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Original article

Synthesis and evaluation of benzimidazole carbamates bearing indole moieties for antiproliferative and antitubulin activities

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ABSTRACT

A series of novel benzimidazole carbamates bearing indole moieties with sulphur or selenium atoms connecting the aromatic rings were synthesised and evaluated for their antiproliferative activities against three human cancer cell lines (SGC-7901, A-549 and HT-1080) using an MTT assay. Compounds **10a**, **10b**, **7a**, **7b** and **7f** showed significant activities against these cell lines. The most potent compound in this series, **10a**, was selected to investigate its antitumour mechanism. In addition, molecular docking studies suggested that compound **10a** interacts very closely with the nocodazole docking pose through hydrogen bonds at the colchicine binding site of tubulin.

1. Introduction

Microtubules formed by the self-assembly of α- and β-tubulin heterodimers are involved in a large number of cellular functions, such as motility, division, shape maintenance, and intracellular transport [1–5]. Due to the multiple functions of microtubules in the cell cycle, tubulin has become an attractive target in anticancer drug discovery. There are at least four binding sites on tubulin: the taxane, laulimalide, vinca, and colchicine binding sites [6]. Many tubulin-binding compounds, such as paclitaxel, vinblastine and eribulin, are used clinically for cancer therapy [7].

Benzimidazole carbamates (BZs), which are active as anthelmintics and fungicides due to their antimicrotubule activities, have been reported to elicit promising antitumour effects. Nocodazole (**1**), which is active against various experimental tumours and leukaemias [8], has often been used as a lead in the discovery of novel antineoplastic drugs. Albendazole (**2**) was recently reported to inhibit the proliferation of cells from a wide variety of cancers, including hepatocellular and colorectal cancer and a variety of other malignant human cell lines [9,10]. Mebendazole (**3**), another anthelmintic BZ, also displays potent antitumour effects against lung cancer cells both *in vitro* and in nude mice [11,12]. MN-029 (**4**), which is structurally related to nocodazole (**1**), is a novel benzoimidazole carbamate that reversibly inhibits microtubule assembly, resulting in the disruption of the cytoskeleton of vascular endothelial cells in tumours [13]. The major target of these agents is tubulin, and the binding site of these agents partly overlaps with that of colchicine [14,15]. As shown in Fig. 1, the BZs described above contain the following three parts: A, B and a connector. Part A, 2-[(methoxycarbonyl)amino]-1*H*-benzoimidazole, is generally considered a privileged structure that is critical for antitumour activity. To promote antitumour activity, a large number of the modifications to BZs involve part B, which is usually an alkyl or aromatic group. The connector between A and B, which includes a carbonyl group or a sulphur atom, is also tolerant of structural modification.

The indole ring may be the most ubiquitous heterocyclic substructure in nature. Owing to its great diversity in both structure and biological activity, it is not surprising that the indole ring is an

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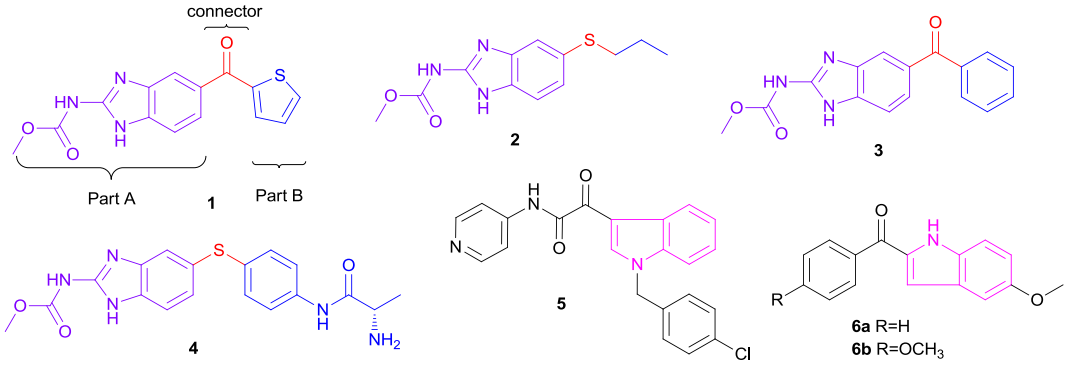


Fig. 1. Structures of some BZs and antitumor agents bearing indole moieties.

important structural component in many pharmaceuticals [16]. The indole scaffold plays an important role in some inhibitors of tubulin polymerisation, including the indolyl-3-glyoxamide D-24851 (**5**) and the 2-aroylindoles D-64131 (**6a**) and D-68144 (**6b**) (Fig. 1) [1].

Inspired by the structural characteristics of BZs and the favourable drug-like properties of the indole ring, we envisioned that the merging of these two bioactive components would afford a compact structure with the potential for antiproliferative activity. We therefore designed a series of benzimidazole carbamates bearing indole moieties (Fig. 2) as antiproliferative and antitubulin agents, in which part A of the BZs was retained but part B was replaced with substituted indole rings. In addition, four isosteres (sulphur, sulfoxide, sulphone and selenium) were introduced as connectors.

2. Results and discussion

2.1. Chemistry

The synthetic routes for the target compounds **7a**–**f**, **8a**–**f**, **9a**–**f** are outlined in Scheme 1. The substituted 1-*H*-indoles (**14a**–**f**) were treated with ammonium thiocyanate and the oxidant cerium ammonium nitrate (CAN) in methanol at room temperature to afford substituted 3-thiocyanato-1*H*-indoles (**15a**–**f**) in 85–97% yields [17]. The key intermediates, substituted 5-[(1-*H*-indol-3-yl) thio]-2-nitro-anilines (**18a**–**f**), were synthesised from **15a**–**f** in "one-pot" procedures. **15a**–**f** were reduced with sodium borohydride in ethanol to give the sodium salt of substituted 3-sulfydryl-1*H*-indoles (**16a**–**f**) [18], which were treated with *N*-(5-chloro-2-nitrophenyl)acetamide to give substituted 5-[(1-*H*-indol-3-yl) thio]-2-nitroacetanilides (**17a**–**f**) via nucleophilic aromatic substitution. Without purification, the alcoholysis of crude **17a**–**f** with sodium and ethanol afforded **18a**–**f**. The reduction of **18a**–**f** by hydrazine hydrate and ferric trichloride in the presence of activated carbon gave substituted 4-[(1-*H*-indol-3-yl)thio]-benzene-1,2-diamines (**19a**–**f**), which were used in the next step without purification [19]. The target compounds, substituted methyl 5-[(1-*H*-indol-3-yl)thio]-1*H*-benzoimidazol-2-ylcarbamates (**7a**–**f**), were prepared in 40–50% yields from **19a**–**f** by condensation with 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea while refluxing in ethanol [20]. Subsequently, the oxidation of **7a**–**f** with *meta*-chloroperbenzoic acid (*m*-CPBA) afforded sulfoxides **8a**–**f** and sulphones **9a**–**f** in modest yields.

The synthetic routes for **10a**–**f** are shown in Scheme 2. After the formation of triselenium dicyanide (TSD) from malononitrile and selenium dioxide in dimethylsulfoxide, substituted 1-*H*-indoles (**14a**–**f**) were added to give substituted 3-selenocyanato-1*H*-indoles (**20a**–**f**) in 85–93% yields [21]. Substituted methyl 5-[(1*H*-indol-3-yl)selanyl]-1*H*-benzoimidazol-2-ylcarbamates **10a**–**f** were obtained from **20a**–**f** employing a similar route as that used to prepare **7a**–**f** from **15a**–**f**.

2.2. Biological evaluation

2.2.1. Antiproliferative activity assay

The *in vitro* antiproliferative activities of all of the target compounds were evaluated using an MTT assay. The following three human cancer cell lines were used: gastric adenocarcinoma (SGC-7901), lung adenocarcinoma (A-549) and fibrosarcoma (HT-1080). Nocodazole (**1**) was the positive control. The antiproliferative activity (IC_{50}) values are given in Table 1. As shown in Table 1, **10a** exhibited the most potent antiproliferative activity, with IC_{50} values of 0.098–0.15 μM against the three cancer cell lines. Nocodazole (**1**), the positive control, showed IC_{50} values of 0.080–0.14 μM **10b**, **7a**, **7b**, and **7f** also significantly inhibited the growth of two or three of the cell lines at submicromolar concentrations. The comparison of the IC_{50} values of **7a**–**f** and **10a**–**f** with **8a**–**f** and **9a**–**f** revealed that a sulphur or selenium connector showed more potential than a sulphone and sulfoxide connector. With sulphur or selenium as the connector, a methyl substituent at the indole C-2 position (**7b**, **10b**) slightly reduced the activities compared with the unsubstituted compounds (**7a**, **10a**). To investigate other positions, halogens (fluorine, chlorine, and bromine) and a cyano group were introduced at C-5 (**7c**–**f**, **10c**–**f**). Interestingly, all of these compounds except for **7f** showed moderately reduced activities compared to the corresponding unsubstituted analogues **7a** and **10a**. Thus, a 5-substituent on the indole skeleton did not improve the antiproliferative activity.

2.2.2. Inhibition of tubulin polymerisation

To investigate whether the antiproliferative activities of the target compounds derived from an interaction with tubulin, the most active **10a** and the moderately active **7e** were evaluated for their inhibition of tubulin polymerisation in comparison to nocodazole (**1**). **7e** and **10a** caused concentration-dependent inhibition of tubulin assembly (Fig. 3), which suggests that these compounds interfere with the microtubule polymerisation. **10a** was slightly less active than the reference compound nocodazole (**1**), with IC_{50} values of 3.6 and 2.3 μM, respectively. **7e**, which exhibited less antiproliferative activity than **10a**, also showed a lower potency (IC_{50} 8.2 μM) than **10a** as an inhibitor of tubulin polymerisation.

