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Efficient Synthesis and Biological Activity of Novel Indole Derivatives as VEGFR-2 Tyrosine Kinase Inhibitors[1]

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Abstract—A series of novel indole derivatives were synthesized as potent inhibitors for the vascular endothelial growth factor receptor 2 (VEGFR-2) tyrosine kinase. Among those, compound **10b** demonstrated the highest growth inhibition rate of 66.7% against the VEGFR-2 tyrosine kinase at 10 μM which indicates that indole-benzothiazole might be the favorable structure. The binding mode of compound **10b** with VEGFR-2 tyrosine kinase was evaluated by molecular docking.

Keywords: indole derivatives, VEGFR-2 tyrosine kinase; growth inhibition, indole-benzothiazole

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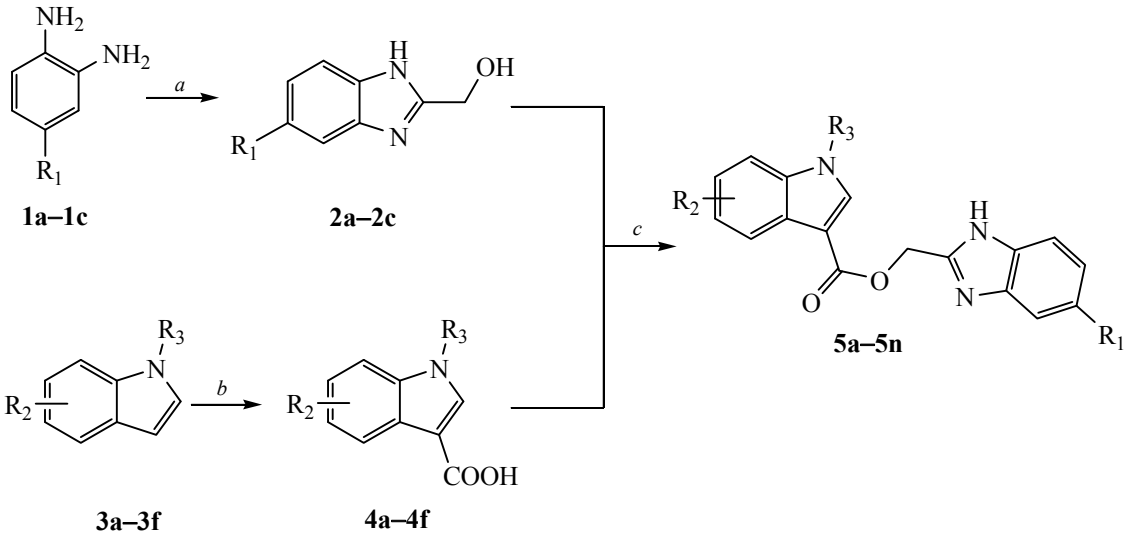
Angiogenesis refers to the process of new blood vessels generation from the vascular system and it is an important mechanism in many physiological and pathological process, and it closely correlates with the proliferation, development and metastasis of tumor cells. Angiogenesis is precisely controlled by a series of signal transduction pathways, and the one mediated by vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor (VEGFR) is crucial to angiogenesis. It has been demonstrated by pathological investigation that upon reaching 1 to 2 mm across, a solid tumor needs to grow its own blood vessels in order to supply oxygen and nutrients and to remove cellular waste [1, 2]. VEGF, secreted by tumor cells, can support new vessels growth and maintenance of a vascular network and culminate in promotion of metastatic spread of primary tumor cells to distant sites in the body [3]. It has been shown that VEGFR-2 is the major receptor for angiogenesis [4]. Inhibiting signal transduction via the VEGF/VEGFR system is a promising approach to starve the tumor cells and thus impede tumor growth [5, 6] and metastasis [7]. Some small molecule inhibitors, such as Sorafenib [8] (Raf kinase and KDR dual inhibitors), Imatinib [9] and Gefitinib [10], targeted VEGFR-2 protein tyrosine kinase (PTK) have been approved in clinical treatment of cancer. Therefore, VEGFR-2 protein tyrosine kinase is recognized as one of the critical targets for anti-cancer drugs.

Indole skeleton is one of privileged molecular scaffolds of pharmaceuticals [11]. In particular, 3-substituted indole derivatives play a key role in many biologically active compounds, especially those with anticancer activity [12–14]. For example, indole derivative Sunitinib [15] is an oral VEGFR-2 protein tyrosine kinase inhibitor approved in 2006 for treatment of patients with renal cell carcinoma (RCC) [16]. Thus, it is important to synthesize new specific indole derivatives for the development of anticancer drugs.

As a part of our ongoing program of discovery of compounds with superior inhibition activity against VEGFR-2 Tyrosine Kinases, linked-fragment strategy was employed to design novel VEGFR-2 tyrosine kinase inhibitors. The compound data base was built on the basis of its crystal structure. After absorption, distribution, metabolism, excretion, and toxicity (ADMET) screening, there was singled out a promising series of indole derivatives of VEGFR-2 tyrosine kinase inhibiting activity. The convenient synthetic method for the novel indole derivatives was developed and VEGFR-2 inhibition activity of those was evaluated. We also explored the binding model and binding free energies of newly synthesized compound 10b with VEGFR-2 tyrosine kinase by molecular docking.

[1] The text was submitted by the authors in English.

Scheme 1. Synthesis of compounds **5a–5n.**



R_1 = H (**1a, 2a**), NO_2 (**1b, 2b**), CH_3 (**1c, 2c**); R_2 = H (**3a, 3f, 4a, 4f**), 5-Br (**3b, 4b**), 5-OCH_3 (**3c, 4c**), 5-NO_2 (**3d, 4d**); 2-CH_3 (**2e, 4e**); R_3 = H (**3a–3e, 4a–4e**); CH_3 (**3f, 4f**); (*a*): $HOCH_2COOH$, concentrated phosphoric acid, 130°C, 3 h; (*b*): $(CF_3CO)_2O$, 0°C, DMF, room temperature, 3.5 h, then 20% NaOH solution, 55°C, 6 h; (*c*): DCC, DMAP, THF, room temperature, 7–9 h.

