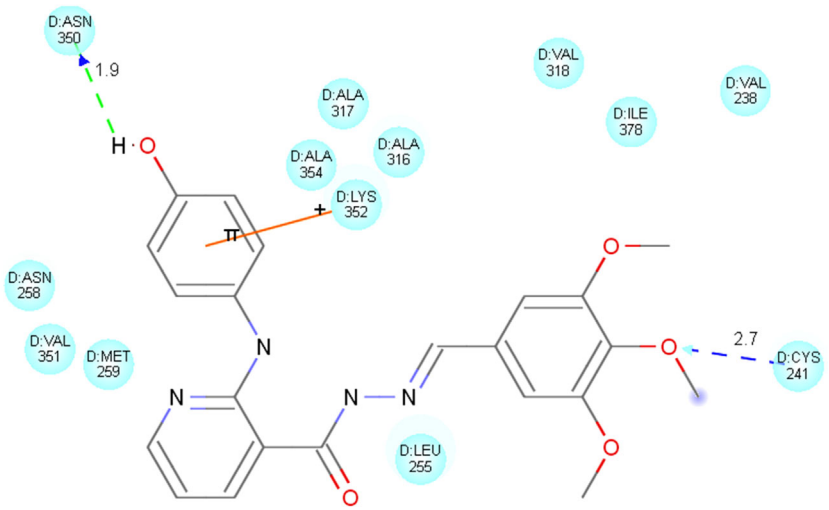

Figure 6. 2D interaction of **5d** with tubulin at the colchicine binding site (3HKC).

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100). Anal. calcd. for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85. Found: C, 65.34; H, 5.52; N, 11.03.

*2-[(4-Hydroxyphenyl)amino]nicotinohydrazide (**3**)*
In an ice bath, hydrazine hydrate (99%, 1.0 g, 20 mmol) was added dropwise while stirring to the compound **2** (1 g, 3.87 mmol), then the stirring was continued at room temperature for an additional 5 h. The reaction mixture was poured onto ice-cold water, the formed precipitate was filtered and crystallized from ethanol. Yield: 74%; m.p.: 250–251°C; IR (KBr) cm^{-1}: 3319, 3286 (NH_2), 3196, 3130 (NH and OH), 3022 (CH aromatic), 1645 (CO), 1622 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 4.56 (s, 2H, NH_2, D_2O exchangeable), 6.70–6.73 (m, 3H, 2ArH and H_5 pyridine), 7.39 (d, 2H, $J = 8.84$ Hz, ArH), 7.94 (dd, 1H, $J_{H4H5} = 7.64$ Hz, $J_{H4H6} = 1.76$ Hz, H_4 pyridine), 8.19 (dd, 1H, $J_{H5H6} = 4.76$ Hz, $J_{H4H6} = 1.76$ Hz, H_6 pyridine), 9.05, 9.93, 10.41 (3s, 3H, 2NH and OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) ppm: 109.93, 112.89, 115.64, 122.10, 132.26, 136.55, 150.96, 153.02, 155.22 (Ar C's), 167.47 (C=O); EIMS *m/z* (% rel. int.): 244.00 ($M^{\neg\dagger}$, 2.59), 79.90 ($C_5H_6N^{\neg+}$, 100). Anal. calcd. for $C_{12}H_{12}N_4O_2$: C, 59.01; H, 4.95; N, 22.94. Found: C, 59.17; H, 5.02; N, 23.15.

*General procedure for the preparation of **4a–c***
An equimolar mixture of **3** and the appropriate arylsulphonyl chloride (1 mmol each) in anhydrous pyridine (5 mL) was stirred at room temperature for 5 h, then poured onto ice-cold water. The formed precipitate was filtered, dried, and crystallized from ethanol.

*N′-[2-(4-Hydroxyphenylamino)nicotinoyl]-benzenesulphonohydrazide (**4a**)*
Yield: 64%; m.p.: 190–191°C; IR (KBr) cm^{-1}: 3396–3174 (NH and OH), 3062 (CH aromatic), 1656 (C=O), 1614 (C=N), 1338, 1157 (SO_2); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.68–6.80 (m, 3H, 2ArH and H_5 pyridine), 7.23 (d, 2H, $J = 8.70$ Hz, ArH), 7.51–7.64 (m, 3H, ArH), 7.84 (d, 2H, $J = 7.20$ Hz, ArH), 7.94 (dd, 1H, $J_{H4H5} = 7.80$ Hz, $J_{H4H6} = 1.80$ Hz, H_4 pyridine), 8.19 (dd, 1H, $J_{H5H6} = 4.80$ Hz, $J_{H4H6} = 1.80$ Hz, H_6 pyridine), 9.14, 9.43, 10.05, 10.84 (4s, 4H, 3NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 384.15 ($M^{\neg\dagger}$, 8.70), 79.95 ($C_5H_6N^{\neg+}$, 67.10), 77.00 ($C_6H_5^{\neg+}$, 100). Anal. calcd. for $C_{18}H_{16}N_4O_4S$: C, 56.24; H, 4.20; N, 14.57. Found: C, 56.37; H, 4.24; N, 14.73.