2.2.3. Analysis of immunofluorescence staining

In addition to *in vitro* tubulin polymerisation, we investigated alterations in the microtubule network induced by **10a** in cultured cells using immunostaining. SGC-7901 and HT-1080 cells were

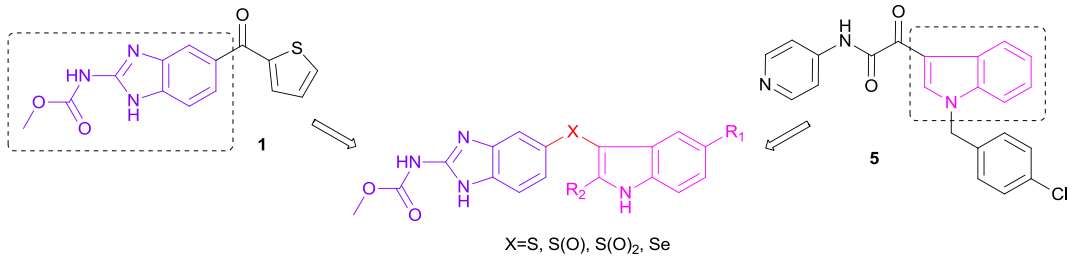
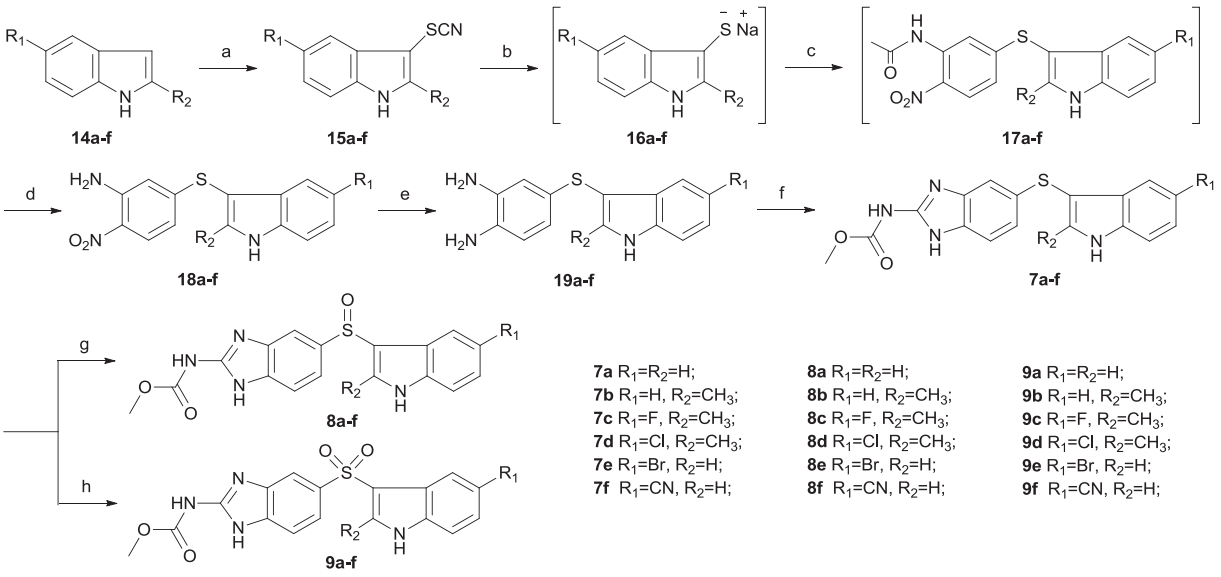
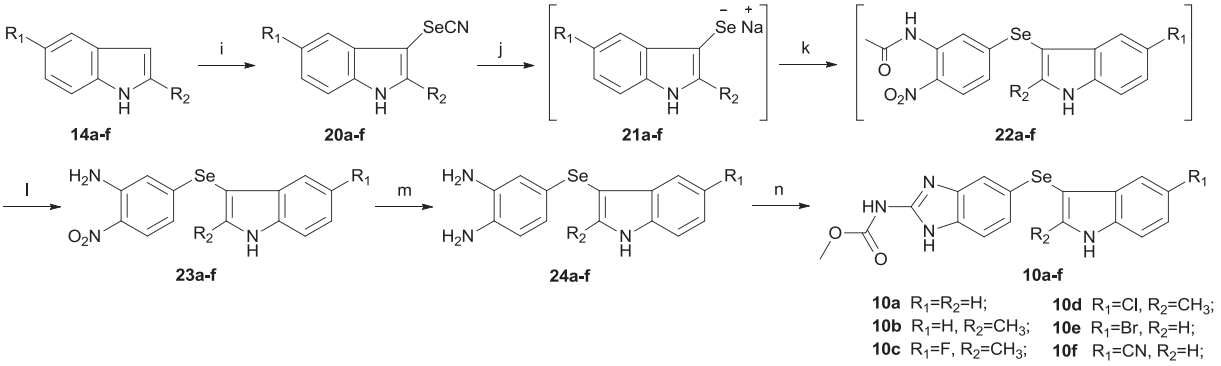


Fig. 2. Design of benzimidazole carbamates bearing indole moieties as antiproliferative agents.



Scheme 1. Synthesis of **7a**–**f**, **8a**–**f** and **9a**–**f**. Reagents and conditions: (a) NH_4SCN, CAN, CH_3OH, 25 °C; (b) $NaBH_4$, EtOH, 40–60 °C; (c) *N*-(5-chloro-2-nitrophenyl)acetamide, N_2, 25 °C; (d) Na, EtOH, 75 °C; (e) $N_2H_4{\cdot}H_2O$, $FeCl_3$, active carbon, CH_3OH, reflux; (f) 1,3-Bis(methoxycarbonyl)-2-methyl-2- thiopseudourea, EtOH, reflux; (g) *m*-CPBA (1.2–1.5 eq.), THF, −20 °C; (h) *m*-CPBA (3.0 eq.), THF, 25 °C.



Scheme 2. Synthesis of **10a**–**f**. Reagents and conditions: (i) $NCCH_2CN$, SeO_2, DMSO, 35 °C; (j) $NaBH_4$, EtOH, 30 °C; (k) *N*-(5-chloro-2-nitrophenyl)acetamide, N_2, 25 °C; (l) Na, EtOH, 75 °C; (m) $N_2H_4{\cdot}H_2O$, $FeCl_3$, active carbon, CH_3OH, reflux; (n) 1,3-Bis(methoxycarbonyl)-2-methyl-2-thiopseudourea, EtOH, reflux.

treated with **10a** and nocodazole (**1**) at their respective 2-fold IC_{50} concentrations and were then stained with the tubulin antibody (Fig. 4). The upper panel represents tubulin protein assembly in treated and untreated cells labelled with FITC. The middle panel represents the DAPI-stained nuclei of the cells, and the lower panel is the result of merging the upper two panels. The results imply that nocodazole (**1**) and **10a** induce significant distortions in the tubulin assembly of the treated cells, leading to the formation of abnormal mitotic spindles in comparison with the control. Moreover, **10a** also significantly disrupted the assembly of tubulin in a manner similar to nocodazole (**1**).

2.3. Molecular modelling

According to our proposed binding mode, in which the binding of the potential drug partially overlaps with the colchicine binding site, described by Rodrigo in 2013 [14,15], **10a** was subjected to molecular docking with tubulin (PDB: 1SA0). To compare the

Table 1
In vitro cell growth inhibition by the target compounds and nocodazole (**1**).

Compound	IC_{50}[a] (μM)		
	SGC-7901	A-549	HT-1080
7a	2.62 ± 0.05	0.39 ± 0.02	0.88 ± 0.01
7b	0.51 ± 0.03	0.83 ± 0.01	0.97 ± 0.02
7c	1.20 ± 0.09	2.52 ± 0.20	3.27 ± 0.02
7d	3.38 ± 0.02	7.33 ± 0.08	4.13 ± 0.14
7e	2.12 ± 0.07	1.19 ± 0.02	1.33 ± 0.05
7f	0.12 ± 0.005	5.14 ± 0.11	0.62 ± 0.01
8a	3.96 ± 0.04	6.23 ± 0.08	5.24 ± 0.02
8b	9.93 ± 0.17	7.98 ± 0.04	9.85 ± 0.15
8c	3.51 ± 0.21	3.27 ± 0.14	>10
8d	>10	>10	>10
8e	5.19 ± 0.08	>10	>10
8f	>10	>10	9.43 ± 0.23
9a	1.26 ± 0.02	4.21 ± 0.09	2.01 ± 0.05
9b	>10	>10	>10
9c	4.18 ± 0.16	9.08 ± 0.24	>10
9d	>10	>10	>10
9e	7.44 ± 0.04	7.42 ± 0.17	3.18 ± 0.12
9f	>10	>10	>10
10a	0.098 ± 0.002	0.15 ± 0.05	0.13 ± 0.07
10b	0.23 ± 0.01	3.83 ± 0.02	0.27 ± 0.01
10c	1.12 ± 0.02	1.15 ± 0.08	1.69 ± 0.03
10d	>10	2.98 ± 0.17	>10
10e	2.15 ± 0.06	2.21 ± 0.14	2.75 ± 0.04
10f	1.11 ± 0.08	5.07 ± 0.02	0.95 ± 0.008
1[b]	0.080 ± 0.01	0.12 ± 0.03	0.14 ± 0.005

[a] IC_{50}: concentration of the compound (μM) producing 50% cell growth inhibition after 72 h of drug exposure, as determined by the MTT assay. Each experiment was carried out in triplicate.
[b] Used as a positive control.

binding poses of **10a** and nocodazole (**1**), the latter compound was also docked. The docked binding poses are shown in Fig. 5. The common moiety of these two compounds overlapped, and several hydrogen bonds formed in this region. The methyl carbamate carbonyl formed a hydrogen bond with Asn-167. The 1-amino group of the benzimidazole ring formed a hydrogen bond with Glu-200. Tyr-202 established hydrogen bonds with the amino groups of both the benzimidazole ring and the methyl carbamate group. On the other hand, the 5-substituent of **10a** also occupied the same pocket as nocodazole (**1**). A hydrogen bond formed between the indole amino group and Lys-352; however, no such interactions were found for nocodazole (**1**) in this region.