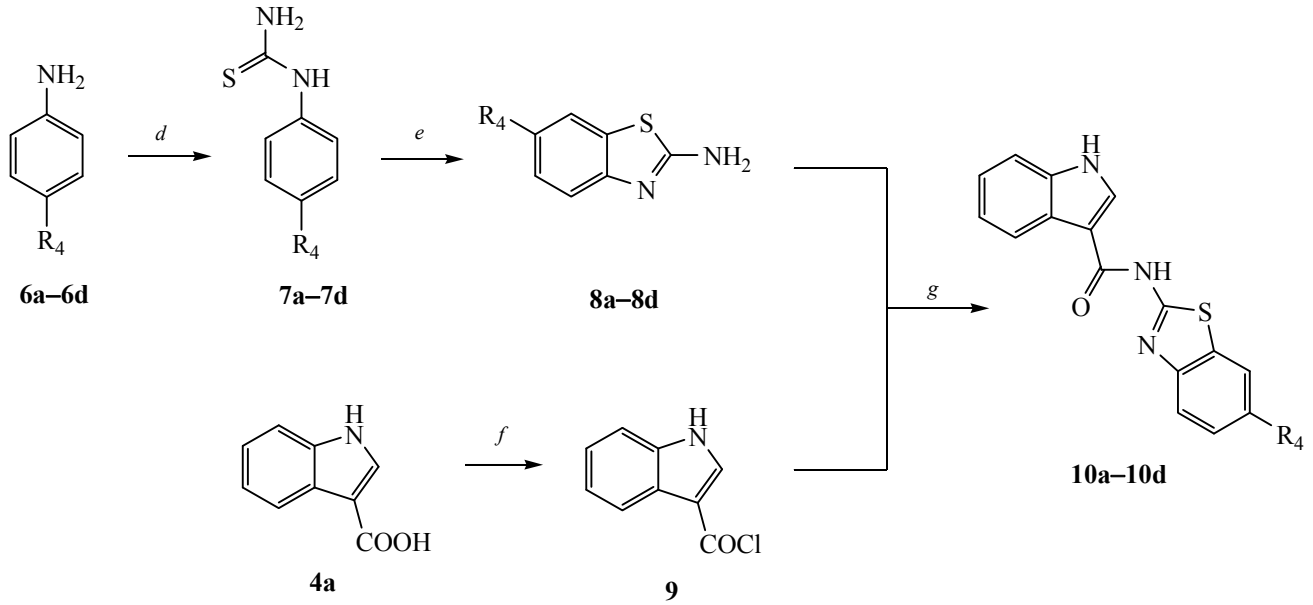
RESULTS AND DISCUSSION

Synthesis. 2-Hydroxymethyl benzimidazole derivatives **2a–2c** were synthesized by the reaction of 4-substituted *o*-phenylenediamine **1a–1c** with hydroxyacetic acid in the presence of concentrated phosphoric acid (Scheme 1). Synthesis of indole-3-carboxylic acid derivatives **4a–4f** involved the reaction of indole derivatives **3a–3f** with trifluoroacetic anhydride in the presence of *N,N*-dimethylformamide (DMF), which was followed by the reaction with 20% NaOH [17]. The compounds **4a–4f** were esterified with **2a–2c** in the presence of dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC) to yield (1*H*-benzoimidazol-2-yl)methyl-1*H*-indole-3-carboxylate derivatives **5a–5n**. As outlined in Scheme 2, *N*-(benzothiazol-2-yl)-1*H*-indole-3-carboxamide derivatives **10a–10d** were obtained from the intermediates 1*H*-indole-3-carbonyl chloride **9** and benzothiazol-2-amine derivatives **8a–8d** via the amidation reaction catalyzed by TEA. Indole derivatives **11a–11d** reacted with phosphorus oxychloride (Scheme 3) giving indole-3-carbaldehyde derivatives (**12a–12d**), that were reduced to (1*H*-indol-3-yl)methanol derivatives **13a–13d** by $NaBH_4$. 2-(Chloromethyl)-1*H*-benzoimidazole derivatives **15a, 15b** were formed upon cyclization of *N*-substituted *o*-phenyl-

Indole derivatives action against VEGFR-2 Tyrosine Kinases at 10 μM

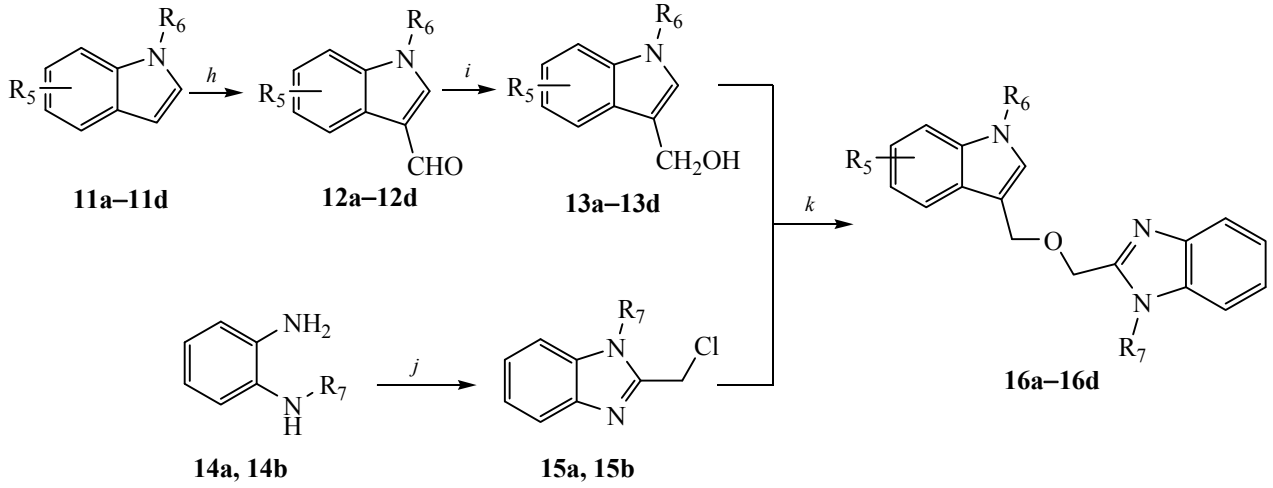
Comp. no.	Inhibition, %	Comp. no.	Inhibition, %	Comp. no.	Inhibition, %
5a	22.2	**5j**	6.0	**16a**	23.3
5b	12.0	**5k**	14.6	**16b**	21.7
5c	8.0	**5l**	29.4	**16c**	18.2
5d	10.0	**5m**	33.4	**16d**	16.1
5e	10.5	**5n**	31.8	**23a**	6.7
5f	21.5	**10a**	21.7	**23b**	8.3
5g	11.7	**10b**	66.7	**24a**	17.4
5h	9.5	**10c**	31.2	**24b**	20.4
5i	32.5	**10d**	51.4	SU11248	98.1

Scheme 2. Synthesis of compounds **10a**–**10d**.



11a: R_5 = H (**11a, 12a, 13a**); 5-OCH_3 (**11b, 12b, 13b**), R_6 = H (**11a, 12a, 13a, 11b, 11d, 12b–12d**); CH_2CH_3 (**11c, 12c, 13c**); CH_2Ph (**11d, 12d, 13d**); R_7 = H (**14a, 15a**); CH_3 (**14b, 15b**). (*d*): $C_6H_5NH_2$, NH_4SCN, CH_3COOH, 4 h; (E). Br_2, 0°C, 15°C, 2 h; (*f*): $SOCl_2$, reflux, 5 h; (*g*): CH_2Cl_2, Et_3N, 30°C, 18 h.