*4-Chloro-N′-[2-(4-hydroxyphenylamino)nicotinoyl]-benzenesulfonohydrazide (**4b**)*
Yield: 70%; m.p.: 226–227°C; IR (KBr) cm^{-1}: 3456–3165 (NH and OH), 3091 (CH aromatic), 1662 (C=O), 1600 (C=N), 1338, 1157 (SO_2); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.68–6.75 (m, 3H, 2ArH and H_5 pyridine), 7.24 (d, 2H, $J = 8.70$ Hz, ArH), 7.62 (d, 2H, $J = 8.40$ Hz, ArH), 7.84 (d, 2H, $J = 8.40$ Hz, ArH), 7.95 (d, 1H, $J = 7.20$ Hz, H_4 pyridine), 8.21 (d, 1H, $J = 3.30$ Hz, H_6 pyridine), 9.09, 9.44, 10.20, 10.87 (4s, 4H, 3NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 420.10 (M+2, 0.09), 418.10 ($M^{\neg\dagger}$, 0.12), 79.95 ($C_5H_6N^{\neg+}$, 100). Anal. calcd. for $C_{18}H_{15}ClN_4O_4S$: C, 51.62; H, 3.61; N, 13.38. Found: C, 51.74; H, 3.67; N, 13.51.

*N′-[2-(4-Hydroxyphenylamino)nicotinoyl]-4-methylbenzenesulfonohydrazide (**4c**)*
Yield: 74%; m.p.: 211–212°C; IR (KBr) cm^{-1}: 3500–3201 (NH and OH), 3039 (CH aromatic), 2958, 2850 (CH aliphatic), 1647 (C=O), 1608 (C=N), 1334, 1157 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.28 (s, 3H, CH_3), 6.69–6.71 (m, 3H, 2ArH and H_5 pyridine), 7.23 (d, 2H, $J = 7.84$ Hz, ArH), 7.32 (d, 2H, $J = 7.36$ Hz, ArH), 7.71 (d, 2H, $J = 7.44$ Hz, ArH), 7.93 (d, 1H, $J = 7.08$ Hz, H_4 pyridine), 8.20 (d, 1H, $J = 5.28$ Hz, H_6 pyridine), 9.12, 9.35, 10.01, 10.86 (4s, 4H, 3NH and OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) ppm: 21.43 (CH_3), 108.76, 112.92, 115.64, 122.30, 128.33, 129.77, 131.73, 136.34, 137.42, 143.91, 151.91, 153.26, 155.00 (Ar C's), 167.19 (C=O); EIMS *m/z* (% rel. int.): 398.00 ($M^{\neg\dagger}$, 3.30), 213.00 ($C_{12}H_9N_2O_2^{\neg+}$, 36.65), 92.00 ($C_7H_8^{\neg+}$, 41.53), 91.00 ($C_7H_7^{\neg+}$, 100), 79.90 ($C_5H_6N^{\neg+}$, 80.70). Anal. calcd. for $C_{19}H_{18}N_4O_4S$: C, 57.27; H, 4.55; N, 14.06. Found: C, 57.49; H, 4.62; N, 14.19.

*General procedure for the preparation of **5a–d***
A solution of equimolar amounts of the hydrazide **3** (0.25 g, 1.02 mmol) and the appropriate aromatic aldehyde (1.02 mmol) in absolute ethanol (15 mL) was heated under reflux for 6 h, cooled, then filtered. The formed precipitate was dried and recrystallized from ethanol.

*(E)-N′-Benzylidene-2-(4-hydroxyphenylamino)-nicotinohydrazide (**5a**)*
Yield: 71%; m.p.: 242–243°C; IR (KBr) cm^{-1}: 3331–3147 (NH and OH), 3064 (CH aromatic), 1645 (C=O), 1614 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.72 (d, 2H, $J = 8.70$ Hz, ArH), 6.78 (dd, 1H, $J_{H4H5} = 7.80$ Hz, $J_{H5H6} = 4.80$ Hz, H_5 pyridine), 7.43–7.47 (m, 5H, ArH), 7.73 (d, 2H, ArH), 8.12 (d, 1H, $J = 7.80$ Hz, H_4 pyridine), 8.26 (dd, 1H, $J_{H5H6} = 4.80$ Hz, $J_{H4H6} = 1.20$ Hz, H_6 pyridine), 8.44 (s, 1H, N=CH), 9.08, 10.17, 11.95 (3s, 3H, 2NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 333.10 (M+H, 16.29), 332.05 ($M^{\neg\dagger}$, 67.56), 213.00 ($C_{12}H_9N_2O_2^{\neg+}$, 100), 76.95($C_6H_5^{\neg+}$, 36.45). Anal. calcd. for $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85; N, 16.86. Found: C, 68.90; H, 4.92; N, 17.04.

*(E)-2-(4-Hydroxyphenylamino)-N′-(4-methoxybenzylidene)nicotinohydrazide (**5b**)*
Yield: 86%; m.p.: 230–231°C; IR (KBr) cm^{-1}: 3319–3219 (NH and OH), 3084 (CH aromatic), 2960, 2850 (CH aliphatic), 1635 (C=O), 1598 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.82 (s, 3H, OCH_3), 6.70 (d, 2H, $J = 8.70$ Hz, ArH), 6.78 (dd, 1H, $J_{H4H5} = 7.50$ Hz, $J_{H5H6} = 4.80$ Hz, H_5 pyridine), 7.02 (d, 2H, $J = 8.40$ Hz, ArH), 7.41 (d, 2H, $J = 8.70$ Hz, ArH), 7.67 (d, 2H, $J = 8.40$ Hz, ArH), 8.09 (d, 1H, $J = 7.50$ Hz, H_4 pyridine), 8.25 (d, 1H, $J = 4.80$ Hz, H_6 pyridine), 8.37 (s, 1H, N=CH), 9.07, 10.17, 11.82 (3s, 3H, 2NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 363.15 (M+H, 12.45), 362.15 ($M^{\neg\dagger}$, 53.02), 213.00 ($C_{12}H_9N_2O_2^{\neg+}$, 100), 79.95 ($C_5H_6N^{\neg+}$, 39.65). Anal. calcd. for

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$C_{20}H_{18}N_4O_3$: C, 66.29; H, 5.01; N, 15.46. Found: C, 66.51; H, 5.09; N, 15.60.

