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Synthesis and Anticancer Activity of Thiophene-2-carboxamide Derivatives and *In Silico* Docking Studies

K. C. Gulipalli[a], P. Ravula[b], S. Bodige[a], S. Endoori[a], P. K. R. Cherukumalli[a], J. N. Narendra Sharath Chandra[c], and N. Seelam[a]*

[a] *Department of Chemistry, Koneru Lakshmaiah Education Foundation, Green Fields, Vaddeswaram, Guntur, 522502 India*
**e-mail: nareshvarma.klu@gmail.com*

[b] *Department of Pharmaceutical Chemistry, Gurunanak Institutions Technical Campus, School of Pharmacy, Hyderabad, 501506 India*

[c] *Department of Pharmaceutical Chemistry, Guruktupa Institute of Pharmacy, Maharashtra, 431129 India*

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Abstract—A novel series of thiophene-2-carboxamide derivatives are designed and synthesized, and their structures are confirmed by ^{1}H and ^{13}C NMR, and mass spectra. The synthesized compounds are evaluated for their *in vitro* cytotoxic activity by MTT assay. Among the tested compounds, the derivative with 4-Cl-phenyl ring exhibits potent inhibitory activity against MCF-7, K562, HepG2, and MDA-MB-231. The molecular docking study performed for the synthesized compounds against PTP1B exhibits essential key interactions.

Keywords: synthesis, thiophene-2-carboxamide, anticancer, molecular docking, protein tyrosine phosphatase inhibitor

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INTRODUCTION

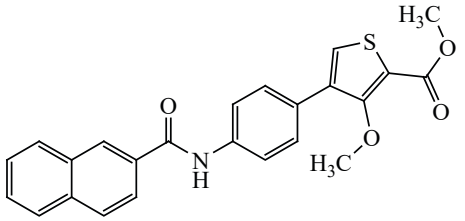
Protein tyrosine phosphatase 1B (PTP1B) has been reported to function as an oncogene in breast cancer making its inhibitors to be potentially important targets in treatment of cancer [1]. In the earlier studies [2] we designed a new series of thiophene analogues that acted against human PTP1B active site and exhibited potent to moderate activity against cancer cell lines, such as MCF-7, MDA-MB-231, K562, HepG2, and HeLa. Among the tested molecular structures, the compound **5b** (Fig. 1) demonstrated the potent cyto-toxicity (IC_{50} = 0.096 μM) against MCF-7 cell line, and also showed the highest PTP1B inhibitory activity (IC_{50} = 5.25 μM).

Many heterocyclic diamides (Fig. 2) were characterized by various biological activities such as anticystic fibrosis [3], antibacterial [4, 5], anti HIV-1 [6], antifungal [7], and anti-cancer [8–10].

Based on the above, we have designed the target molecules by attaching various alicyclic, aliphatic and aromatic amide linkages to the 2nd position of biologically active molecule **5b** for promoting its activity. The designed compounds were synthesized and characterized by ^{1}H and ^{13}C NMR, and mass spectra. All the synthesized compounds were evaluated for their *in vitro* cytotoxicity against cancer cell lines, such as MCF-7, MDA-MB-231, K562, HepG2, HeLa, and HEK293. The following molecular docking study was performed for the synthesized compounds against PTP1B.

RESULTS AND DISCUSSION

Synthesis of methyl 4-[4-(2-naphthamido)phenyl]-3-methoxythiophene-2-carboxylate **5b** was carried out by using the protocol described in our previous paper [2] (Scheme 1). Compound **5b** was treated with $LiOH \cdot H_2O$



5b

MCF-7 cell line: IC_{50} = 0.09 μM
PTP1B: IC_{50}= 5.25 μM

Fig. 1. Active compound **5b**.