3. Conclusion

A series of novel benzimidazole carbamates (BZs) bearing indole moieties and sulphur or selenium atoms connecting the aromatic rings were synthesised. The compounds were investigated for their inhibition of the proliferation of three human cancer cell lines (SGC-7901, A-549 and HT-1080) using an MTT assay. Sulphur or selenium connectors showed more potential than sulphone or sulfoxide connectors. **10a** showed the strongest antiproliferative activity, with IC_{50} values of 0.098–0.15 μM in the three human cancer cell lines, which are comparable to those of nocodazole (**1**). **10b**, **7a**, **7b**, and **7f** also exhibited significant antiproliferative activities against some cell lines. *In vitro* tubulin polymerisation and immunostaining experiments revealed that **10a** significantly inhibits tubulin polymerisation and disrupts tubulin-microtubule dynamics in a manner similar to that of nocodazole (**1**).

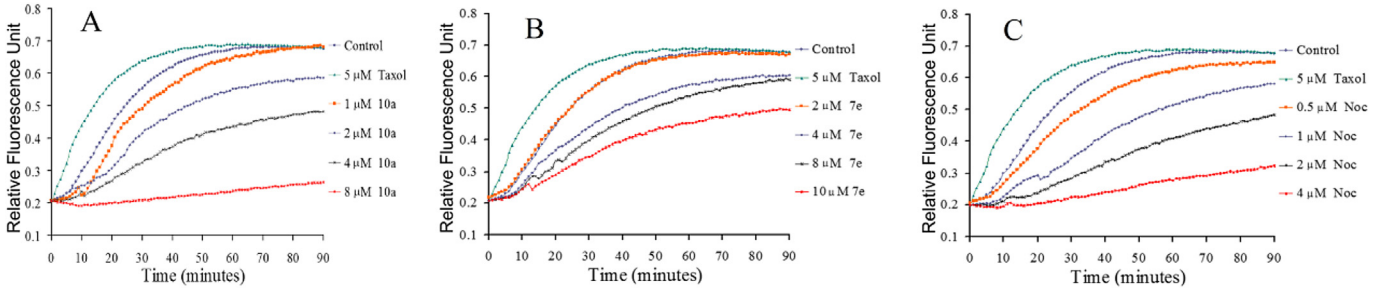


Fig. 3. Dose-response curves of compounds **10a** (A), **7e** (B) and nocodazole (C) for the inhibition of tubulin polymerisation. Purified bovine tubulin and GTP were mixed in a 96-well plate. The reaction was started by warming the solution from 4 °C to 37 °C. DMSO was used as a control. The effect on the assembly of tubulin was monitored using a plate reader at 1 min intervals for 90 min at 37 °C.

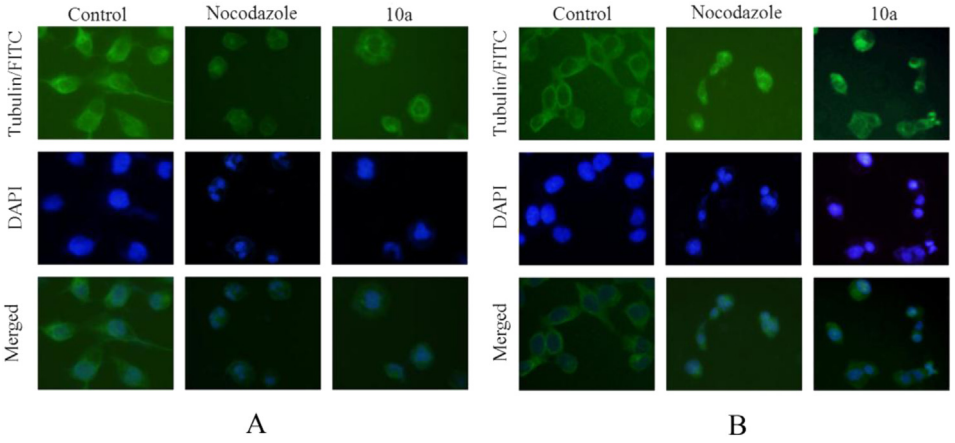


Fig. 4. Immunostaining of the assembly of tubulin in SGC-7901 (A) and HT-1080 (B) cells. SGC-7901 and HT-1080 cells were treated with nocodazole or **10a** for 48 h. The upper, middle and lower panels represent the tubulin assembly stained with FITC, DAPI and the merged upper panels, respectively. Images were taken under a fluorescence microscope.

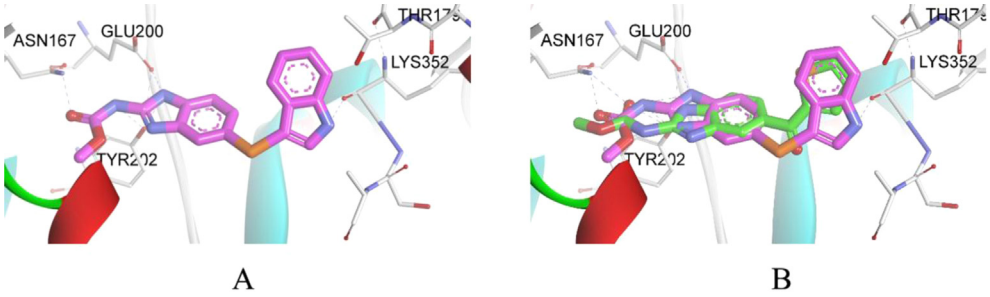


Fig. 5. (A) Docking conformation of **10a** (pink) in the colchicine binding site of tubulin. (B) Overlay of **10a** (pink) and nocodazole (green) in the binding site. The backbone of tubulin is shown using a ribbon representation, and the interacting amino acids are shown as stick models. Hydrogen bond interactions are shown as dotted lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Molecular simulations of the docking of **10a** to tubulin showed that **10a** binds to the colchicine site of tubulin with a binding mode very similar to that of nocodazole (**1**). These results may prove important for the design of structurally related tubulin inhibitors or BZs.

4. Experimental

4.1. Chemistry

4.1.1. General

Unless otherwise noted, all of the materials were obtained from commercially available sources and were used without purification. The boiling range of the light petroleum is 60–90 °C. The reaction process was monitored by TLC with silica gel plates (thickness 250 μm, Indicator F-254) under UV light. The purification of the products was performed using column chromatography (60 Å, 200–300 mesh, Qingdao Ocean Chemicals) or silica gel plates (0.25 mm layer, Qingdao Ocean Chemicals) with the designated solvents. Melting points were measured on a hot-stage microscope (X-4, Beijing Taike Ltd.) and were uncorrected. Mass spectra (MS) were measured on an Agilent 1100-sl mass spectrometer with an electrospray ionisation source from Agilent Co. Ltd. High resolution accurate mass determinations (HRMS) for all of the final target compounds were obtained on a Bruker Micromass Time of Flight mass spectrometer equipped with electrospray ionisation (ESI). NMR spectra were performed on a Bruker AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) or a Bruker AVANCE 600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) in DMSO-d_6 (internal standard tetramethylsilane). Infrared spectra were recorded using KBr plates on a PE Spectrum-100 instrument.

4.1.2. General procedure for the preparation of compounds ***15a–f***

The substituted indoles **14a–f** (1 mmol) and ammonium thiocyanate (1.2 mmol) were dissolved in 4 mL of methanol and treated with cerium (IV) ammonium nitrate (CAN; 2.3 mmol) in methanol (25 mL) at room temperature. The reaction mixture was stirred for 15 min. It was then diluted with water (100 mL) and extracted with ethyl acetate (20 mL × 4). The combined organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product, which was purified by column chromatography on silica gel with light petroleum/AcOEt (v/v = 3:1).

*4.1.2.1. 3-Thiocyanato-1H-indole (**15a**).* Colourless solid; yield: 96%; M.p.: 57–61 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.03 (s, 1H), 8.00 (d, J = 3.0 Hz, 1H), 7.68 (d, J = 7.2 Hz, 1H), 7.54 (d, J = 7.2 Hz, 1H), 7.26 (m, 2H).

*4.1.2.2. 2-Methyl-3-thiocyanato-1H-indole (**15b**).* Colourless solid; yield: 97%; M.p.: 96–100 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 1H), 7.56 (m, 1H), 7.43 (m, 1H), 7.19 (m, 2H), 2.54 (s, 3H).