*(E)-N′-(3,4-Dimethoxybenzylidene)-2-(4-hydroxyphenylamino)nicotinohydrazide (**5c**)*
Yield: 87%; m.p.: 245–246°C; IR (KBr) cm^{-1}: 3448–3180 (NH and OH), 3028 (CH aromatic), 2970, 2854 (CH aliphatic), 1626 (C=O), 1602 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.78 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.71 (d, 2H, J = 8.70 Hz, ArH), 6.78 (dd, 1H, J_{H4H5} = 7.20 Hz, J_{H5H6} = 5.10 Hz, H_5 pyridine), 7.02 (d, 1H, J = 8.40 Hz, ArH), 7.20 (d, 1H, J = 8.40 Hz, ArH), 7.35 (s, 1H, ArH), 7.42 (d, 2H, J = 8.70 Hz, ArH), 8.09 (d, 1H, J = 7.20 Hz, H_4 pyridine), 8.25 (d, 1H, J = 5.10 Hz, H_6 pyridine), 8.35 (s, 1H, NCH), 9.07, 10.17, 11.84 (3s, 3H, 2NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 393.15 (M+H, 12.38), 392.15 ($M^{\neg\ddagger}$, 52.96), 213.00 ($C_{12}H_9N_2O_2^{\neg+}$, 100), 79.95 ($C_5H_6N^{\neg+}$, 44.10). Anal. calcd. for $C_{21}H_{20}N_4O_4$: C, 64.28; H, 5.14; N, 14.28. Found: C, 64.52; H, 5.23; N, 14.35.

*(E)-2-(4-Hydroxyphenylamino)-N′-(3,4,5-trimethoxybenzylidene)nicotinohydrazide (**5d**)*
Yield: 90%; m.p.: 248–250°C; IR (KBr) cm^{-1}: 3331–3172 (NH and OH), 3064 (CH aromatic), 2939, 2854 (CH aliphatic), 1631 (C=O), 1581 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.72 (s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 6.71 (d, 2H, J = 8.72 Hz, ArH), 6.80 (dd, 1H, J_{H4H5} = 7.68 Hz, J_{H5H6} = 4.84 Hz, H_5 pyridine), 7.05 (s, 2H, ArH), 7.43 (d, 2H, J = 8.72 Hz, ArH), 8.10 (d, 1H, J = 7.68 Hz, H_4 pyridine), 8.26 (d, 1H, J = 4.80 Hz, H_6 pyridine), 8.35 (s, 1H, N=CH), 9.12, 10.13, 11.97 (3s, 3H, 2NH and OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) ppm: 56.43, 60.60 ($3OCH_3$), 104.83, 109.97, 112.84, 115.59, 122.44, 130.14, 132.09, 137.62, 139.81, 148.66, 151.65, 153.18, 153.68, 155.58 (Ar C′s), 164.70 (C=O); EIMS *m/z* (% rel. int.): 423.20 (M+H, 10.75), 422.25 ($M^{\neg\ddagger}$, 43.90), 213.05 ($C_{12}H_9N_2O_2^{\neg+}$, 100), 79.95 ($C_5H_6^{\neg+}$, 28.74). Anal. calcd. for $C_{22}H_{22}N_4O_5$: C, 62.55; H, 5.25; N, 13.26. Found: C, 62.72; H, 5.34; N, 13.54.

*General procedure for the preparation of **6a–d***
A mixture of an appropriate Schiff's base **5a–d** (1 mmol) and acetic anhydride (5 mL) was heated under reflux for 6–8 h. After cooling, the reaction mixture was poured onto ice-cold water, then triturated with conc. ammonium hydroxide solution. The precipitated solid product was filtered and crystallized from ethanol/H_2O.

*4-[3-(4-Acetyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-pyridin-2-ylamino]phenyl acetate (**6a**)*
Yield: 61%; m.p.: 118–119°C; IR (KBr) cm^{-1}: 3327 (NH), 3084 (CH aromatic), 2950, 2850 (CH aliphatic), 1749, 1668 (CO), 1622 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.27 (s, 3H, $NCOCH_3$), 2.35 (s, 3H, $OCOCH_3$), 6.14–8.35 (m, 13H, ArH, pyridine Hs and CH oxadiazoline), 9.67 (s, 1H, NH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 416.20 ($M^{\neg\ddagger}$, 0.12), 79.95 ($C_5H_6N^{\neg+}$, 100), 63.95 ($C_5H_4^{\neg+}$, 85.41). Anal. calcd. for $C_{23}H_{20}N_4O_4$: C, 66.34; H, 4.84; N, 13.45. Found: C, 66.52; H, 4.93; N, 13.62.