Scheme 3. Synthesis of compounds **16a**–**16d.**

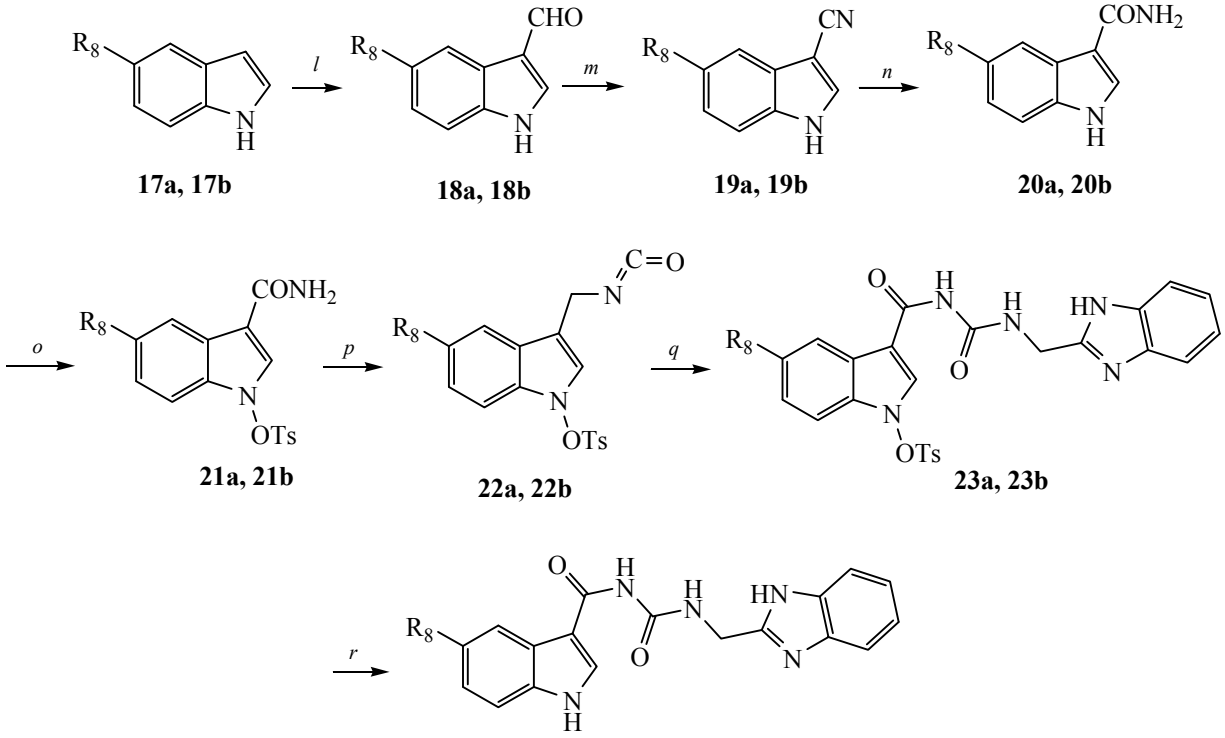


R_5 = R_6 = H (**11a, 11c, 11d, 12a, 12c, 12d, 13a, 13c, 13d**), **b**: R_5 = 5-OCH_3 (**11b, 12b, 13b**), R_6 = CH_2CH_3 (**11c, 12c, 13c**); R_6 = CH_2Ph (**11d, 12d, 13d**); **14a**: R_7= H (**14a, 15a**); CH_3 (**14b, 15b**); (*h*): $POCl_3$, DMF, 0°C, 55°C, 2 h; (*i*): $NaBH_4$, CH_3CH_2OH/THF, room temperature; (*j*): $ClCH_2COOH$, 4 N HCl, reflux, 3 h; (*k*): $(CH_3)_2CO$, K_2CO_3, reflux, 8–20 h.

enediamine **14a, 14b** under the action of chloro-acetic acid. The desired 2-{[(1*H*-indol-3-yl)methoxy]methyl}-1*H*-benzoimidazole derivatives **16a–16d** were synthesized by a reaction of **15a, 15b** with **13a–13d** using potassium carbonate as the absorb acid agent. *N*-{[(1*H*-Benzoimidazol-2-yl)methyl]carbamoyl}-1*H*-indole-3-carboxamide derivatives **24a, 24b** were obtained upon hydrolysis of the corresponding tosyl derivatives **23a, 23b** (Scheme 4).

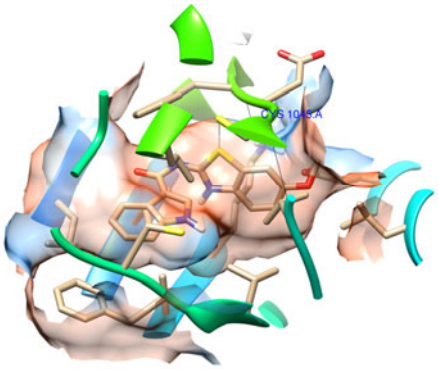
In vitro inhibition activity of VEGFR-2. The newly synthesized indole derivatives (5a–**5n, 10a–10d, 16a–16d**, and **24a, 24b**) were evaluated for their ability to inhibit VEGFR-2 tyrosine kinase (see the table).

Scheme 4. Synthesis of compounds **20a**, **20b**.



R_8 = H (**17a–23a**); OCH_3 (**17b–23b**); (*l*): $POCl_3$, DMF, 0°C, then 55°C, 2 h; (*m*): $NH_4OH \cdot HCl$, DMF, 110°C; (*n*): 40% NaOH, 30% H_2O_2, EtOH, room temperature; (*o*): $CH_3C_6H_4SO_2Cl$, $(C_4H_9)_2NHSO_4$, NaOH, CH_2Cl_2; (*p*): $(COCl)_2$, $ClCH_2 \cdot CH_2Cl$, 0°C, then 45°C, 3 h, 70°C, 2 h: (*q*). (1*H*-benzo[*d*]imidazol-2-yl)methanamine, DMF, 70°C, 1 h; (*r*): 30% NaOH, $(C_4H_9)_2NHSO_4$, THF, 35°C.

Among them, compounds **10b** and **10d** demonstrated the highest inhibition with the proliferative inhibition rates of 66.7 and 51.4% at 10 μM, respectively. The presence of the methoxy group in the benzothiazole ring 10b coursed a significant increase in VEGFR-2 inhibition activity relative to **10a**, while the presence of chlorine in the same position **10c** promoted a small increase in VEGFR-2 inhibition activity. Compounds **5a–5n, 16a–16d, 24a, 24b** showed poor VEGFR-2 inhibition activity, which indicated that indole-benzothiazole derivatives were more favorable than the indole-benzimidazole derivatives. So, the structure optimization might be carried out on the basis of indole-benzothiazole rings for developing compounds with superior VEGFR-2 inhibition activity.



Docking model of compound **10b** with VEGFR-2 tyrosine kinase.

Molecular docking study. Molecular docking was used to examine the binding mode of compound **10b** to VEGFR-2 tyrosine kinase (see the figure). The docking results demonstrated that the VEGFR-2 tyrosine kinase-10b complex was stabilized by the hydrogen bond between Cys 1045 amino acid residues of the protein and compound 10b. Free energy change (DG) obtained for the lowest energy conformation was determined to be –8.8 kcal/mol.

EXPERIMENTAL

All solvents and reagents used were obtained from commercial sources without further purification. TLC

and silica gel column chromatography were carried out with silica gel GF_{254} and 200-300 mesh, respectively (Qingdao Haiyang Chemical Co., Ltd., China). Melting points were determined on a digital melting-point apparatus and were uncorrected. ^{1}H NMR spectra were measured on a Bruker Avance DMX 500 M Hz spectrometer, using TMS as the internal standard and DMSO-d_6 as the solvent. High-resolution electronic ionization mass spectra were measured on a Waters GCT Premie spectrometer. Purity of compounds was determined by HPLC). All products had purity higher than 95%.

Synthesis of 2-hydroxymethyl benzimidazole derivatives (2a–2c). To a mixture of **1a–1c** (10 mmol) with glycolic acid (2.28 g, 30 mmol) was added concentrated H_3PO_4 (20 mL). The reaction mixture was refluxed at 130°C for 3 h, then quenched with 20% NaOH. The respective solid product was collected by filtration.

(1*H*-Benzoimidazole-2-yl)methanol (2a). White solid, yield 81%, mp 169–171°C (177–179°C [18]). ^{1}H NMR spectrum, δ, ppm: 4.69 s (2H, CH_2), 5.69 s (1H, OH), 7.12–7.52 m (4H, ArH), 12.31 s (1H, NH).

(5-Nitro-1*H*-benzoimidazol-2-yl)methanol (2b). Brown solid, yield 87%, mp 191–194°C (196–198°C [19]). ^{1}H NMR spectrum, δ, ppm: 4.77 s (2H, CH_2), 7.66 d (J = 8.5 Hz, 1H, ArH), 8.08 d (J = 8.5 Hz, 1H, ArH), 8.38 s (1H, ArH), 13.12 s (1H, NH).

(5-Methyl-1*H*-benzoimidazol-2-yl)methanol (2c). Yellow solid, yield 72%, mp 200–201°C (202–203°C [19]). ^{1}H NMR spectrum, δ, ppm: 2.38 s (3H, CH_3), 4.65 s (2H, CH_2), 5.72 s (1H, OH), 6.94 s (1H, ArH), 7.32 d (J = 8.5 Hz, 2H, ArH), 12.17 s (1H, NH).

Synthesis of indole-3-carboxylic acid derivatives (4a–4f). One of compounds **3a–3f** (20 mmol) was dissolved in DMF (10 mL). Trifluoroacetic anhydride (4.2 mL, 30 mmol) was added dropwise at 0°C. After stirring for 3.5 h, water was added, the solid filtered off and treated with 20% NaOH (40 mL, 0.2 mol) at 55°C overnight. Upon cooling down, the solution was extracted with Et_2O. The aqueous phase was acidified with concentrated HCl and the residue was filtered off to give one of compounds **4a–4f**.

1*H*-Indole-3-carboxylic acid (4a). White solid, yield 81%, mp 216–218°C (214°C [20]). ^{1}H NMR spectrum, δ, ppm: 7.15 t (J = 8.0 Hz, 2H, ArH), 7.45 d (J = 8.0 Hz, 2H, ArH), 8.00 s (1H, CH–N), 11.81 s (1H, COOH), 11.93 s (1H, NH).