*4.1.2.3. 5-Fluoro-2-methyl-3-thiocyanato-1H-indole (**15c**).* Colourless solid; yield: 93%; M.p.: 116–119 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H), 7.43 (dd, J_1 = 4.3 Hz, J_2 = 8.7 Hz, 1H), 7.29 (dd, J_1 = 2.3 Hz, J_2 = 9.3 Hz, 1H), 7.03 (td, J_1 = 2.3 Hz, J_2 = 9.3 Hz, 1H), 2.54 (s, 3H).

*4.1.2.4. 5-Chloro-2-methyl-3-thiocyanato-1H-indole (**15d**).* Colourless solid; yield: 91%; M.p.: 150–153 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (s, 1H), 7.54 (d, J = 1.8 Hz, 1H), 7.43 (d, J = 8.7 Hz, 1H), 7.19 (dd, J_1 = 1.8 Hz, J_2 = 8.7 Hz, 1H), 2.54 (s, 3H).

*4.1.2.5. 5-Bromo-3-thiocyanato-1H-indole (**15e**).* Colourless solid; yield: 97%; M.p.: 105–107 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.21 (s, 1H), 8.06 (s, 1H), 7.80 (d, J = 1.7 Hz, 1H), 7.51 (d, J = 8.6 Hz, 1H), 7.39 (dd, J_1 = 1.7 Hz, J_2 = 8.6 Hz, 1H).

*4.1.2.6. 5-Cyano-3-thiocyanato-1H-indole (**15f**).* Colourless solid; yield: 96%; M.p.: 179–180 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.51 (s, 1H), 8.23 (s, 1H), 8.19 (s, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.63 (d, J = 8.3 Hz, 1H).

4.1.3. General procedure for the preparation of compounds ***18a–f***

To a stirred solution of ethanol (20 mL) and **15a–f** (1 mmol), obtained as described above, sodium borohydride (2 mmol) was carefully added, and then, the reaction mixture was stirred at 40–60 °C under a nitrogen atmosphere. Upon completion (monitored by TLC), *N*-(5-chloro-2-nitrophenyl)acetamide (1 mmol) was added and reacted for another 2–4 h at room temperature. Then, sodium (0.05 mmol) was added to the mixture, and the temperature was increased to 75 °C. After 1.5 h, the solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate, washed with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product, which was purified by column chromatography on silica gel with light petroleum/AcOEt (v/v = 3:1).

*4.1.3.1. 5-[(1H-indol-3-yl)thio]-2-nitroaniline (**18a**).* Yellow solid; yield: 51%; M.p.: 172–176 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.85 (s, 1H), 7.83 (d, J = 2.3 Hz, 1H), 7.81 (d, J = 4.1 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.42 (s, 1H), 7.37 (s, 2H), 7.22 (t, J_1 = 7.2 Hz, J_2 = 14.6 Hz, 1H), 7.11 (t, J_1 = 7.2 Hz, J_2 = 14.6 Hz, 1H),6.51 (d, J = 1.9 Hz, 1H), 6.30 (dd, J_1 = 1.9 Hz, J_2 = 9.0 Hz, 1H).

*4.1.3.2. 5-[(2-Methyl-1H-indol-3-yl)thio]-2-nitroaniline (**18b**).* Yellow solid; yield: 47%; M.p.: 189–192 °C; ^{1}H NMR (400 MHz,

DMSO-d_6): δ 11.79 (s, 1H), 7.82 (d, J = 9.1 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.37 (s, 2H), 7.31 (d, J = 7.7 Hz, 1H), 7.13 (m, 1H), 7.04 (m, 1H), 6.45 (d, J = 1.9 Hz, 1H), 6.28 (dd, J_1 = 1.9 Hz, J_2 = 9.2 Hz, 1H), 2.43 (s, 3H).

*4.1.3.3. 5-[(5-Fluoro-2-methyl-1H-indol-3-yl)thio]-2-nitroaniline (**18c**).* Yellow solid; yield: 54%; M.p.: 165–168 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.92 (s, 1H), 7.82 (d, J = 9.1 Hz, 1H), 7.43 (m, 1H), 7.38 (s, 2H), 6.99 (m, 2H), 6.44 (d, J = 1.8 Hz, 1H), 6.28 (dd, J_1 = 1.8 Hz, J_2 = 9.1 Hz, 1H), 2.42 (s, 3H).

*4.1.3.4. 5-[(5-Chloro-2-methyl-1H-indol-3-yl)thio]-2-nitroaniline (**18d**).* Yellow solid; yield: 57%; M.p.: 62–65 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (s, 1H), 7.83 (d, J = 9.1 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.39 (s, 2H), 7.27 (d, J = 1.9 Hz, 1H), 7.14 (dd, J_1 = 1.9 Hz, J_2 = 8.5 Hz, 1H), 6.43 (d, J = 1.8 Hz, 1H), 6.28 (dd, J_1 = 1.8 Hz, J_2 = 9.1 Hz, 1H), 2.43 (s, 3H).

*4.1.3.5. 5-[(5-Bromo-1H-indol-3-yl)thio]-2-nitroaniline (**18e**).* Yellow solid; yield: 62%; M.p.: 168–171 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.06 (s, 1H), 7.90 (d, J = 2.7 Hz, 1H), 7.84 (d, J = 9.1 Hz, 1H), 7.51 (d, 1H), 7.50 (s, 1H), 7.40 (s, 2H), 7.34 (dd, J_1 = 1.9 Hz, J_2 = 8.6 Hz, 1H), 6.47 (d, J = 2.0 Hz, 1H), 6.30 (dd, J_1 = 2.0 Hz, J_2 = 9.1 Hz, 1H).

*4.1.3.6. 5-[(5-Cyano-1H-indol-3-yl)thio]-2-nitroaniline (**18f**).* Yellow solid; yield: 68%; M.p.: 238–240 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.39 (s, 1H), 8.06 (s, 1H), 7.87 (d, J = 0.6 Hz, 1H), 7.83 (d, J = 9.0 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.58 (dd, J_1 = 1.4 Hz, J_2 = 8.4 Hz, 1H), 7.38 (s, 2H), 6.46 (d, J = 1.9 Hz, 1H), 6.30 (dd, J_1 = 1.9 Hz, J_2 = 9.0 Hz, 1H).

*4.1.4. General procedure for the preparation of compounds **19a–f***

A mixture of **18a–f** (0.5 mmol), active carbon (3 mmol), ferric chloride anhydrous (0.1 mmol), and methanol (20 mL) was refluxed for 10 min with stirring, and then hydrazine hydrate (80% purity, 0.7 mL) was added dropwise to the mixture. The mixture was stirred for an additional 3 h under refluxing until **18a–f** disappeared. The reaction mixture was filtered to remove the active carbon, and the filtrate was concentrated under reduced pressure. Next, the filtrate was extracted with ethyl acetate, washed with water and brine, and concentrated under vacuum to afford **19a–f**, which were used without further purification.

*4.1.5. General procedure for the preparation of compounds **7a–f***

A mixture of **19a–f** (0.5 mmol) and 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea (0.5 mmol) in ethanol (5 mL) was refluxed for 2–4 h. The solvent was removed under reduced pressure to afford the crude product, and the crude product was purified by chromatography on silica gel plates to afford pure product **7a–f**.

*4.1.5.1. Methyl 5-[(1H-indol-3-yl)thio]-1H-benzoimidazol-2-ylcarbamate (**7a**).* White solid; yield: 44%; M.p.: 112–115 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.61 (s, 3H), 7.75 (s, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.26 (m, 1H), 7.16 (t, J_1 = 7.4 Hz, J_2 = 15.3 Hz, 1H), 7.13 (s, 1H), 7.04 (t, J_1 = 7.4 Hz, J_2 = 14.9 Hz, 1H), 6.96 (m, 1H), 3.70 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 155.01, 147.86, 136.88, 131.97, 131.67, 130.58, 128.93, 128.84, 122.15, 120.51, 120.07, 118.65, 114.30, 112.39, 111.97, 101.85, 52.54; HRMS (ESI): calcd for $C_{17}H_{15}N_4O_2S$ $[M+H]^+$: 339.0910, found: 339.0912; IR ν_{max}/cm^{-1} 3335 (N–H), 1710, 1642 (C=O), 1273, 1099 (C–O), 2950, 2854 (C–H), 3057, 1593, 1524 (Ar) (KBr).

*4.1.5.2. Methyl 5-[(2-methyl-1H-indol-3-yl)thio]-1H-benzoimidazol-2-ylcarbamate (**7b**).* White solid; yield: 47%; M.p.: 170–172 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.56 (s, 3H), 7.39 (d, J = 5.1 Hz, 1H), 7.35 (d, J = 5.1 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.08 (m, 1H), 7.04 (s, 1H), 7.00 (m, 1H), 6.89 (d, J = 8.4 Hz, 1H), 3.70 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.13, 147.85, 141.77, 136.57, 135.76, 134.94, 130.72, 130.00, 121.41, 120.15, 119.90, 117.75, 114.36, 111.38, 98.78, 52.54, 11.93; HRMS (ESI): calcd for $C_{18}H_{17}N_4O_2S$ $[M+H]^+$: 353.1067, found: 353.1082; IR ν_{max}/cm^{-1} 3384 (N–H), 1712, 1642 (C=O), 1273, 1100 (C–O), 2951, 2850 (C–H), 3058, 1594, 1524 (Ar) (KBr).