*4-{3-[4-Acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]pyridin-2-ylamino}phenyl acetate (**6b**)*
Yield: 73%; m.p.: 130–131°C; IR (KBr) cm^{-1}: 3419 (NH), 3084 (CH aromatic), 2926, 2830 (CH aliphatic), 1751, 1672 (C=O), 1614 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.27 (s, 3H, $NCOCH_3$), 2.37 (s, 3H, $OCOCH_3$), 3.77 (s, 3H, OCH_3), 6.92–6.95 (m, 1H, H_5 pyridine), 6.97 (d, 2H, J = 8.70 Hz, ArH), 7.11–7.15 (m, 3H, 2ArH and CH oxadiazoline), 7.44 (d, 2H, J = 8.40 Hz, ArH), 7.77 (d, 2H, J = 9 Hz, ArH), 7.98 (d, 1H, J = 6 Hz, H_4 pyridine), 8.35 (dd, 1H, J_{H5H6} = 4.80 Hz, J_{H4H6} = 2.10 Hz, H_6 pyridine), 9.56 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) ppm: 21.30, 22.05 ($2CH_3$), 55.75 (OCH_3), 91.57 (CH oxadiazoline), 114.51, 114.62, 114.82, 121.28, 122.57, 128.76, 128.88, 137.47, 137.57, 145.92, 151.16, 153.54, 159.50, 160.95 (Ar C′s), 167.24, 169.90 (2CO); EIMS *m/z* (% rel. int.): 446.10 ($M^{\neg\ddagger}$, 0.31), 79.95 ($C_5H_6N^{\neg+}$, 63.56), 63.95 (C_5H_4, 100). Anal. calcd. for $C_{24}H_{22}N_4O_5$: C, 64.57; H, 4.97; N, 12.55. Found: C, 64.71; H, 4.95; N, 12.79.

*4-{3-[4-Acetyl-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]pyridin-2-ylamino}phenyl acetate (**6c**)*
Yield: 75%; m.p.: 122–123°C; IR (KBr) cm^{-1}: 3329 (NH), 3074 (CH aromatic), 2931, 2835 (CH aliphatic), 1705, 1670 (C=O), 1593 (CN); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.26 (s, 3H, $NCOCH_3$), 2.32 (s, 3H, $OCOCH_3$), 3.71 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 7.01–8.35 (m, 11H, ArH, pyridine Hs and CH oxadiazoline), 9.92 (s, 1H, NH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 475.20 (M−H, 0.28), 79.95 ($C_5H_6N^{\neg+}$, 43.65), 63.95 ($C_5H_4^{\neg+}$, 100). Anal. calcd. for $C_{25}H_{24}N_4O_6$: C, 63.02; H, 5.08; N, 11.76. Found: C, 63.18; H, 5.14; N, 11.93.

*4-{3-[4-Acetyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl]pyridin-2-ylamino}phenyl acetate (**6d**)*
Yield: 77%; m.p.: 180–181°C; IR (KBr) cm^{-1}: 3329 (NH), 3068 (CH aromatic), 2933, 2848 (CH aliphatic), 1749, 1662 (C=O), 1595 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.27 (s, 3H, $NCOCH_3$), 2.33 (s, 3H, $OCOCH_3$), 3.71 (s, 3H, OCH_3), 3.78 (s, 6H, $2OCH_3$), 6.78–7.80 (m, 8H, ArH, H_5 pyridine and CH oxadiazoline), 7.99 (d, 1H, J = 7.04 Hz, H_4 pyridine), 8.36 (d, 1H, J = 3.50 Hz, H_6 pyridine), 9.56 (s, 1H, NH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 506.00 ($M^{\neg\ddagger}$, 0.29), 212.95 ($C_{12}H_9N_2O_2^{\neg+}$, 60.14), 79.95 ($C_5H_6N^{\neg+}$, 22.74), 63.90 ($C_5H_4^{\neg+}$, 36.03). Anal. calcd. for $C_{26}H_{26}N_4O_7$: C, 61.65; H, 5.17; N, 11.06. Found: C, 61.81; H, 5.23; N, 11.14.

*General procedure for the preparation of **7a–f***
A mixture of equimolar amounts of the hydrazide **3** and the appropriate phenyl isothiocyanate (1 mmol each) in absolute ethanol (15 mL) was heated under reflux for 6 h then cooled. The precipitated crystalline solid was filtered and recrystallized from ethanol.

*2-[2-(4-Hydroxyphenylamino)nicotinoyl]-N-phenylhydrazinecarbothioamide (**7a**)*
Yield: 77%; m.p.: 165–166°C; IR (KBr) cm^{-1}: 3323–3167 (NH and OH), 3084 (CH aromatic), 1639 (C=O), 1602 (C=N), 1236

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(C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.70–6.79 (m, 3H, 2ArH and H_5 pyridine), 7.14 (t, 1H, J = 7.20 Hz, ArH), 7.31–7.43 (m, 6H, ArH), 8.16 (d, 1H, J = 7.50 Hz, H_4 pyridine), 8.26 (d, 1H, J = 4.50 Hz, H_6 pyridine), 9.08, 9.68, 9.86, 10.27, 10.68 (5s, 5H, 4NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 379.20 ($M^{\neg\ddagger}$, 0.15), 244.15 ($C_{12}H_{12}N_4O_2^{\neg+}$, 46.83), 213.10 ($C_{12}H_9N_2O_2^{\neg+}$, 100), 79.95 ($C_5H_6N$, 30.10). Anal. calcd. for $C_{19}H_{17}N_5O_2S$: C, 60.14; H, 4.52; N, 18.46. Found: C, 60.31; H, 4.63; N, 18.72.