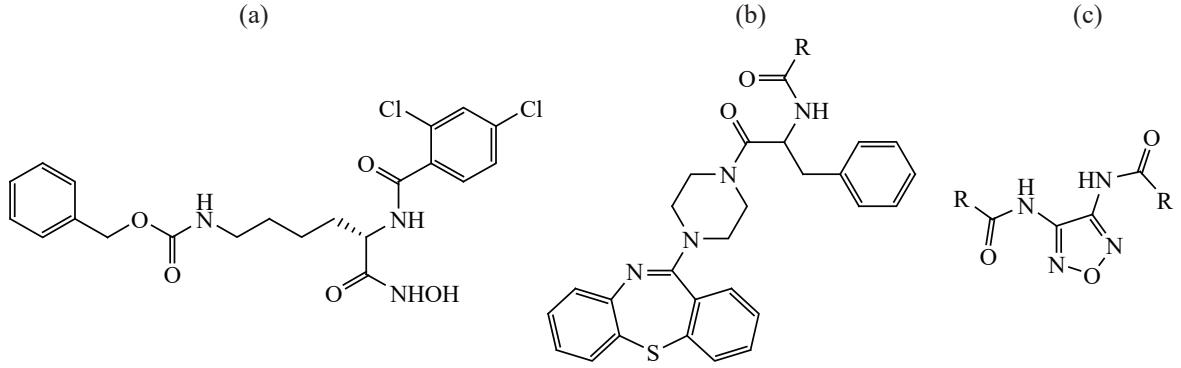


Fig. 2. Biologically active diamides: (a) L-lysine derivative, (b) 11-(piperazin-1-yl)dibenzo[*b*,*f*][1,4]thiazepine, and (c) furazan-3,4-diamide.

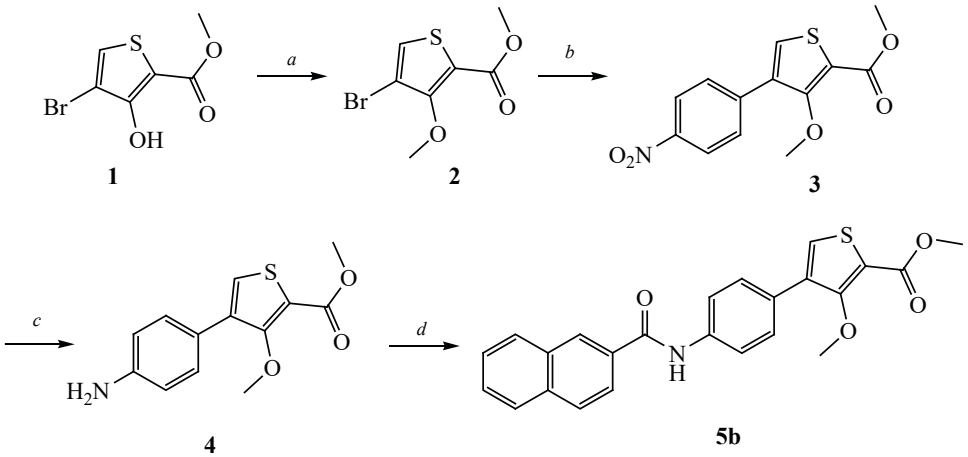
to afford the acid intermediate **6**, formation of which was confirmed by a broad singlet at 13.01 in its ^{1}H NMR spectrum.

The intermediate 6 was introduced in the reaction with different amines to afford the corresponding amide derivatives **7a**–**7p** (Scheme 2, Table 1). Their structures were confirmed by ^{1}H and ^{13}C NMR and mass spectra.

The coupling reaction of acid compound **6** with aromatic amines was carried out under HATU/DIPEA conditions. Reaction of aliphatic amines with HATU/DIPEA in DMF, even after lengthy process contained a high rate of initial compounds identified by LC-MS. The alternative coupling reaction of aliphatic amines with acid was carried out using EDC and HOBT in DMF, but it did not proceed to completion. Upon the action of TBTU the presence of starting material was recorded after 12 h. Eventually, the coupling went smoothly to completion with T_3P and Et_3N in THF at room temperature within 12 h.