*4.1.5.3. Methyl 5-[(5-fluoro-2-methyl-1H-indol-3-yl)thio]-1H-benzoimidazol-2-ylcarbamate (**7c**).* White solid; yield: 42%; M.p.: 147–150 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.71 (s, 3H), 7.37 (dd, J_1 = 4.4 Hz, J_2 = 8.8 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H), 7.03–7.06 (m, 2H), 6.89–6.95 (m, 2H), 3.71 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.68–158.99 (d, J = 233.0 Hz), 155.12, 148.03, 144.01, 132.35, 130.79–130.89 (d, J = 10.3 Hz), 130.37, 120.45, 112.50–112.60 (d, J = 10.3 Hz), 111.64, 109.25–109.51 (d, J = 26.6 Hz), 102.69–102.93 (d, J = 26.6 Hz), 99.33, 99.28, 52.09, 12.10; HRMS (ESI): calcd for $C_{18}H_{16}FN_4O_2S$ $[M+H]^+$: 371.0973, found: 371.0973; IR ν_{max}/cm^{-1} 3391, 3371 (N–H), 1732, 1644 (C=O), 1259, 1099 (C–O), 2960, 2865 (C–H), 3057, 1594, 1524 (Ar) (KBr).

*4.1.5.4. Methyl 5-[(5-chloro-2-methyl-1H-indol-3-yl)thio]-1H-benzoimidazol-2-ylcarbamate (**7d**).* White solid; yield: 40%; M.p.: 162–165 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.81 (s, 1H), 11.64 (s, 2H), 7.40 (d, J = 8.6 Hz, 1H), 7.33 (d, J = 1.7 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 7.10 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H), 7.06 (s, 1H), 6.91 (dd, J_1 = 1.6 Hz, J_2 = 8.2 Hz, 1H), 3.72 (s, 3H), 2.50 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 154.97, 147.89, 143.80, 134.28, 131.65, 131.37, 130.13, 128.84, 124.77, 121.40, 120.35, 117.00, 114.55, 113.04, 111.56, 98.97, 52.56, 12.00; HRMS (ESI): calcd for $C_{18}H_{16}ClN_4O_2S$ $[M+H]^+$: 387.0677, found: 387.0684; IR ν_{max}/cm^{-1} 3389 (N–H), 1713, 1640 (C=O), 1270, 1099 (C–O), 2951, 2865 (C–H), 3066, 1593, 1524 (Ar) (KBr).

*4.1.5.5. Methyl 5-[(5-bromo-1H-indol-3-yl)thio]-1H-benzoimidazol-2-ylcarbamate (**7e**).* White solid; yield: 39%; M.p.: 166–172 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.84 (s, 3H), 7.84 (s, 1H), 7.53 (s, 1H), 7.46 (d, J = 8.7 Hz, 1H), 7.27–7.30 (m, 2H), 7.15 (s, 1H), 6.97 (d, J = 8.3 Hz, 1H), 3.71 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 154.95, 147.91, 136.66, 135.62, 135.20, 133.63, 130.85, 129.92, 124.80, 120.68, 120.61, 114.57, 114.45, 112.88, 112.08, 101.78, 52.57; HRMS (ESI): calcd for $C_{17}H_{16}BrN_4O_2S$ $[M+H]^+$: 419.0172, found: 419.0184; IR ν_{max}/cm^{-1} 3337 (N–H), 1704, 1638 (C=O), 1272, 1100 (C–O), 2949, 2869 (C–H), 3072, 1594, 1523 (Ar) (KBr).

*4.1.5.6. Methyl 5-[(5-cyano-1H-indol-3-yl)thio]-1H-benzoimidazol-2-ylcarbamate (**7f**).* White solid; yield: 45%; M.p.: 263–266 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (s, 1H), 11.61 (s, 2H), 7.99 (d, J = 1.9 Hz, 1H), 7.86 (d, J = 0.6 Hz, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.52 (dd, J_1 = 1.4 Hz, J_2 = 8.5 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 7.18 (d, J = 1.3 Hz, 1H), 7.00 (dd, J_1 = 1.7 Hz, J_2 = 8.3 Hz, 1H), 3.72 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 154.93, 148.00, 138.74, 134.57, 129.31, 128.81, 125.01, 123.90, 121.20, 120.42, 114.51, 113.90, 112.75, 103.97, 102.39, 52.58; HRMS (ESI): calcd for $C_{18}H_{14}N_5O_2S$ $[M+H]^+$: 364.0863, found: 364.0864; IR ν_{max}/cm^{-1} 3336 (N–H), 1752, 1640 (C=O), 1277, 1103 (C–O), 2951, 2857 (C–H), 3037, 1589, 1524 (Ar), 2225 (C≡N) (KBr).

*4.1.6. General procedure for the preparation of compounds **8a–f***

A stirred solution of **7a–f** (0.2 mmol) in THF (15 mL) was cooled to −20 °C, treated with *meta*-chloroperbenzoic acid (0.25 mmol)

and stirred for 4 h. The reaction was sequentially washed with water and saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product, which was purified by column chromatography on silica gel with dichloromethane/MeOH (v/v = 20:1).

*4.1.6.1. Methyl 5-[(1H-indol-3-yl)sulfinyl]-1H-benzoimidazol-2-ylcarbamate (**8a**).* White solid; yield: 61%; M.p.: 249–252 °C; ^{1}H NMR (600 MHz, DMSO-d_6); δ 11.94 (s, 3H), 8.03 (s, 1H), 7.77 (s, 1H), 7.49 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H), 7.25–7.28 (m, 2H), 7.12 (t, J_1 = 7.4 Hz, J_2 = 14.7 Hz, 1H), 6.92 (t, J_1 = 7.4 Hz, J_2 = 14.7 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 154.61, 148.77, 137.22, 137.19, 131.67, 130.20, 123.51, 122.85, 120.64, 119.24, 117.61, 114.30, 112.76, 110.50, 52.75; HRMS (ESI): calcd for $C_{17}H_{15}N_4O_3S$ $[M+H]^+$: 355.0859, found: 355.0852; IR ν_{max}/cm^{-1} 3359 (N–H), 1728, 1642 (C=O), 1261, 1101 (C–O), 2954, 2854 (C–H), 3039, 1593, 1523 (Ar) (KBr).

*4.1.6.2. Methyl 5-[(2-methyl-1H-indol-3-yl)sulfinyl]-1H-benzoimidazol-2-ylcarbamate (**8b**).* White solid; yield: 59%; M.p.: 258–260 °C; ^{1}H NMR (100 MHz, DMSO-d_6): δ 12.06 (s, 1H), 11.89 (s, 2H), 8.00 (s, 1H), 7.85 (m, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.49 (d, J = 8.3 Hz, 1H), 7.36 (m, 1H), 7.13–7.16 (m, 2H), 3.76 (s, 3H), 2.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.66, 148.69, 141.40, 138.06, 137.29, 135.83, 129.08, 124.53, 122.08, 120.43, 118.71, 117.49, 114.31, 113.07, 111.74, 110.37, 52.78, 11.95; HRMS (ESI): calcd for $C_{18}H_{17}N_4O_3S$ $[M+H]^+$: 369.1016, found: 369.1018; IR ν_{max}/cm^{-1} 3301 (N–H), 1732, 1645 (C=O), 1274, 1086 (C–O), 2953, 2854 (C–H), 3067, 1594, 1524 (Ar) (KBr).

*4.1.6.3. Methyl 5-[(5-fluoro-2-methyl-1H-indol-3-yl)sulfinyl]-1H-benzoimidazol-2-ylcarbamate (**8c**).* White solid; yield: 68%; M.p.: 275–280 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.94 (s, 3H), 7.75 (s, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.34 (dd, J_1 = 4.5 Hz, J_2 = 8.8 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 6.89 (td, J_1 = 2.1 Hz, J_2 = 9.3 Hz, 1H), 6.77 (dd, J_1 = 2.1 Hz, J_2 = 9.8 Hz, 1H), 3.76 (s, 3H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.10–158.42 (d, J = 233.3 Hz), 154.63, 148.78, 143.08, 136.90, 132.45, 124.83–124.94 (d, J = 10.9 Hz), 117.42, 114.45, 113.41, 113.36, 112.89–112.99 (d, J = 10.9 Hz), 109.95–110.21 (d, J = 23.6 Hz), 103.53–103.77 (d, J = 23.6 Hz), 52.79, 12.06; HRMS (ESI): (calcd for $C_{18}H_{16}FN_4O_3S$ $[M+H]^+$: 387.0922, found: 387.0925; IR ν_{max}/cm^{-1} 3363 (N–H), 1723, 1645 (C=O), 1260, 1108 (C–O), 2960, 2850 (C–H), 3033, 1593, 1527 (Ar) (KBr).

*4.1.6.4. Methyl 5-[(5-chloro-2-methyl-1H-indol-3-yl)sulfinyl]-1H-benzoimidazol-2-ylcarbamate (**8d**).* White solid; yield: 62%; M.p.: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (s, 1H), 11.86 (s, 2H), 7.76 (s, 1H), 7.50 (d, J = 8.2 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.13 (s, 1H), 7.06 (d, J = 8.6 Hz, 1H), 3.77 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 154.64, 148.80, 142.71, 136.87, 134.36, 131.68, 128.83, 125.54, 124.82, 122.04, 117.77, 117.25, 113.4, 112.99, 52.73, 12.01; HRMS (ESI): calcd for $C_{18}H_{16}ClN_4O_3S$ $[M+H]^+$: 403.0626, found: 403.0627; IR ν_{max}/cm^{-1} 3389 (N–H), 1720, 1645 (C=O), 1259, 1106 (C–O), 2961, 2868 (C–H), 3081, 1595, 1527 (Ar) (KBr).