*N-(4-Bromophenyl)-2-[2-(4-hydroxyphenylamino)-nicotinoyl]hydrazinecarbothioamide (**7b**)*
Yield: 79%; m.p.: 200–201°C; IR (KBr) cm^{-1}: 3286–3174 (NH and OH), 3082 (CH aromatic), 1633 (C=O), 1610 (C=N), 1236 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.70 (d, 2H, J = 8.70 Hz, ArH), 6.76 (dd, 1H, J_{H4H5} = 6.90 Hz, J_{H5H6} = 5.10 Hz, H_5 pyridine), 7.40–7.53 (m, 6H, ArH), 8.15 (d, 1H, J = 7 Hz, H_4 pyridine), 8.26 (d, 1H, J = 5.10 Hz, H_6 pyridine), 9.08, 9.80, 9.90, 10.27, 10.69 (5s, 5H, 4NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 458.90 (M+2, 0.38), 456.90 ($M^{\neg\ddagger}$, 0.27), 213.00 ($C_{12}H_9N_2O_2^{\neg+}$, 29.19), 79.95 ($C_5H_6N^{\neg+}$, 66.78), 63.95 ($C_5H_4^{\neg+}$, 100). Anal. calcd. for $C_{19}H_{16}BrN_5O_2S$: C, 49.79; H, 3.52; N, 15.28. Found: C, 50.08; H, 3.50; N, 15.36.

*N-(4-Chlorophenyl)-2-[2-(4-hydroxyphenylamino)-nicotinoyl]hydrazinecarbothioamide (**7c**)*
Yield: 83%; m.p.: 196–197°C; IR (KBr) cm^{-1}: 3564–3263 (NH and OH), 3080 (CH aromatic), 1635 (C=O), 1612 (C=N), 1234 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.70 (d, 2H, J = 8.10 Hz, ArH), 6.76 (dd, 1H, J_{H4H5} = 7.50 Hz, J_{H5H6} = 4.50 Hz, H_5 pyridine), 7.37–7.51 (m, 6H, ArH), 8.15 (d, 1H, J = 7.50 Hz, H_4 pyridine), 8.26 (d, 1H, J = 4.80 Hz, H_6 pyridine), 9.08, 9.79, 9.90, 10.24, 10.69 (5s, 5H, 4NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 415.10 (M+2, 0.26), 413.10 ($M^{\neg\ddagger}$, 0.24), 244.10 ($C_{12}H_{12}N_4O_2^{\neg+}$, 32.67), 213.10 ($C_{12}H_9N_2O_2^{\neg+}$, 62.32), 79.95 ($C_5H_6N^{\neg+}$ 69.13), 63.95 ($C_5H_4^{\neg+}$, 100). Anal. calcd. for $C_{19}H_{16}ClN_5O_2S$: C, 55.14; H, 3.90; N, 16.92. Found: C, 55.27; H, 3.93; N, 17.13.

*2-[2-(4-Hydroxyphenylamino)nicotinoyl]-N-(4-methoxyphenyl)hydrazinecarbothioamide (**7d**)*
Yield: 86%; m.p.: 186–187°C; IR (KBr) cm^{-1}: 3323–3196 (NH and OH), 3084 (CH aromatic), 2929, 2850 (CH aliphatic), 1653 (C=O), 1602 (C=N), 1247 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.74 (s, 3H, OCH_3), 6.70 (d, 2H, J = 9 Hz, ArH), 6.75 (dd, 1H, J_{H4H5} = 7.80 Hz, J_{H5H6} = 4.80 Hz, H_5 pyridine), 6.88 (d, 2H, J = 8.70 Hz, ArH), 7.27 (d, 2H, J = 8.70 Hz, ArH), 7.41 (d, 2H, J = 9 Hz, ArH), 8.16 (d, 1H, J = 7.80 Hz, H_4 pyridine), 8.25 (dd, 1H, J_{H5H6} = 4.80 Hz, J_{H4H6} = 1.50 Hz, H_6 pyridine), 9.08, 9.57, 9.75, 10.23, 10.64 (5s, 5H, 4NH and OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) ppm: 55.69 (OCH_3), 112.72, 114.04, 115.64, 122.69, 123.88, 125.49, 127.82, 131.34, 151.87, 153.21, 155.51, 156.72, 159.06 (Ar C's), 187.21 (C=O), 188.59 (C=S); EIMS *m/z* (% rel. int.): 409.20 ($M^{\neg\ddagger}$, 0.18), 244.15 ($C_{12}H_{12}N_4O_2^{\neg+}$, 16.39), 213.05 ($C_{12}H_9N_2O_2^{\neg+}$, 37.60), 79.95 ($C_5H_6N^{\neg+}$, 70.75), 63.95 ($C_5H_4^{\neg+}$, 100). Anal. calcd. for $C_{20}H_{19}N_5O_3S$: C, 58.67; H, 4.68; N, 17.10. Found: C, 58.82; H, 4.73; N, 17.29.