5-Bromo-1*H*-indole-3-carboxylic acid (4b). Light yellow solid, yield 89%, mp 236–238°C (238–240°C [20]). ^{1}H NMR spectrum, δ, ppm: 7.31 d (J = 8.5 Hz, 1H, ArH), 7.44 d (J = 8.5 Hz, 1H, ArH), 8.05 s (1H, CH–N), 8.14 s (1H, ArH), 12.01 s (1H, COOH), 12.18 s (1H, NH).

5-Methoxy-1*H*-indole-3-carboxylic acid (4c). White solid, yield 82%, mp 173–174°C. ^{1}H NMR spectrum, δ, ppm: 3.78 s (3H, OCH_3), 6.81 d (J = 6.5 Hz, 1H, ArH), 7.35 d (J = 8.5 Hz, 1H, ArH), 7.48 s (1H, CH–N), 7.93 s (1H, ArH), 11.69 s (1H, COOH), 11.88 s (1H, NH).

5-**Nitro-1*H*-indole-3-carboxylic acid (4d).** Light green solid, yield 82%, mp 277–279°C (276–278°C [20]). ^{1}H NMR spectrum, δ, ppm: 7.66 d (J = 8.5 Hz, 1H, ArH), 8.09 d (J = 7.5 Hz, 1H, ArH), 8.28 s (1H, CH–N), 8.89 s (1H, ArH), 12.46 s (1H, COOH), 12.50 s (1H, NH).

2-Methyl-1*H*-indole-3-carboxylic acid (4e). Pink solid, yield 68%, mp 173–174°C (175–176°C [21]). ^{1}H NMR spectrum, δ, ppm: 2.64 s (3H, CH_3), 7.08 t (J = 8.5 Hz, 2H, ArH), 7.32 d (J = 8.5 Hz, 1H, ArH), 7.92 d (J = 8.5 Hz, 1H, ArH), 11.75 s (1H, COOH), 11.87 s (1H, NH).

1-Methyl-1*H*-indole-3-carboxylic acid (4f). Pink solid, yield 81%, mp 204–206°C (200–201°C [22]). ^{1}H NMR spectrum, δ, ppm: 3.74 s (3H, CH_3), 7.12 t (J = 8.5 Hz, 2H, ArH), 7.39 d (J = 8.5 Hz, 1H, ArH), 7.64 d (J = 8.5 Hz, 1H, ArH), 8.35 s (1H, CH–N), 11.50 s (1H, COOH).

Synthesis of (1*H*-benzoimidazol-2-yl)methyl-1*H*-indole-3-carboxylate derivatives (5a–5n). One of compounds **4a–4f** (5 mmol) was dissolved in THF (25 mL) and DCC (1.24 g, 6 mmol), and DMAP (0.12 g, 1 mmol) was slowly added and the mixture was stirred for 30 min. Compounds **2a–2c** (5 mmol) was added and the mixture was stirred for 7–9 h. The by-product, *N,N*'-dicyclohexylurea, was removed by filtration. The filtrate was concentrated in vacuum to give a solid which was dissolved in CH_2Cl_2 and the product **5a–5n** was filtered off.

(1*H*-Benzoimidazol-2-yl)methyl-1*H*-indole-3-carboxylate (5a). Light green solid, yield 90%, mp 190–192°C. ^{1}H NMR spectrum, δ, ppm: 5.49 s (2H, CH_2), 7.18 m (4H, ArH), 7.50 t (J = 5.0 Hz, 2H, ArH), 7.61 d (J = 7.5 Hz, 1H, ArH), 8.01 d (J = 7.2 Hz, 1H, ArH), 8.21 s (1H, CH–N), 12.02 s (1H, NH), 12.60 s

(1H, NH). HRMS (EI): *m/z:* calculated 291.1008 for $C_{17}H_{13}N_3O_2$ $[M]^+$, found 291.1005.

(5-Methyl-1*H*-benzoimidazol-2-yl)methyl-1*H*-indole-3-carboxylate (5b). White solid, yield 82%, mp 117–120°C. ^{1}H NMR spectrum, δ, ppm: 2.41 s (3H, CH_3), 5.46 s (2H, CH_2), 7.01 d (J = 8.0 Hz, 1H, ArH), 7.20 m (2H, ArH), 7.34 s (1H, ArH), 7.44 (s,1H, CH–N), 7.50 d (J = 7.5 Hz, 1H, ArH), 8.00 d (J = 7.5 Hz, 1H, ArH), 8.25 d (J = 3.0 Hz, 1H, ArH), 12.03 s (1H, NH), 12.50 s (1H, NH). HRMS (EI): *m/z:* calculated 305.1164 for $C_{18}H_{15}N_3O_2$ $[M]^+$, found 305.1162.

(1*H*-Benzoimidazol-2-yl)methyl-5-methoxy-1*H*-indole-3-carboxylate (5c). White solid, yield 71%, mp 115–117°C. ^{1}H NMR spectrum, δ, ppm: 3.75 s (3H, OCH_3), 5.48 s (2H, CH_2), 6.85 d (J = 8.75 Hz, 1H, ArH), 7.19 m (2H, ArH), 7.39 d (J = 8.75 Hz, 1H, ArH), 7.51 d (J = 7.9 Hz, 2H, ArH), 7.61 d (J = 7.6 Hz, 1H, CH–N), 8.15 s (1H, ArH), 11.93 s (1H, NH), 12.65 s (1H, NH). HRMS (EI): *m/z*: calculated 321.1113 for $C_{18}H_{15}N_3O_3$ $[M]^+$, found 321.1110.

(5-Nitro-1*H*-benzoimidazol-2-yl)methyl-1*H*-indole-3-carboxylate (5d). Khaki color solid, yield 89%, mp 143–145°C. ^{1}H NMR, δ, ppm: 5.57 s (2H, CH_2), 7.21 m (2H, ArH), 7.50 d (J = 7.5 Hz, 1H, ArH), 7.74 d (J = 8.5 Hz, 1H, ArH), 8.01 d (J = 8.5 Hz, 1H, ArH), 8.11 d (J = 7.5 Hz, 1H, ArH), 8.39 s (1H, CH–N), 8.48 s (1H, ArH), 12.07 s (1H,NH). HRMS (EI): *m/z:* calculated 336.0859 for $C_{17}H_{12}N_4O_4$ $[M]^+$, found 336.0855.

(1*H*-Benzoimidazol-2-yl)methyl-5-bromo-1*H*-indole-3-carboxylate (5e). Khaki color solid, yield 86%, mp 149–153°C. ^{1}H NMR spectrum, δ, ppm: 5.51 s (2H, CH_2), 7.20 t (J = 7.5 Hz, 2H, ArH), 7.35 d (J = 7.5 Hz, 1H, ArH), 7.47 d (J = 7.5 Hz, 2H, ArH), 7.52 d (J = 7.5 Hz, 1H, ArH), 8.15 s (1H, CH–N), 8.26 s (1H, ArH), 12.23 s (1H, NH), 12.61 s (1H, NH). HRMS (EI): *m/z:* calculated 369.0113 for $C_{17}H_{12}N_3O_2Br$ $[M]^+$, found 369.0112.

(5-Nitro-1*H*-benzoimidazol-2-yl)methyl-5-methoxy-1*H*-indole-3-carboxylate (5f). Khaki color solid, yield 35%, mp 290–293°C. ^{1}H NMR spectrum, δ, ppm: 3.76 s (3H, OCH_3), 5.56 s (2H, CH_2), 6.85 d (J = 6.5 Hz, 1H, ArH), 7.40 d (J = 9.0 Hz, 1H, ArH), 7.51 s (1H, ArH), 7.75 s (1H, CH–N), 8.13 d (J = 6.0 Hz, 1H, ArH), 8.18 d (J = 3.0 Hz, 1H, ArH), 8.48 s (1H, ArH), 11.95 s (1H, NH), 13.30 s (1H, NH). HRMS (EI): *m/z:* calculated 336.0964 for $C_{18}H_{14}N_4O_5$ $[M]^+$, found 336.0965.