*4.1.6.5. Methyl 5-[(5-bromo-1H-indol-3-yl)sulfinyl]-1H-benzoimidazol-2-ylcarbamate (**8e**).* White solid; yield: 63%; M.p.: 263–267 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H), 11.84 (s, 2H), 8.10 (s, 1H), 7.78 (s, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.44–7.45 (m, 2H), 7.26–7.31 (m, 2H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.16, 154.57, 148.83, 136.90, 136.00, 131.91, 131.69, 131.34, 128.86, 125.55, 125.20, 121.42, 117.52, 117.31, 114.91, 113.17, 52.80; HRMS (ESI): calcd for $C_{17}H_{14}BrN_4O_3S$ $[M+H]^+$: 432.9965, found: 432.9959; IR ν_{max}/cm^{-1} 3387 (N–H), 1730, 1646 (C=O), 1262, 1097 (C–O), 2957, 2860 (C–H), 3103, 1592, 1523 (Ar) (KBr).

*4.1.6.6. Methyl 5-[(5-cyano-1H-indol-3-yl)sulfinyl]-1H-benzoimidazol-2-ylcarbamate (**8f**).* White solid; yield: 55%; M.p.: 247–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.39 (s, 1H), 11.91 (s, 2H), 8.24 (s, 1H), 7.82 (s, 1H), 7.77 (s, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.51–7.54 (m, 2H), 7.34 (dd, J_1 = 1.5 Hz, J_2 = 8.3 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.55, 148.87, 139.04, 136.71, 132.06, 125.67, 124.33, 123.25, 120.01, 118.98, 117.48, 114.33, 110.20, 102.89, 52.77; HRMS (ESI): calcd for $C_{18}H_{14}N_5O_3S$ $[M+H]^+$: 380.0812, found: 380.0826; IR ν_{max}/cm^{-1} 3387, 3237 (N–H), 1721, 1646 (C=O), 1264, 1105 (C–O), 2955, 2850 (C–H), 3113, 1595, 1525 (Ar), 2225 (C≡N) (KBr).

*4.1.7. General procedure for the preparation of compounds **9a–f***

A stirred solution of **7a–f** (0.2 mmol) in THF (15 mL) was treated with *meta*-chloroperbenzoic acid (0.6 mmol) and stirred for 2 h at room temperature. The reaction was sequentially washed with water and saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product, which was purified by column chromatography on silica gel with dichloromethane/MeOH (v/v = 50:1).

*4.1.7.1. Methyl 5-[(1H-indol-3-yl)sulfonyl]-1H-benzoimidazol-2-ylcarbamate (**9a**).* White solid; yield: 72%; M.p.: 222–224 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.33 (s, 3H), 8.12 (s, 1H), 8.06 (s, 1H), 7.77 (d, J = 7.1 Hz, 1H), 7.66 (d, J = 5.4 Hz, 1H), 7.48–7.50 (d, J = 7.4 Hz, 2H), 7.16–7.22 (m, 2H), 3.73 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 154.42, 149.74, 136.60, 136.02, 131.01, 123.29, 123.21, 119.77, 118.84, 116.27, 112.97, 52.88; HRMS (ESI): calcd for $C_{17}H_{15}N_4O_4S$ $[M+H]^+$: 371.0809, found: 371.0805; IR ν_{max}/cm^{-1} 3412, 3348 (N–H), 1735, 1651 (C=O), 1275, 1097 (C–O), 2955, 2854 (C–H), 3105, 1595, 1527 (Ar) (KBr).

*4.1.7.2. Methyl 5-[(2-methyl-1H-indol-3-yl)sulfonyl]-1H-benzoimidazol-2-ylcarbamate (**9b**).* White solid; yield: 69%; M.p.: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.78 (s, 3H), 7.75 (s, 1H), 7.46 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.20 (d, J = 8.1 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 7.03 (t, J_1 = 7.9 Hz, J_2 = 14.9 Hz, 1H), 6.83 (t, J_1 = 7.9 Hz, J_2 = 14.9 Hz, 1H), 3.76 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.45, 149.71, 141.42, 136.92, 134.69, 125.07, 122.56, 121.53, 119.21, 118.79, 114.61, 112.40, 111.84, 111.37, 52.94; HRMS (ESI): calcd for $C_{18}H_{17}N_4O_4S$ $[M+H]^+$: 385.0965, found: 385.0962; IR ν_{max}/cm^{-1} 3390 (N–H), 1734, 1643 (C=O), 1260, 1087 (C–O), 2953, 2855 (C–H), 3067, 1592, 1528 (Ar) (KBr).

*4.1.7.3. Methyl 5-[(5-fluoro-2-methyl-1H-indol-3-yl)sulfonyl]-1H-benzoimidazol-2-ylcarbamate (**9c**).* White solid; yield: 62%; M.p.: 240–242 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ12.22 (s, 3H), 8.01 (s, 1H), 7.66 (dd, J_1 = 1.5 Hz, J_2 = 8.4 Hz, 1H), 7.55 (dd, J_1 = 2.5 Hz, J_2 = 9.9 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.37 (dd, J_1 = 4.6 Hz, J_2 = 8.8 Hz, 1H), 7.02 (td, J_1 = 2.5 Hz, J_2 = 9.3 Hz, 1H), 3.77 (s, 3H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.10–159.43 (d, J = 233.3 Hz), 154.45, 149.81, 143.14, 136.54, 131.26, 125.59–125.70 (d, J = 11.2 Hz), 119.19, 114.76, 113.15–113.25 (d, J = 11.2 Hz), 111.78, 111.74, 110.52–110.77 (d, J = 25.4 Hz), 103.75–104.00 (d, J = 25.4 Hz), 52.91, 12.79; HRMS (ESI): calcd for $C_{18}H_{16}FN_4O_4S$ $[M+Na]^+$: 425.0696, found: 425.0741; IR ν_{max}/cm^{-1} 3392, 3302 (N–H), 1731, 1642 (C=O), 1260, 1101 (C–O), 2955, 2871(C–H), 3073, 1587, 1528 (Ar) (KBr).

*4.1.7.4. Methyl 5-[(5-chloro-2-methyl-1H-indol-3-yl)sulfonyl]-1H-benzoimidazol-2-ylcarbamate (**9d**).* White solid; yield: 66%; M.p.: 262–264 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.30 (s, 1H), 11.90 (s,

2H), 8.01 (s, 1H), 7.83 (s, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 7.39 (d, J = 8.5 Hz, 1H), 7.18 (d, J = 8.5 Hz, 1H), 3.77 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.40, 149.75, 143.05, 136.35, 133.17, 131.66, 128.83, 126.19, 126.15, 122.56, 119.13, 117.81, 114.71, 113.52, 111.59, 111.33, 52.87, 12.69; HRMS (ESI): calcd for $C_{18}H_{16}ClN_4O_4S$ $[M+H]^+$: 419.0575, found: 419.0566; IR ν_{max}/cm^{-1} 3406 (N–H), 1733, 1643 (C=O), 1258, 1090 (C–O), 2957, 2850 (C–H), 3177, 1594, 1528 (Ar) (KBr).

*4.1.7.5. Methyl 5-[(5-bromo-1H-indol-3-yl)sulfonyl]-1H-benzoimidazol-2-ylcarbamate (**9e**).* White solid; yield: 60%; M.p.: 261–265 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.37 (s, 1H), 12.01 (s, 2H), 8.22 (s, 1H), 8.03 (s, 1H), 7.89 (d, J = 1.5 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 8.6 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.41, 149.92, 135.55, 135.32, 132.45, 125.93, 124.97, 120.87, 119.67, 115.91, 115.19, 114.58, 114.43, 112.56, 52.85; HRMS (ESI): calcd for $C_{17}H_{14}BrN_4O_4S$ $[M+H]^+$: 448.9914, found: 448.9926; IR ν_{max}/cm^{-1} 3386, 3286 (N–H), 1728, 1657 (C=O), 1266, 1095 (C–O), 2957, 2851 (C–H), 3080, 1594, 1523 (Ar) (KBr).

*4.1.7.6. Methyl 5-[(5-cyano-1H-indol-3-yl)sulfonyl]-1H-benzoimidazol-2-ylcarbamate (**9f**).* White solid; yield: 61%; M.p.: 272–276 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.64 (s, 1H), 11.90 (s, 2H), 8.38 (s, 1H), 8.22 (s, 1H), 8.09 (s, 1H), 7.76 (dd, J_1 = 1.4 Hz, J_2 = 8.4 Hz, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.61 (dd, J_1 = 1.2 Hz, J_2 = 8.5 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.40, 149.90, 138.38, 133.70, 126.11, 123.99, 123.02, 119.94, 117.50, 114.60, 112.38, 104.26, 52.89; HRMS (ESI): calcd for $C_{18}H_{14}N_5O_4S$ $[M+H]^+$: 396.0761, found: 396.0768; IR ν_{max}/cm^{-1} 3330 (N–H), 1729, 1653 (C=O), 1267, 1099 (C–O), 2955, 2854 (C–H), 3027, 1595, 1527 (Ar), 2225 (C≡N) (KBr).

*4.1.8. General procedure for the preparation of compounds **20a–f***

Selenium dioxide (6 mmol) was added with stirring to a solution of malononitrile (3 mmol) in DMSO (2 mL) at 35 °C. The mixture became reddish after 5 min, and an exothermic reaction with the evolution of gas began during the next 30 min. **14a–f** (4.5 mmol) were added to the reaction mixture after the termination of the exothermic reaction. The homogeneous solution was diluted with water (20 mL) after 10 min and extracted with ethyl acetate (20 mL × 4). The combined organic layers were washed with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product, which was purified by column chromatography on silica gel with light petroleum/AcOEt (v/v = 3:1).