*2-[2-(4-Hydroxyphenylamino)nicotinoyl]-N-(4-methylphenyl)hydrazinecarbothioamide (**7e**)*
Yield: 87%; m.p.: 199–200°C; IR (KBr) cm^{-1}: 3278–3190 (NH and OH), 3084 (CH aromatic), 2960, 2850 (CH aliphatic), 1637 (C=O), 1610 (C=N), 1236 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.28 (s, 3H, CH_3), 6.71 (d, 2H, J = 8.80 Hz, ArH), 6.76 (dd, 1H, J_{H4H5} = 7.76 Hz, J_{H5H6} = 4.80 Hz, H_5 pyridine), 7.13 (d, 2H, J = 8.08 Hz, ArH), 7.29 (d, 2H, J = 8.08 Hz, ArH), 7.41 (d, 2H, J = 8.80 Hz, ArH), 8.16 (d, 1H, J = 7.76 Hz, H_4 pyridine), 8.25 (dd, 1H, J_{H5H6} = 4.80 Hz, J_{H4H6} = 1.48 Hz, H_6 pyridine), 9.12, 9.62, 9.80, 10.28, 10.66 (5s, 5H, 4NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 393.00 ($M^{\neg\ddagger}$, 0.27), 79.95 ($C_5H_6N^{\neg+}$, 66.42), 63.95 ($C_5H_4^{\neg+}$, 100). Anal. calcd. for $C_{20}H_{19}N_5O_2S$: C, 61.05; H, 4.87; N, 17.80. Found: C, 61.31; H, 4.95; N, 17.94.

*N-(2-Chloro-6-methylphenyl)-2-(2-(4-hydroxyphenylamino)nicotinoyl) hydrazinecarbothioamide (**7f**)*
Yield: 69%; m.p.: 163–164°C; IR (KBr) cm^{-1}: 3323–3246 (NH and OH), 3016 (CH aromatic), 2926, 2854 (CH aliphatic), 1647 (C=O), 1606 (C=N), 1244 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.25 (s, 3H, CH_3), 6.71–6.73 (m, 3H, 2ArH and H_5 pyridine), 7.21–7.46 (m, 5H, ArH), 8.19 (d, 1H, H_4 pyridine), 8.24 (d, 1H, H_6 pyridine), 9.10, 9.62, 9.70, 10.15, 10.75 (5s, 5H, 4NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 429.00 (M+2, 0.48), 427.00 ($M^{\neg\ddagger}$, 0.81). Anal. calcd. for $C_{20}H_{18}ClN_5O_2S$: C, 56.14; H, 4.24; N, 16.37. Found: C, 56.27; H, 4.27; N, 16.53.

*General procedure for the preparation of **8a–f***
A mixture of the thiosemicarbazide derivatives **7a–f** (1 mmol), pyridine (0.165 g, 2.10 mmol) and *p*-toluenesulphonyl chloride (0.23 g, 1.20 mmol) in dry THF (5 mL) was heated under reflux for 6 h. The solvent was distilled off under reduced pressure. The residue was triturated with ethanol, and the precipitated solid product was filtered and crystallized from DMF/ethanol.

*4-{3-[5-(Phenylamino)-1,3,4-oxadiazol-2-yl]pyridin-2-ylamino}phenol (**8a**)*
Yield: 83%; m.p.: 250–251°C; IR (KBr) cm^{-1}: 3369–3170 (NH and OH), 3068 (CH aromatic), 1625 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 6.82 (d, 2H, J = 8.72 Hz, ArH), 6.98 (dd, 1H, J_{H4H5} = 7.60 Hz, J_{H5H6} = 5.24 Hz, H_5 pyridine), 7.03 (t, 1H, J = 7.36 Hz, ArH), 7.37 (t, 2H, J = 7.68 Hz, ArH), 7.43 (d, 2H, J = 8.76 Hz, ArH), 7.64 (d, 2H, J = 7.88 Hz, ArH), 8.09 (dd, 1H, J_{H4H5} = 7.60 Hz, J_{H4H6} = 1.50 Hz, H_4 pyridine), 8.21 (dd, 1H, J_{H5H6} = 5.08 Hz, J_{H4H6} = 1.50 Hz, H_6 pyridine), 9.90 (s, 1H, NH, D_2O exchangeable), 10.97 (s, 1H, NH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 346.00 (M+H, 23.67), 345.05 ($M^{\neg\ddagger}$, 100), 213.00 ($C_{12}H_9N_2O_2^{\neg+}$, 34.30), 79.95 ($C_5H_6N^{\neg+}$, 18.08), 63.95 ($C_5H_4^{\neg+}$, 44.70). Anal. calcd. for $C_{19}H_{15}N_5O_2$: C, 66.08; H, 4.38; N, 20.28. Found: C, 66.17; H, 4.46; N, 20.47.

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*4-{3-[5-(4-Bromophenylamino)-1,3,4-oxadiazol-2-yl]-pyridin-2-ylamino}phenol (**8b**)*
Yield: 82%; m.p.: 275–276°C; IR (KBr) cm^{-1}: 3441–3197 (NH and OH), 3032 (CH aromatic), 1612 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 6.78 (d, 2H, $J = 8.72$ Hz, ArH), 6.93 (dd, 1H, $J_{H4H5} = 7.60$ Hz, $J_{H5H6} = 5$ Hz, H_5 pyridine), 7.43–7.47 (m, 4H, ArH), 7.66 (d, 2H, $J = 8.84$ Hz, ArH), 8.02 (dd, 1H, $J_{H4H5} = 7.60$ Hz, $J_{H4H6} = 1.44$ Hz, H_4 pyridine), 8.24 (dd, 1H, $J_{H5H6} = 5$ Hz, $J_{H4H6} = 1.44$ Hz, H_6 pyridine), 9.79 (s, 1H, NH, D_2O exchangeable), 11.08 (s, 1H, NH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 425.00 (M+2, 0.19), 423.00 ($M^{\neg\ddagger}$, 0.26), 79.95 ($C_5H_6N^{\neg+}$, 100), 63.95 ($C_5H_4^{\neg+}$, 48.92). Anal. calcd. for $C_{19}H_{14}BrN_5O_2$: C, 53.79; H, 3.33; N, 16.51. Found: C, 53.94; H, 3.31; N, 16.70.