(1*H*-Benzoimidazol-2-yl)methyl-2-methyl-1*H*-indole-3-carboxylate (5g). White solid, yield 22%, mp 202–206°C. ^{1}H NMR spectrum, δ, ppm: 2.67 s (3H, CH_3), 5.49 s (2H, CH_2), 7.11 t (J = 7.5 Hz, 2H, ArH), 7.19 t (J = 7.5 Hz, 2H, ArH), 7.36 d (J = 7.5 Hz, 1H, ArH), 7.56 d (J = 5.5 Hz, 1H, ArH), 7.92 d (J = 7.5 Hz, 2H, ArH), 12.08 s (1H, NH). HRMS (EI): *m/z:* calculated 305.1164 for $C_{18}H_{15}N_3O_2$ $[M]^+$, found 305.1168.

(5-Methyl-1*H*-benzoimidazol-2-yl)methyl-5-methoxy-1*H*-indole-3-carboxylate (5h). White solid, yield 32%, mp 138–141°C. ^{1}H NMR spectrum, δ, ppm: 2.41 s (3H, CH_3), 3.75 s (3H, OCH_3), 5.45 s (2H, CH_2), 6.84 d (J = 6.5 Hz, 1H, ArH), 7.01 s (1H, ArH), 7.38 d (J = 8.5 Hz, 2H, ArH), 7.51 d (J = 6.5 Hz, 1H, ArH), 7.82 s (1H, CH–N), 8.14 s (1H, ArH), 11.92 s (1H, NH), 12.48 s (1H, NH). HRMS (EI): *m/z:* calculated 355.1270 for $C_{19}H_{17}N_3O_3$ $[M]^+$, found 305.1265.

(5-Nitro-1*H*-benzoimidazol-2-yl)methyl-2-methyl-1*H*-indole-3-carboxylate (5i). Khaki color solid, yield 35%, mp 248–249°C. ^{1}H NMR spectrum, δ, ppm: 2.67 s (3H, CH_3), 5.58 s (2H, CH_2), 7.12 d (J = 7.5 Hz, 2H, ArH), 7.37 d (J =7.5 Hz, 1H, ArH), 7.74 d (J = 8.0 Hz, 1H, ArH), 7.94 d (J = 7.5 Hz, 1H, ArH), 8.13 d (J = 8.5 Hz, 1H, ArH), 8.48 (s,1H, ArH), 11.96 s (1H, NH), 13.29 s (1H, NH). HRMS (EI): *m/z:* calculated 350.1015 for $C_{18}H_{14}N_4O_4$ $[M]^+$, found 350.1012.

(1*H*-Benzoimidazol-2-yl)methyl-N-methylindole-3-carboxylate (5j). White solid, yield 54%, mp 221–224°C. ^{1}H NMR spectrum, δ, ppm: 3.88 s (3H, CH_3), 5.48 s (2H, CH_2), 7.22 t (J = 8.5 Hz, 2H, ArH), 7.28 t (J = 8.5 Hz, 2H, ArH), 7.57 d (J = 7.5 Hz, 2H, ArH), 8.02 d (J = 7.5 Hz, 2H, ArH), 8.24 s (1H, CH–N), 12.63 s (1H, NH). HRMS (EI): *m/z:* calculated 305.1164 for $C_{18}H_{15}N_3O_2$ $[M]^+$, found 305.1162.

(5-Methyl-1*H*-benzoimidazol-2-yl)methyl-5-bromo-1*H*-indole-3-carboxylate (5k). White solid, yield 77%, mp 212–215°C. ^{1}H NMR spectrum, δ, ppm: 2.50 s (3H, CH_3), 5.47 s (2H, CH_2), 7.00 s (1H, ArH), 7.35 d (J = 6.5 Hz, 2H, ArH), 7.48 d (J = 8.0 Hz, 2H, ArH), 8.15 s (1H, CH–N), 8.26 s (1H, ArH), 12.24 s (1H, NH), 12.47 s (1H, NH). HRMS (EI): *m/z:* calculated 383.0269 for $C_{18}H_{14}N_3O_2Br$ $[M]^+$, found 382.0273.

(5-Nitro-1*H*-benzoimidazol-2-yl)methyl-5-bromo-1*H*-indole-3-carboxylate (5l). Khaki color solid, yield 75%, mp 157–159°C. ^{1}H NMR spectrum, δ, ppm: 5.59

s (2H, CH_2), 7.36 s (1H, CH–N), 7.46 d (J = 7.5 Hz, 1H, ArH), 7.57 d (J = 8.5 Hz, 1H, ArH), 7.74 d (J = 8.5 Hz, 2H, ArH), 8.29 s (1H, ArH), 8.47 s (1H, ArH), 12.27 s (1H, NH), 13.28 s (1H, NH). HRMS (EI): *m/z:* calculated 413.9964 for $C_{17}H_{11}N_4O_4Br$ $[M]^+$, found 413.9965.

(5-Methyl-1*H*-benzoimidazol-2-yl)methyl-*N*-methylindole-3-carboxylate (5m). White solid, yield 49%, mp 200–202°C. ^{1}H NMR spectrum, δ, ppm: 3.27 s (3H, CH_3), 3.87 s (3H, CH_3), 5.45 s (2H, CH_2), 7.01 d (J = 8.0 Hz, 1H, ArH), 7.25 m (2H, ArH), 7.34 d (J = 8.0 Hz, 2H, ArH), 7.57 s (1H, ArH), 8.01 d (J = 7.5 Hz, 1H, ArH), 8.22 s (1H, CH–N), 12.48 s (1H, NH). HRMS (EI): *m/z:* calculated 319.1321 for $C_{19}H_{17}N_3O_2$ $[M]^+$, found 319.1326.

(5-Nitro-1*H*-benzoimidazol-2-yl)methyl-*N*-methylindole-3-carboxylate (5n). Khaki color solid, yield 35%, mp 187–191°C. ^{1}H NMR spectrum, δ, ppm: 3.88 s (3H, CH_3), 5.57 s (2H, CH_2), 7.27 m (2H, ArH), 7.57 d (J = 8.0 Hz, 1H, ArH), 7.74 d (J = 8.5 Hz, 1H, ArH), 8.03 d (J = 8.5 Hz, 1H, ArH), 8.13 d (J = 7.5 Hz, 1H, ArH), 8.26 s (1H,CH–N), 8.48 s (1H,ArH). HRMS (EI): *m/z:* calculated 350.1015 for $C_{18}H_{14}N_4O_4$ $[M]^+$, found 350.1014.

Synthesis of 2-aminobenzothiazole derivatives (8a–8d). One of compounds **6a–6d** (10 mmol) and ammonium thiocyanate (0.91 g, 12 mmol) were dissolved in glacial acetic acid and the reaction mixture was stirred for 4 h. Bromine (0.6 mL, 11 mmol) in glacial acetic acid (8 mL) was added dropwise to the reaction mixture and it was stirred at 15°C for 2 h. Then, the solid was filtered off and dissolved in hot water. The filtrate was quenched with saturated aqueous sodium bicarbonate. The solid residue of the corresponding product **8a–8d** was filtered off.

2-Aminebenzothiazol (8a). Light yellow solid, yield 86%, 160–162°C (163°C [23]). ^{1}H NMR spectrum, δ, ppm: 7.15 d (2H, J = 8.0 Hz, ArH), 7.57 t (2H, J = 8.5 Hz, ArH).

2-Amino-6-methoxybenzothiazole (8b). Black solid, yield 76%, mp 184–186°C (183°C [23]). ^{1}H NMR spectrum, δ, ppm: 3.76 s (3H, OCH_3), 7.26 d (1H, J = 8.5 Hz, ArH), 7.38 d (1H, J = 8.5 Hz, ArH), 7.77 s (2H, NH_2), 7.96 s (1H, ArH).

2-Amino-6-chlorobenzothiazole (8c). White solid, yield 42%, mp 198–200°C (192°C [23]). ^{1}H NMR spectrum, δ, ppm: 7.27 d (1H, J = 8.5 Hz, ArH), 7.38 d (1H, J = 8.5 Hz, ArH), 7.65 s (2H, NH_2), 7.89 s (1H, ArH).