*4.1.8.1. 3-Selenocyanato-1H-indole (**20a**).* Colourless solid; yield: 89%; M.p.: 73–75 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.89 (s, 1H), 7.88 (d, J = 2.6 Hz, 1H), 7.61 (d, J = 7.0 Hz, 1H), 7.52 (dd, J_1 = 7.1 Hz, J_2 = 1.1 Hz, 1H), 7.24 (m, 2H).

*4.1.8.2. 2-Methyl-3-Selenocyanato-1H-indole (**20b**).* Colourless solid; yield: 85%; M.p.: 110–115 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.83 (s, 1H), 7.49 (m, 1H), 7.41 (m, 1H), 7.17 (m, 2H), 2.55 (s, 3H).

*4.1.8.3. 5-Fluoro-2-methyl-3-selenocyanato-1H-indole (**20c**).* Colourless solid; yield: 88%; M.p.: 113–115 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.94 (s, 1H), 7.40 (dd, J_1 = 4.4 Hz, J_2 = 8.7 Hz, 1H), 7.18 (dd, J_1 = 2.7 Hz, J_2 = 9.6 Hz, 1H), 7.00 (td, J_1 = 2.5 Hz, J_2 = 9.6 Hz, 1H), 2.54 (s, 3H).

*4.1.8.4. 5-Chloro-2-methyl-3-selenocyanato-1H-indole (**20d**).* Colourless solid; yield: 83%; M.p.: 146–148 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.02 (s, 1H), 7.44 (d, J = 2.0 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.17 (dd, J_1 = 2.2 Hz, J_2 = 8.5 Hz, 1H), 2.54 (s, 3H).

*4.1.8.5. 5-Bromo-3-selenocyanato-1H-indole (**20e**).* Colourless solid; yield: 93%; M.p.: 140–142 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.06 (s, 1H), 7.93 (d, J = 1.48 Hz, 1H), 7.70 (d, J = 1.7 Hz, 1H), 7.49 (d, J = 8.6 Hz, 1H), 7.36 (dd, J_1 = 1.9 Hz, J_2 = 8.6 Hz, 1H).

*4.1.8.6. 5-Cyano-3-selenocyanato-1H-indole (**20f**).* Colourless solid; yield: 92%; M.p.: 161–163 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.38 (s, 1H), 8.09 (s, 1H), 8.07 (s, 1H), 7.70 (d, J = 7.9 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H).

*4.1.9. General procedure for the preparation of compounds **23a–f***

To a stirred solution of **20a–f** (1 mmol), obtained as described above, in ethanol (20 mL), sodium borohydride (2 mmol) was carefully added, and then the reaction mixture was stirred at 30 °C. Upon completion (monitored by TLC), *N*-(5-chloro-2-nitrophenyl) acetamide (1 mmol) was added and reacted for another 2–4 h at room temperature under a nitrogen atmosphere. Then, sodium (0.05 mmol) was added to the mixture, and the temperature was increased to 75 °C. After 1.5 h, the solvent was removed under reduced pressure. Then, the residue was extracted with ethyl acetate, washed with water, dried over anhydrous $MgSO_4$ and concentrated under vacuum to afford the crude product, which was purified by column chromatography on silica gel with light petroleum/AcOEt (v/v = 3:1).

*4.1.9.1. 5-[(1H-indol-3-yl)selanyl]-2-nitroaniline (**23a**).* Yellow solid; yield: 53%; M.p.: 127–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.82 (s, 1H), 7.79 (d, J = 2.7 Hz, 1H), 7.76 (d, J = 9.1 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.41 (d, 1H), 7.36 (s, 2H), 7.20 (m, 1H), 7.10 (m, 1H), 6.71 (d, J = 1.8 Hz, 1H), 6.38 (dd, J_1 = 1.8 Hz, J_2 = 9.1 Hz, 1H).

*4.1.9.2. 5-[(2-Methyl-1H-indol-3-yl)selanyl]-2-nitroaniline (**23b**).* Yellow solid; yield: 49%; M.p.: 50–54 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (s, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.37 (s, 2H), 7.30 (d, J = 7.8 Hz, 1H), 7.12 (m, 1H), 7.03 (m, 1H), 6.65 (d, J = 1.8 Hz, 1H), 6.35 (dd, J_1 = 1.8 Hz, J_2 = 9.0 Hz, 1H), 2.47 (s, 3H).

*4.1.9.3. 5-[(5-Fluoro-2-methyl-1H-indol-3-yl)selanyl]-2-nitroaniline (**23c**).* Yellow solid; yield: 45%; M.p.: 52–56 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.89 (s, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.41 (m, 1H), 7.36 (s, 2H), 6.96 (m, 2H), 6.63 (d, J = 1.7 Hz, 1H), 6.36 (dd, J_1 = 1.7 Hz, J_2 = 9.0 Hz, 1H), 2.46 (s, 3H).

*4.1.9.4. 5-[(5-Chloro-2-methyl-1H-indol-3-yl)selanyl]-2-nitroaniline (**23d**).* Yellow solid; yield: 51%; M.p.: 157–160 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.98 (s, 1H), 7.77 (d, J = 9.1 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H), 7.38 (s, 2H), 7.25 (d, J = 2.0 Hz, 1H), 7.12 (dd, J_1 = 2.0 Hz, J_2 = 8.6 Hz, 1H), 6.62 (d, J = 1.8 Hz, 1H), 6.36 (dd, J_1 = 1.8 Hz, J_2 = 9.1 Hz, 1H), 2.47 (s, 3H).

*4.1.9.5. 5-[(5-Bromo-1H-indol-3-yl)selanyl]-2-nitroaniline (**23e**).* Yellow solid; yield: 63%; M.p.: 153–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.03 (s, 1H), 7.86 (d, J = 2.3 Hz, 1H), 7.78 (d, J = 9.1 Hz, 1H), 7.51 (d, 1H), 7.49 (s, 1H), 7.38 (s, 2H), 7.32 (dd, J_1 = 1.9 Hz, J_2 = 8.6 Hz, 1H), 6.67 (d, J = 1.8 Hz, 1H), 6.38 (dd, J_1 = 1.8 Hz, J_2 = 9.1 Hz, 1H).

*4.1.9.6. 5-[(5-Cyano-1H-indol-3-yl)selanyl]-2-nitroaniline (**23f**).* Yellow solid; yield: 61%; M.p.: 194–198 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.37 (s, 1H), 8.02 (s, 1H), 7.83 (d, J = 0.7 Hz, 1H), 7.77 (d, J = 9.1 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.56 (dd, J_1 = 1.5 Hz, J_2 = 8.5 Hz, 1H), 7.37 (s, 2H), 6.66 (d, J = 1.8 Hz, 1H), 6.39 (dd, J_1 = 1.8 Hz, J_2 = 9.1 Hz, 1H).

*4.1.10. General procedure for the preparation of compounds **24a–f***

A mixture of **23a–f** (0.5 mmol), active carbon (2 mmol), ferric chloride anhydrous (0.1 mmol), and methanol (20 mL) was refluxed for 10 min with stirring, and then, hydrazine hydrate (80% purity, 0.7 mL) was added dropwise to the mixture. The mixture was stirred for an additional 3 h under refluxing until **23a–f** disappeared. The reaction mixture was filtered to remove the active carbon, and the filtrate was concentrated under reduced pressure. Next, the filtrate was extracted with ethyl acetate, washed with water, and concentrated under vacuum to afford the crude product, which was used without further purification.

*4.1.11. General procedure for the preparation of compounds **10a–f***

A mixture of **24a–f** (0.5 mmol) and 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseud-ourea (0.5 mmol) in ethanol (5 mL) was refluxed for 2–4 h. The solvent was removed under reduced pressure to afford the crude product, which was purified by chromatography on silica gel plates to afford pure product.

*4.1.11.1. Methyl 5-[(1H-indol-3-yl)selanyl]-1H-benzoimidazol-2-ylcarbamate (**10a**).* White solid; yield: 45%; M.p.: 125–128 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 3H), 7.71 (s, 1H), 7.44–7.47 (m, 2H), 7.27 (s, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.15 (t, J_1 = 7.4 Hz, J_2 = 15.0 Hz, 1H), 7.03–7.05 (m, 2H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.99, 147.71, 136.78, 132.38, 131.69, 129.78, 128.84, 124.29, 122.71, 122.06, 120.04, 119.36, 114.67, 112.18, 97.03, 52.57; HRMS (ESI): calcd for $C_{17}H_{15}N_4O_2Se$ $[M+H]^+$: 387.0355, found: 387.348; IR ν_{max}/cm^{-1} 3336 (N–H), 1709, 1634 (C=O), 1272, 1099 (C–O), 3073, 2950, 2850 (C–H), 1591, 1523 (Ar) (KBr).

*4.1.11.2. Methyl 5-[(2-methyl-1H-indol-3-yl)selanyl]-1H-benzoimidazol-2-ylcarbamate (**10b**).* White solid; yield: 49%; M.p.: 157–160 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.58 (s, 3H), 7.39 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.22 (d, J = 8.3 Hz, 1H), 7.19 (s, 1H), 7.08 (m, 1H), 7.00 (m, 2H), 3.72 (s, 3H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.02, 147.71, 141.46, 136.96, 136.12, 135.37, 130.92, 124.50, 122.47, 121.36, 119.86, 118.78, 114.58, 114.21, 111.24, 95.56, 52.57, 12.96; HRMS (ESI): calcd for $C_{18}H_{17}N_4O_2Se$ $[M+H]^+$: 401.0511, found: 401.0507; IR ν_{max}/cm^{-1} 3353 (N–H), 1711, 1639 (C=O), 1272, 1100 (C–O), 2950, 2851 (C–H), 3057, 1592, 1524 (Ar) (KBr).