*4-{3-[5-(4-Chlorophenylamino)-1,3,4-oxadiazol-2-yl]-pyridin-2-ylamino}phenol (**8c**)*
Yield: 87%; m.p.: 280–281°C; IR (KBr) cm^{-1}: 3223–3203 (NH and OH), 3084 (CH aromatic), 1614 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.76 (d, 2H, $J = 9$ Hz, ArH), 6.90 (dd, 1H, $J_{H4H5} = 7.80$ Hz, $J_{H5H6} = 4.80$ Hz, H_5 pyridine), 7.42–7.48 (m, 4H, ArH), 7.65 (d, 2H, $J = 9$ Hz, ArH), 7.98 (dd, 1H, $J_{H4H5} = 7.50$ Hz, $J_{H4H6} = 1.80$ Hz, H_4 pyridine), 8.26 (dd, 1H, $J_{H5H6} = 4.80$ Hz, $J_{H4H6} = 1.80$ Hz, H_6 pyridine), 9.72 (s, 1H, NH, D_2O exchangeable), 11.00 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) ppm: 104.19, 113.78, 116.17, 119.34, 124.38, 126.31, 129.46, 129.96, 137.37, 137.81, 147.58, 151.82, 154.95, 156.69, 159.61 (Ar C's); EIMS *m/z* (% rel. int.): 381.10 (M+2, 35.23), 380.10 (M+H, 31.61), 379.10 ($M^{\neg\ddagger}$, 100), 378.10 (M−H, 28.45), 213.05 ($C_{12}H_9N_2O_2^{\neg+}$, 24.42), 111.05 ($C_6H_4Cl^{\neg+}$, 12.59), 91.05 ($C_7H_7^{\neg+}$, 20.60), 80.00 ($C_5H_6N^{\neg+}$, 17.06), 63.95 ($C_5H_4^{\neg+}$, 39.84). Anal. calcd. for $C_{19}H_{14}ClN_5O_2$: C, 60.09; H, 3.72; N, 18.44. Found: C, 60.32; H, 3.75; N, 18.72.

*4-{3-[5-(4-Methoxyphenylamino)-1,3,4-oxadiazol-2-yl]pyridin-2-ylamino}phenol (**8d**)*
Yield: 92%; m.p.: 266–267°C; IR (KBr) cm^{-1}: 3300–3142 (NH and OH), 3028 (CH aromatic), 2956, 2856 (CH aliphatic), 1627 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.74 (s, 3H, OCH_3), 6.76 (d, 2H, $J = 8.80$ Hz, ArH), 6.89 (dd, 1H, $J_{H4H5} = 7.68$ Hz, $J_{H5H6} = 4.80$ Hz, H_5 pyridine), 6.96 (d, 2H, $J = 9.04$ Hz, ArH), 7.47 (d, 2H, $J = 8.80$ Hz, ArH), 7.54 (d, 2H, $J = 9$ Hz, ArH), 7.96 (dd, 1H, $J_{H4H5} = 7.68$ Hz, $J_{H4H6} = 1.80$ Hz, H_4 pyridine), 8.26 (dd, 1H, $J_{H5H6} = 4.80$ Hz, $J_{H4H6} = 1.80$ Hz, H_6 pyridine), 9.21, 9.73, 10.61 (3s, 3H, 2NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 376.10 (M+H, 24.31), 375.10 ($M^{\neg\ddagger}$, 87.07), 374.10 (M−H, 20.68), 213.00 ($C_{12}H_9N_2O_2^{\neg+}$, 34.76), 211.00 ($C_{12}H_9N_3O^{\neg+}$, 50.50), 210.00 ($C_{12}H_8N_3O^{\neg+}$, 100), 108.05 ($C_7H_8O^{\neg+}$, 48.49), 91.00 ($C_7H_7^{\neg+}$, 25.22), 79.95 ($C_5H_6N^{\neg+}$, 22.93), 77.00 ($C_6H_5^{\neg+}$, 30.61), 63.95 ($C_5H_4^{\neg+}$, 25.37). Anal. calcd. for $C_{20}H_{17}N_5O_3$: C, 63.99; H, 4.56; N, 18.66. Found: C, 64.18; H, 4.62; N, 18.91.