2-Amino-6-bromobenzothiazole (8d). White solid, yield 35%, mp 207–209°C (203°C [23]). ^{1}H NMR spectrum, δ, ppm: 7.24 d (1H, J = 8.5 Hz, ArH), 7.33 d (1H, J = 8.5 Hz, ArH), 7.61 s (2H, NH_2), 7.88 s (1H, ArH).

Synthesis of *N*-(benzothiazol-2-yl)-1*H*-indole-3-carboxamide derivatives (10a–10d). Thionylchloride (8 mL) was added dropwise to a solution of **4a** (2.5 mmol) and the mixture was refluxed for 5 h to afford **9**. After removal of the solvent in vacuum, the solid was dissolved in CH_2Cl_2 (20 mL) and **8a–8d** (2.5 mmol) in Et_3N was added to the mixture at 0°C. The reaction mixture was stirred for 18 h at 30°C. The solid residue was collected by filtration. The crude product was recrystallized from absolute ethanol.

***N*-(Benzothiazol-2-yl)-1*H*-indole-3-carboxamide (10a).** Khaki color solid, yield 75%, mp 153–154°C. ^{1}H NMR spectrum, δ, ppm: 7.55 t (J = 8.0 Hz, 2H, ArH), 7.61 s (1H, CH–N), 7.67 t (J = 8.5 Hz, 2H, ArH), 7.96 d (J = 8.5 Hz, 4H, ArH), 10.53 s (1H, NH). HRMS (EI): *m/z:* calculated 293.0623 for $C_{16}H_{11}N_3OS$ $[M]^+$, found 293.0619.

***N*-(6-Methoxybenzothiazol-2-yl)-1*H*-indole-3-carboxamide (10b).** Khaki color solid, yield 73%, mp 199–202°C. ^{1}H NMR spectrum, δ, ppm: 2.83 s (3H, CH_3), 7.06 d (J = 6.5 Hz, 1H, ArH), 7.56 t (J = 7.5 Hz, 2H, ArH), 7.61 d (J = 5.5 Hz, 1H, ArH), 7.65 d (J = 8.5 Hz, 1H, ArH), 7.69 d (J = 8.5 Hz, 1H, ArH), 8.02 d (1H, CH–N), 8.13 s (1H, ArH), 12.77 s (1H, NH). HRMS (EI): *m/z:* calculated 323.0728 for $C_{17}H_{13}N_3O_2S$ $[M]^+$, found 323.0725.

***N*-(6-Chlorobenzothiazol-2-yl)-1*H*-indole-3-carboxamide (10c).** White solid, yield 23%, mp 348–350°C. ^{1}H NMR spectrum, δ, ppm: 7.24 m (2H, ArH), 7.46 d (J = 8.5 Hz, 1H, ArH), 7.51 d (J = 8.0 Hz, 1H, ArH), 7.73 d (J = 8.5 Hz, 1H, ArH), 8.13 s (1H, CH–N), 8.24 d (J = 8.0 Hz, 1H, ArH), 8.63 s (1H, ArH), 12.04 s (1H, NH), 12.50 s (1H, NH). HRMS (EI): *m/z:* calculated 327.0233 for $C_{16}H_{10}N_3OSCl$ $[M]^+$, found 327.0231.

***N*-(6-Bromobenzothiazol-2-yl)-1*H*-indole-3-carboxamide (10d).** White solid, yield 31%, mp 327–330°C. ^{1}H NMR spectrum, δ, ppm: 7.22 t (J = 7.0 Hz, 2H, ArH), 7.51 d (J = 8.0 Hz, 1H, ArH), 7.58 d (J = 9.0 Hz, 1H, ArH), 7.67 d (J = 9.0 Hz, 1H, CH–N), 8.25 d (J = 7.5 Hz, 2H, ArH), 8.63 s (1H, ArH), 12.05

s (1H, NH), 12.50 s (1H, NH). HRMS (EI): *m/z:* calculated 370.9728 for $C_{16}H_{10}N_3OSBr$ $[M]^+$, found 370.9727.

Synthesis of *N*-substitued indole-3-formaldehyde derivatives (13a–13d). To a solution of a compound **11a–11d** (10 mmol) in 20 mL of DMF, phosphorus oxychloride (1.4 mL, 15 mmol) was added dropwise at 0°C. Upon 2 h of the process at 55°C, the mixture was poured into 150 mL of ice water. Then 20% NaOH solution was added to adjust pH at 8.0. The crude product was filtered off and recrystallized from anhydrous ethanol to give the corresponding product. Compounds **12a–12d** (10 mmol) were dissolved in 20 mL of EtOH/THF (1: 1), and sodium boron hydride was added to the reaction mixture in portions. Upon stirring the mixture for 3 h, 20 mL of saturated Na_2CO_3 solution was added to quench the reaction. In 10 min the mixture was extracted by 30 mL of ethyl acetate, then the organic layer was washed with 30 mL of saturated sodium chloride solution. The organic layer was concentrated in vacuum to give the corresponding solid product **13a–13d**.

(1*H*-Indol-3-yl)methanol (13a). White solid, yield 91%, mp 97–98°C (99–100°C [24]). ^{1}H NMR spectrum, δ, ppm: 4.64 d (J = 5.0 Hz, 2H, CH_2), 4.74 t (J = 5.0 Hz, 1H, OH), 6.98 t (J = 7.5 Hz, 1H, ArH), 7.07 t (J = 7.5 Hz, 1H, ArH), 7.23 s (1H, CH–N), 7.35 d (J = 8.5 Hz, 1H, ArH), 7.59 d (J = 7.5 Hz, 1H, ArH), 10.90 s (1H, NH).

(5-Methoxy-1*H*-indol-3-yl)methanol (13b). White solid, yield 84%, mp 79–80°C. ^{1}H NMR spectrum, δ, ppm: 3.75 s (3H, OCH_3), 4.60 d (J = 5.5 Hz, 2H, CH_2), 4.72 t (J = 5.5 Hz, 1H, OH), 6.72 d (J = 7.0 Hz, 1H, ArH), 7.09 d (J = 7.0 Hz, 1H, ArH), 7.19 s (1H, CH–N), 7.24 s (1H, ArH), 10.74 s (1H, NH).

(***N*-Ethylindol-3-yl)methanol (13c).** Red brown solid, yield 80%, mp 61–62°C (61–63°C [25]). ^{1}H NMR spectrum, δ, ppm:1.33 t (J = 5.0 Hz, 3H, CH_3), 4.14–4.17 m (2H, CH_2), 4.63 d (J = 5.0 Hz, 2H, CH_2), 4.79 t (J = 5.0 Hz, 1H, OH), 7.02 t (J = 7.5 Hz, 1H, ArH), 7.13 t (J = 7.5 Hz, 1H, ArH), 7.28 s (1H, CH–N), 7.42 d (J = 7.5 Hz, 1H, ArH), 7.61 d (J = 7.5 Hz, 1H, ArH).

(*N*-Benzylindol-3-yl)methanol (13d). Light yellow solid, yield 86%, mp 87–89°C (88–89°C [25]). ^{1}H NMR spectrum, δ, ppm: 4.65 d (J = 5.5 Hz, 2H, CH_2), 4.85 t (J = 5.5 Hz, 1H, OH), 5.37 s (2H, CH_2), 7.01 t (J = 7.5 Hz,1H, ArH), 7.09 t (J = 7.5 Hz, 1H, ArH), 7.23 t (J = 7.5 Hz, 3H, ArH), 7.29 d (J = 7.5 Hz, 2H, ArH), 7.39 s (1H, ArH), 7.42 d (J = 8.0 Hz, 1H, ArH), 7.61 d (J = 7.5 Hz, 1H, ArH).

Synthesis of *N*-substituted-2-chloromethylbenzimidazole derivates (15a, 15b). To a mixture of **14a, 14b** (10 mmol) with chloroacetic acid (1.42 g, 15 mmol) was added 4 N HCl (30 mL). The reaction mixture was refluxed for 3 h, then quenched with 20% NaOH. The solid product was collected by filtration.