*4.1.11.3. Methyl 5-[(5-fluoro-2-methyl-1H-indol-3-yl)selanyl]-1H-benzoimidazol-2-ylcarbamate (**10c**).* White solid; yield: 44%; M.p.: 154–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.64 (s, 3H), 7.35 (dd, J_1 = 4.5 Hz, J_2 = 8.7 Hz, 1H), 7.25 (d, J = 8.2 Hz, 1H), 7.20 (s, 1H), 7.01–7.02 (m, 2H), 6.91 (td, J_1 = 2.6 Hz, J_2 = 9.5 Hz, 1H), 3.71 (s, 3H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.74, 156.58–158.89 (d, J = 232.7 Hz), 155.11, 147.88, 143.62, 132.63, 131.62–131.72 (d, J = 10.1 Hz), 124.02, 122.61, 114.66, 114.34, 112.26–112.36 (d, J = 10.1 Hz), 109.08–109.34 (d, J = 24.5 Hz), 103.36–103.60 (d, J = 24.5 Hz), 95.82, 95.78, 52.59, 13.05; HRMS (ESI): calcd for $C_{18}H_{16}FN_4O_2Se$ $[M+H]^+$: 419.0417, found: 419.0408; IR ν_{max}/cm^{-1} 3388, 3371 (N–H), 1730, 1639 (C=O), 1258, 1097 (C–O), 2957, 2863 (C–H), 3055, 1591, 1527 (Ar) (KBr).

*4.1.11.4. Methyl 5-[(5-chloro-2-methyl-1H-indol-3-yl)selanyl]-1H-benzoimidazol-2-ylcarbamate (**10d**).* White solid; yield: 46%; M.p.: 170–175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.82 (s, 1H), 11.55 (s, 2H), 7.37 (d, J = 8.5 Hz, 1H), 7.32 (s, 1H), 7.24 (d, J = 8.2 Hz, 1H), 7.20 (s, 1H), 7.07 (d, J = 8.5 Hz, 1H), 7.00 (d, J = 8.2 Hz, 1H), 3.71 (s, 3H), 2.53 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.04, 147.99, 143.51, 137.07, 135.75, 134.63, 132.28, 124.62, 123.89, 122.51, 121.27, 117.77, 114.73, 114.32, 112.90, 95.43, 52.58, 12.99; HRMS (ESI): calcd for $C_{18}H_{16}ClN_4O_2Se$ $[M+H]^+$: 435.0122, found: 435.0134; IR ν_{max}/cm^{-1} 3346 (N–H), 1710, 1639 (C=O), 1271, 1100 (C–O), 2950, 2850 (C–H), 3068, 1591, 1523 (Ar) (KBr).

*4.1.11.5. Methyl 5-[(5-bromo-1H-indol-3-yl)selanyl]-1H-benzoimidazol-2-ylcarbamate (**10e**).* White solid; yield: 50%; M.p.: 144–146 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.82 (s, 1H), 11.51 (s, 2H), 7.80 (d, J = 2.1 Hz, 1H), 7.54 (d, J = 1.6 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 7.24–7.28 (m, 3H), 7.05 (dd, J_1 = 1.6 Hz, J_2 = 8.2 Hz, 1H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.44, 154.99, 147.89, 137.05, 135.60, 134.09, 131.80, 124.72, 123.88, 122.85, 121.47, 114.79, 114.67, 114.41, 112.84, 96.77, 52.64; HRMS (ESI): calcd for $C_{17}H_{14}BrN_4O_2Se$ $[M+H]^+$: 464.9460, found: 464.9451; IR ν_{max}/cm^{-1} 3335 (N–H), 1707, 1635 (C=O), 1270, 1099 (C–O), 2949, 2868 (C–H), 3067, 1591, 1523 (Ar) (KBr).

*4.1.11.6. Methyl 5-[(5-cyano-1H-indol-3-yl)selanyl]-1H-benzoimidazol-2-ylcarbamate (**10f**).* White solid; yield: 46%; M.p.: 250–253 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.16 (s, 1H), 11.56 (s, 2H), 7.96 (s, 1H), 7.87 (s, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.32 (s, 1H), 7.26 (d, J = 8.2 Hz, 1H), 7.10 (d, J = 8.2 Hz, 1H), 3.72 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 154.96, 147.86, 138.75, 137.11, 135.87, 135.03, 129.73, 124.93, 124.67, 123.35, 123.30, 120.51, 115.40, 114.65, 113.73, 102.32, 98.70, 52.63; HRMS (ESI): calcd for $C_{18}H_{14}N_5O_2Se$ $[M+H]^+$: 412.0307, found: 412.0318; IR ν_{max}/cm^{-1} 3382 (N–H), 1722, 1641 (C=O), 1262, 1097 (C–O), 2955, 2869 (C–H), 3020, 1591, 1525 (Ar), 2225 (C≡N) (KBr).

4.2. Biological evaluation

4.2.1. Cell culture

The human gastric adenocarcinoma cell line SGC-7901, the human fibrosarcoma cell line HT-1080 and the human pulmonary carcinoma cell line A-549 were cultured in RPMI-1640 medium containing 10% FBS, 100 U/mL streptomycin and 100 U/mL penicillin at 37 °C in humidified atmosphere with 5% CO_2. All of the cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA).

4.2.2. Antiproliferative activity assay

The *in vitro* antiproliferative activities of nocodazole and all of the target compounds were determined by an MTT (Sigma) assay. Briefly, cells were seeded into 96-well plates at a density of 1–3 × 10^4/well (depends on the cell growth rate). 24 h later, triplicate wells were treated with media and the agents. After 72 h of incubation at 37 °C in 5% CO_2, the drug containing medium was removed and replaced by 100 μL of fresh medium with 5 mg/mL MTT solution. After 4 h of incubation, the medium with MTT was removed, and 100 μL of dimethyl sulfoxide (DMSO) was added to each well. The plates were gently agitated until the purple formazan crystals were dissolved, and the OD_{490} was determined using a microplate reader (MK3, Thermo, Germany). The data were calculated and plotted as the percent viability compared to the control. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the vehicle in the MTT assay.

4.2.3. In vitro tubulin polymerisation assay

The effects of compounds **10a** and **7e** on the polymerisation of tubulin were determined by employing a fluorescence-based tubulin polymerisation assay kit (Cytoskeleton-Cat.# BK011P) according to the manufacturer's protocol. Tubulin was re-suspended in ice cold G-PEM buffer (80 mM PIPES, 2 mM $MgCl_2$, 0.5 mM EGTA, 1 mM GTP, 20% (v/v) glycerol) and added to wells on a 96-well plate containing the designated concentration of the drug or

vehicle. Samples were mixed well, and tubulin assembly was monitored (emission wavelength of 420 nm; excitation wavelength pf 360 nm) at 1 min intervals for 90 min at 37 °C using a plate reader (FASCalibur, BD Biosciences, USA). IC_{50} values were calculated at 20 min using SPSS software.

4.2.4. Immunofluorescence staining

Immunostaining was carried out to detect microtubule associated tubulin protein after exposure to nocodazole and the investigated compound **10a**. The SGC-7901 and HT-1080 cells were seeded at 1×10^4 per well on a 24-well plate and grown for 24 h. Cells were treated with the vehicle or the 2-fold IC_{50} concentration of nocodazole or **10a** for 48 h. The control and treated cells were fixed with 4% formaldehyde in PBS for 30 min at −20 °C, washed twice with PBS and permeabilised with 0.1% (*v*/*v*) Triton X-100 in PBS for 5 min. Then, the cells were blocked with 5% bovine serum albumin (BSA) in PBS for 10 min. The primary α-tubulin antibody (Santa Cruz, CA) was diluted (1:100) with 2% BSA in PBS and incubated overnight at 4 °C. The cells were washed with PBS to remove unbound primary antibody, and then, the cells were incubated with FITC-conjugated anti-mouse secondary antibody, diluted (1:1000) with 2% BSA in PBS, for 3 h at 37 °C. The cells were washed with PBS to remove unbound secondary antibody, the nuclei were stained with 4,6-diamino-2-phenolindol dihydrochloride (DAPI) and then, immunofluorescence was detected using a fluorescence microscope (Olympus, Tokyo, Japan).

4.3. Molecular modelling

The molecular modelling studies were performed with Accelrys Discovery Studio 3.0. The crystal structure of tubulin complexed with DAMA-colchicine (PDB: 1SA0) was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb). In the docking process, the protein protocol was prepared by several operations, including standardization of atom names, insertion of missing atoms in residues and removal of alternate conformations, insertion of missing loop regions based on SEQRES data, optimization of short and medium size loop regions with Looper Algorithm, minimization of remaining loop regions, calculation of pK, and protonation of the structure. Then, the receptor model was typed with the CHARMm force field and a binding sphere with radius of 15.0 Å was defined through the original ligand (DAMA-colchicine) as the binding site. The nocodazole and **10a** were drawn with Chemdraw and fully minimized using the CHARMm force field. Finally, they were docked into the binding site using the CDOCKER protocol with the default settings.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.071. These data include MOL files and InChiKeys of the most important compounds described in this article.

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