*4-{3-[5-(4-Methylphenylamino)-1,3,4-oxadiazol-2-yl]-pyridin-2-ylamino}phenol (**8e**)*
Yield: 88%; m.p.: 260–261°C; IR (KBr) cm^{-1}: 3300–3136 (NH and OH), 3053 (CH aromatic), 2960, 2854 (CH aliphatic), 1618 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.27 (s, 3H, CH_3), 6.76 (d, 2H, $J = 8.76$ Hz, ArH), 6.89 (dd, 1H, $J_{H4H5} = 7.68$ Hz, $J_{H5H6} = 4.84$ Hz, H_5 pyridine), 7.18 (d, 2H, $J = 8.40$ Hz, ArH), 7.47 (d, 2H, $J = 8.76$ Hz, ArH), 7.51 (d, 2H, $J = 8.40$ Hz, ArH), 7.96 (dd, 1H, $J_{H4H5} = 7.68$ Hz, $J_{H4H6} = 1.68$ Hz, H_4 pyridine), 8.26 (dd, 1H, $J_{H5H6} = 4.84$ Hz, $J_{H4H6} = 1.68$ Hz, H_6 pyridine), 9.20, 9.74, 10.71 (3s, 3H, 2NH and OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) ppm: 20.80 (CH_3), 104.68, 113.77, 116.28, 117.83, 124.69, 125.96, 127.83, 128.56, 129.98, 131.63, 136.28, 151.24, 155.25, 156.26, 159.96 (Ar C's); EIMS *m/z* (% rel. int.): 360.15 (M+H, 27.51), 359.20 ($M^{\neg\ddagger}$, 100), 358.15 (M−H, 26.06), 213.05 ($C_{12}H_9N_2O_2^{\neg+}$, 15.67). Anal. calcd. for $C_{20}H_{17}N_5O_2$: C, 66.84; H, 4.77; N, 19.49. Found: C, 66.97; H, 4.80; N, 19.68.

*4-{3-[5-(2-Chloro-6-methylphenylamino)-1,3,4-oxadiazol-2-yl]pyridin-2-ylamino}phenol (**8f**)*
Yield: 74%; m.p.: 204–205°C; IR (KBr) cm^{-1}: 3290–3116 (NH and OH), 3068 (CH aromatic), 2980, 2854 (CH aliphatic), 1624 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.31 (s, 3H, CH_3), 6.73 (d, 2H, $J = 8.10$ Hz, ArH), 6.84 (dd, 1H, $J_{H4H5} = 7.80$ Hz, $J_{H5H6} = 4.80$ Hz, H_5 pyridine), 7.25–7.34 (m, 3H, ArH), 7.42 (d, 2H, $J = 8.10$ Hz, ArH), 7.86 (d, 1H, $J = 7.80$ Hz, H_4 pyridine), 8.23 (d, 1H, $J = 4.80$ Hz, H_6 pyridine), 9.16, 9.65, 10.04 (3s, 3H, 2NH and OH, D_2O exchangeable); EIMS *m/z* (% rel. int.): 395.20 (M+2, 1.52), 393.20 ($M^{\neg\ddagger}$, 4.29), 79.95 ($C_5H_6N^{\neg+}$, 54.26), 63.95 ($C_5H_4^{\neg+}$, 100). Anal. calcd. for $C_{20}H_{16}ClN_5O_2$: C, 60.99; H, 4.09; N, 17.78. Found: C, 61.14; H, 4.13; N, 18.01.

Biological studies

Growth inhibition assay (MTT assay)
Cancer cells from different cancer cell lines; human hepatocellular carcinoma (HepG-2) and human colon adenocarcinoma (HCT-116), were purchased from American Type Cell Culture Collection (ATCC, Manassas, USA) and grown on the appropriate growth medium Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 100 mg/mL of streptomycin, 100 U/mL of penicillin, and 10% of heat-inactivated fetal bovine serum in a humidified 5% (v/v) CO_2 atmosphere at 37°C. Exponentially growing cells from different cancer cell lines were trypsinized, counted, and seeded at the appropriate densities (1000–2000 cells/0.33 cm^2 well) into 96-well microtiter plates.

Cells were incubated in a humidified atmosphere at 37°C for 24 h, then exposed to different concentrations of tested compounds (0.1, 10, 100, 1000 μM) for 72 h. The viability of treated cells was determined using MTT technique: media were removed; cells were incubated with 200 μL of 5% MTT solution/well (Sigma–Aldrich, MO) and were allowed to metabolize the dye into colored insoluble formazan crystals for 2 h. The remaining MTT solution was discarded from the wells and the formazan crystals were dissolved in 200 μL/well acidified isopropanol for 30 min, then covered with aluminum foil with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc., MI) at room temperature.

Absorbance was measured at 570 nm using a Stat FaxR 4200 plate reader (Awareness Technology, Inc., FL). The cell

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viability was expressed as percentage of control and the concentration that induces 50% of maximum inhibition of cell proliferation (IC_{50}) was determined using GraphPad Prism version 5 Software (GraphPad software Inc., CA) [34, 35].

In vitro tubulin polymerization assay

HCT-116 and HepG-2 cells were obtained from American Type Culture Collection, and were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone), 10 μg/mL of insulin (Sigma), and 1% penicillin–streptomycin. All other chemicals and reagents were obtained from Sigma or Invitrogen. The cells were cultured in 96 well plate (10000 cells/well, cell density 1.2–1.8) and incubated with the tested compounds at IC_{50} concentration of each compound 24 h before enzyme assay (Table 2). Tubulin polymerization inhibitory activity was determined using human β-tubulin SEB870HU assay kit (Cloud-Clone Corp., USA). The procedure of the used kit was performed according to the manufacturer's instructions.

Molecular docking

The X-ray crystal structure of ABT751-tubulin complex (PDB code 3HKC) was downloaded from http://www.rscb.org/pdb. All molecular modeling calculations and docking studies were carried out using Discovery Studio software (version 2.55). Automatic protein preparation was done using CHARMm forcefield. 3D structures of target compounds were built using ChemBioDraw Ultra 11.0. Our ligands were prepared using Accelry's Discovery Studio Prepare Ligands protocol. The validation results showed the same binding interactions of the co-crystallized and the re-docked ligand with RMSD of 0.65 Å and docking score of −13.72 kcal/mol.

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