2-(Chloromethyl)-1*H*-benzoimidazole (15a). Yellow solid, yield 82%, mp 154–155°C (153–154°C [26]). ^{1}H NMR spectrum, δ, ppm: 4.85 s (2H, CH_2), 7.27 t (J = 6.5 Hz, 2H, ArH), 7.35 d (J = 6.0 Hz, 2H, ArH).

2-(Chloromethyl)-*N*-methylbenzoimidazole (15b). Gray solid, yield 92%, mp 94°C (94–95°C [27]). ^{1}H NMR spectrum, δ, ppm: 3.86 s (3H, CH_3), 5.08 s (2H, CH_2), 7.23 t (J = 8.5 Hz, 1H, ArH), 7.30 t (J = 7.5 Hz, 1H, ArH), 7.56 d (J = 8.0 Hz, 1H, ArH), 7.63 d (J = 8.0 Hz, 1H, ArH).

Synthesis of 2-{[(1H-indol-3-yl)methoxy]methyl}-1*H*-benzoimidazole derivates (16a–16d). Compounds **13a–13d** (5 mmol), anhydrous K_2CO_3 (1.38g, 10 mmol) and acetone (25 mL) were mixed in 100 mL three-neck round bottomed flask. After heating up to 100°C, the solution of compounds **15a, 15b** (5 mmol) in 10 mL of acetone was added dropwise, then the mixture was stirred for 8–20 h. Anhydrous K_2CO_3 was filtered off. The filtrate was concentrated in vacuum. The final products **16a–16d** were isolated by column chromatography by the eluent ethyl acetate – petroleum ether (1 : 2).

2-{[(1*H*-Indol-3-yl)methoxy]methyl}-1*H*-benzoimidazole (16a). Khaki color solid, yield 35%, mp 139–140°C. ^{1}H NMR spectrum, δ, ppm: 3.75 s (3H, CH_3), 4.64 s (2H, CH_2), 5.72 s (2H, CH_2), 7.02 t (J = 7.5 Hz, 1H, ArH), 7.11 t (J = 7.5 Hz, 1H, ArH), 7.18 t (J = 7.5 Hz, 1H, ArH), 7.24 t (J = 7.5 Hz, 1H, ArH), 7.37 s (1H, CH–N), 7.51 d (J = 8.0 Hz, 1H, ArH), 7.55 d (J = 8.0 Hz, 1H, ArH), 7.60 d (J = 8.0 Hz, 2H, ArH). HRMS (EI): *m/z:* calculated 291.1372 for $C_{18}H_{17}N_3O$ $[M]^+$, found 291.1377.

2-{[(5-Methoxy-1*H*-indol-3-yl)methoxy]methyl}-1*H*-benzoimidazole (16b). Khaki color solid, yield 29%, mp 143–146°C. ^{1}H NMR spectrum, δ, ppm: 3.71 s (3H, CH_3), 3.74 s (3H, OCH_3), 4.60 s (2H, CH_2), 5.66 s (2H, CH_2), 6.75 d (J = 7.0 Hz, 1H, ArH), 7.10 s (1H, CH–N), 7.19 t (J = 7.5 Hz, 2H, ArH), 7.33 (s,1H, ArH), 7.47 d (J = 7.5 Hz, 2H, ArH), 7.59 d (J =

7.5 Hz, 1H, ArH). HRMS (EI): *m/z:* calculated 321.1477 for $C_{19}H_{19}N_3O_2$ $[M]^+$, found 321.1482.

2-{[(*N*-Ethylindol-3-yl)methoxy]methyl}-1*H*-benzoimidazole (16c). Khaki color solid, yield 43%, mp 159–162°C. ^{1}H NMR spectrum, δ, ppm: 1.30 t (J = 7.0 Hz, 3H, CH_3), 4.16 m (2H, CH_2), 5.10 s (2H, CH_2), 5.70 s (2H, CH_2), 6.97 d (J = 7.5 Hz, 1H, ArH), 7.12 s (1H, CH–N), 7.21 t (J = 7.5 Hz, 2H, ArH), 7.36 t (J = 7.5 Hz, 1H, ArH), 7.42 t (J = 7.0 Hz, 1H, ArH), 7.45 d (J = 8.5 Hz, 1H, ArH), 7.60 d (J = 7.0 Hz, 1H, ArH), 7.62 d (J = 8.0 Hz, 1H, ArH). HRMS (EI): *m/z:* calculated 305.1528 for $C_{19}H_{19}N_3O$ $[M]^+$, found 305.1531.

2-{[(1-Benzylindol-3-yl)methoxy]methyl}-1*H*-benzoimidazole (16d). Khaki color solid, yield 32%, mp 176–178°C. ^{1}H NMR spectrum, δ, ppm: 5.13 s (2H, CH_2), 5.39 s (2H, CH_2), 5.73 s (2H, CH_2), 6.95 t (J = 7.5 Hz, 1H, ArH), 7.08 t (J = 7.5 Hz, 1H, ArH), 7.14 d (J = 7.5 Hz, 2H, ArH), 7.22 t (J = 7.5 Hz, 3H, ArH), 7.27 d (J = 7.5 Hz, 2H, ArH), 7.40 t (J = 8.0 Hz, 2H, ArH), 7.55 (s,1H, CH–N), 7.58 d (J = 6.5 Hz, 1H, ArH), 7.64 d (J = 7.5 Hz, 1H, ArH). HRMS (EI): *m/z:* calculated 367.1685 for $C_{24}H_{21}N_3O$ $[M]^+$, found 367.1688.

Synthesis of indole-3-carbaldehyde derivatives (18a, 18b). To a solution of compounds **17a, 17b** (10 mmol) in 20 mL of DMF, phosphorus oxychloride (1.4 mL, 15 mmol) was added dropwise at 0°C. In 2 h of the reaction at 55°C, the mixture was poured into 150 mL of ice water. 20% NaOH solution was added to adjust pH at 8.0. The crude product was filtered off and recrystallized from anhydrous ethanol to give the corresponding product.

1*H*-Indole-3-carbaldehyde (18a). Light yellow solid, yield 98%, mp 201–203°C (197–198°C [28]). ^{1}H NMR spectrum, δ, ppm: 7.20–7.27 m (2H, ArH), 7.50 d (J = 8.0 Hz, 1H, ArH), 8.09 d (J = 7.5 Hz, 1H, ArH), 8.28 s (1H, ArH), 9.93 s (1H, CHO), 12.13 s (1H, NH).

5-Methoxy-1*H*-indole-3-carbaldehyde (18b). Light yellow solid, yield 92%, mp 177–179°C (178–179°C [29]). ^{1}H NMR spectrum, δ, ppm: 3.79 s (3H, OCH_3), 6.88 d (1H, J = 8.5 Hz, ArH), 7.40 d (J = 9.0 Hz, 1H, ArH), 7.58 s (1H, ArH), 8.22 s (1H, ArH), 9.90 s (1H, CHO), 12.02 s (1H, NH).

Synthesis of indole-3-carbonitrile derivatives (19a, 19b). To a solution of **18a, 18b** (30 mmol) in DMF (13 mL) was added $NH_2OH{\cdot}HCl$ (45 mmol) upon stirring. The reaction mixture was stirred at 110°C and its progress was monitored by TLC. Upon cooling down the reaction mixture, it was poured into ice cold water. The precipitated solid was filtered off and washed with water and recrystallized from anhydrous ethanol to give the respective product.

1*H*-Indole-3-carbonitrile (19a). Purple red solid, yield 93%, mp 176–178°C (177–178°C [30]). ^{1}H NMR spectrum, δ, ppm: 7.22–7.30 m (2H, ArH), 7.55 d (J = 8.0 Hz, 1H, ArH), 7.63 d (1H, J = 7.5 Hz, ArH), 8.25 s (1H, ArH), 12.19 s (1H, NH).

5-Methoxy-1*H*-indole-3-carbonitrile (19b). Purple solid, yield 85%, mp 191–193°C. ^{1}H NMR spectrum, δ, ppm: 3.87 s (3H, OCH_3), 7.42 d (J = 8.0 Hz, 1H, ArH), 7.52 d (J = 8.5 Hz, 1H, ArH), 7.80 s (1H, ArH), 8.31 s (1H, ArH), 12.40 s (1H, NH).

Synthesis of indole-3-carboxamide derivatives (20a, 20b). To a stirred solution of **19a, 19b** (15 mmol) in anhydrous ethanol was added 40% NaOH (5 mL), then 10.5mL of 30% H_2O_2 (104.7 mmol) was slowly added to the mixture upon monitoring by TLC. The precipitated solid was filtered off, washed with water, recrystallized from anhydrous ethanol, and dried in vacuum to give the respective product.

1*H*-Indole-3-carboxamide (20a). White solid, yield 75%, mp 195–197°C (197–199°C [31]). ^{1}H NMR spectrum, δ, ppm: 6.84 s (1H, NH), 7.13~7.21 m (2H, ArH), 7.47 d (J = 8.0 Hz, 2H, ArH), 8.08 s (1H, NH), 8.20 d (J = 7.5 Hz, 1H, ArH), 11.60 s (1H, NH).

5-Methoxy-1*H*-indole-3-carboxamide (20b). Light yellow solid, yield 62%, 228–230°C. ^{1}H NMR spectrum, δ, ppm: 3.75 (s,3H,OCH_3), 6.75(d, J = 8.5 Hz, 1H, ArH), 6.78 s (1H, NH), 7.29 d (J = 8.5 Hz,1H, NH), 7.37 s (1H, NH), 7.64 s (1H, ArH), 7.97 s (1H, ArH), 11.41 s (1H, ArH).

Synthesis of *N*-tosylindole-3-carboxamide derivatives (21a, 21b). To a stirred mixture of **20a, 20b** (10 mmol), NaOH (1.40 g) with $(C_4H_9)_2NHSO_4$ (0.15 g) and dichloromethane (20 mL) in 10 min was added *p*-toluenesulfonyl chloride 2.28 g (12.0 mmol). The reaction mixture was cooled down and the solid residue was filtered off, and washed with water, then with anhydrous ethanol and dried in vacuum giving the respective product.

***N*-Tosylindole-3-carboxamide (21a).** White solid, yield 77%, mp 174–177°C. ^{1}H NMR spectrum, δ, ppm: 2.29 s (3H, CH_3), 7.30 t (J = 7.5 Hz, 2H, ArH), 7.37 d (J = 7.5 Hz, 1H, ArH), 7.91 d (J = 7.5 Hz, 4H, ArH), 8.16 d (J = 7.5 Hz, 1H, ArH), 8.56 s (1H, ArH).

5-Methoxy-*N*-tosylindole-3-carboxamide (21b). White solid, yield 76%, mp 201–203°C. ^{1}H NMR spec-

trum, δ, ppm: 2.29 s (3H, CH_3), 3.73 s (3H, OCH_3), 7.27 d (*J* = 8.5 Hz, 1H, ArH), 7.35 d (*J* = 8.5 Hz, 1H, ArH), 7.82 d (*J* = 7.5 Hz, 4H, ArH), 8.26 s (1H, ArH), 8.58 s (1H, ArH).

Synthesis of *N*-{[(1*H*-benzoimidazol-2-yl)methyl]-carbamoyl}-1*H*-indole-3-carboxamide derivatives (24a, 24b). To 2.0 mmol of **21a, 21b** (2.00 mmol) in dichloroethane was added oxalyl chloride 0.3 mL (3.55 mmol) at 0–2°C. After 1 h of low temperature process, the mixture was heated to 45°C and kept at this temperature for ca 3 h. Then it was heated up to 70°C for 2 h upon monitoring the process by TLC. The mixture was concentrated in vacuum to give a solid, to which was added a solution of (1*H*-benzoimidazol-2-yl)methanamine (2.00 mmol) in DMF (5.0 mL). Then the reaction mixture was stirred for 1 h at 70°C upon TLC monitoring. The resulting solid was cooled down, filtered off, after that it was washed with $ClCH_2CH_2Cl$ to give the respective compound **23a, 23b**.

To a stirred solution of **23a, 23b** (1.0 mmol) in THF (4.0 mL) was added 30% NaOH (1.0 mL) in anhydrous ethanol (3.0 mL) and a small amount of tetrabutylammonium hydrogen sulfate phase transfer catalyst. Then the reaction mixture was stirred at 35°C upon TLC monitoring. The filtrate was concentrated to give the residual solid which was filtered off and washed with water. The final products **24a, 24b** were obtained by column chromatography with the eluent ethyl acetate – petroleum ether (1: 3).

***N*-{[(1*H*-Benzoimidazol-2-yl)methyl]carbamoyl}-1*H*-indole-3-carboxamide (24a).** White solid, yield 73%, mp 237–240°C. ^{1}H NMR spectrum, δ, ppm: 4.79 s (2H, CH_2), 7.16 m (4H, ArH), 7.47 d (*J* = 7.0 Hz, 3H, ArH), 8.19 d (*J* = 7.5 Hz, 1H, ArH), 8.56 s (1H, CH–N), 9.46 s (1H, NH), 10.41 s (1H, NH), 12.20 s (2H, NH). HRMS (EI): *m/z:* calculated 333.1226 for $C_{18}H_{15}N_5O_2$ $[M]^+$, found 333.1229.

***N*-{[(1*H*-Benzoimidazol-2-yl)methyl]carbamoyl}-5-methoxy-1*H*-indole-3-carboxamide (24b).** White solid, yield 64%, mp 212–213°C. ^{1}H NMR spectrum, δ, ppm: 3.76 s (3H, OCH_3), 4.48 s (2H, CH_2), 6.51 d (*J* = 8.5 Hz, 1H, ArH), 6.76 d (*J* = 8.5 Hz, 2H, ArH), 7.21 d (*J* = 7.5 Hz, 1H, ArH), 7.30 t (*J* = 7.5 Hz, 2H, ArH), 7.54 s (1H, CH–N), 8.24 s (1H, ArH). HRMS (EI): *m/z:* calculated 363.1331 for $C_{19}H_{17}N_5O_3$ $[M]^+$, found 363.1327.

VEGFR−2 Tyrosine kinase activity. The enzyme linked immunosorbent assay (ELISA) was conducted to measure the VEGFR-2 kinase activity (see the table). Kinase substrate was diluted using PBS without potassium ion to 20 μg/mL and enzyme labeled board was coated with kinase substrate, then reacted for 12–16 h at 37°C. The liquid was abandoned in the well. Enzyme labeled board was washed with T-PBS and dried at 37°C. Each test sample and SU11248 were added to the well. 5 μM ATP and diluted VEGFR-2 kinase were added to each well and a negative control well together with the control well without ATP were set up and incubated at 37°C for 1h. Then 100 μL antibody PY99 was added to each well and incubated at 37°C for 30 min. Peroxidase,100 μL, laveled goat-anti-mouse IgG was added to each well and incubated for 10 min. 100 μL OPD color solution was added to each well and incubated at 37°C for 30 min. The reaction was stopped by adding 50 μL of 2 M H_2SO_4 and absorbance at 490 nm was measured with a microplate reader.

Molecular docking. Molecular docking study was performed using AutoDock Vina 1.1.2 [32]. The crystal structure of VEGFR-2 tyrosine kinase (PDB ID: 3VHE) was obtained from RCSB Protein Data Bank. Ligand and receptor were prepared and the binding model was analyzed using Chimera 1.9 [33].

CONCLUSIONS

A series of indole derivatives was synthesized efficiently. Several of those made a novel class of potent and selective inhibitors for the VEGFR-2 tyrosine kinase. Compounds 10b and 10d displayed the highest inhibition with the proliferative inhibition rates of 66.7 and 51.4% at 10 μM, respectively. The presence of the benzothiazole ring clearly increased the inhibitory activity toward the VEGFR-2 tyrosine kinase, however, the other compounds did not demonstrate such high effect. Therefore, additional studies of indole-benzothiazole derivatives are important for developing further the novel class of VEGFR-2 tyrosine kinase inhibitors. Such studies are currently under way.

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