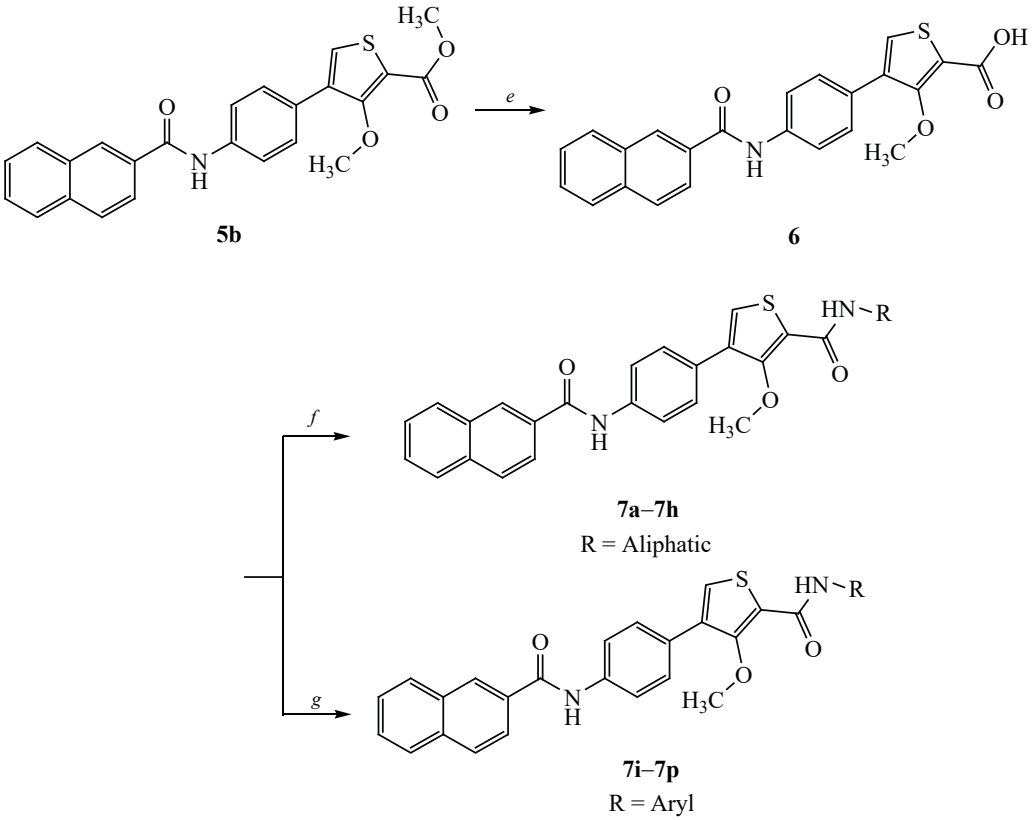
Anticancer activity. The synthesized compounds **7a**–**7p** were evaluated for their anticancer activity against MCF-7, MDA-MB-231, K562, HepG2, HeLa, and HEK293 cell lines using the MTT assay [11, 12]. Doxorubicin was used as a standard drug. The calculated IC_{50} (μM) and presented in Table 2. The synthesized compounds showed potent to moderate anticancer activity. Among the tested compounds, the product **7m** with 4-Cl-phenyl ring exhibited potent inhibitory activity against MCF-7, K562, HepG2, and MDA-MB-231 cell lines with IC_{50} values of 2.25, 5.83, 6.43, and 5.36 μM respectively. Replacement 4-Cl-phenyl ring with 4-CH_3O-phenyl in 6g led to decrease in anticancer activity, but essentially it still retained. Compounds with F-phenyl ring (**7n** and **7o**) exhibited moderate antiproliferative activity against

Scheme 1. Synthesis of the active core **5b**.



Reagents and conditions: (*a*) NaH (1.2 eq.), MeI (3.0 eq.), THF, 0°C, 16 h; (*b*) (4-nitrophenyl)boronic acid (1.2 eq.), K_2CO_3 (3.0 eq.), $Pd(PPh_3)_4$ (0.05 eq.), DME, 85°C; (*c*) Zn (3.0 eq.), NH_4Cl (3.0 eq.), EtOH, 85°C, 16 h; (*d*) 2-Naphthoic acid (1.2 eq.), HATU (1.5 eq.), DIPEA (3.0 eq.), CH_2Cl_2, room temperature, 3 h.

Scheme 2. Synthesis of the title compounds.



Reagents and conditions: (*e*) LiOH·H_2O (1.5 eq., MeOH : THF = 1 : 1), room temperature, 4 h; (*f*) R–NH_2 (1.2 eq., 50% T_3P in EtOAc (2.0 eq.), Et_3N (2.5 eq.), THF, room temperature, 12 h; (*g*) HATU (1.5 eq.), DIPEA (2.0 eq.), DMF, room temperature, 5 h.

cancer cell lines. Replacement of F-phenyl ring with saturated cycles (**7f**, **7g**, **7h**) led to decreased anti-cancer activity. The aryl ring, when replaced with aliphatic side chain **7a**–**7e**, caused decrease in inhibitory activity.

All the synthesized compounds were assessed against non-cancerous HEK293 cells. It is evident from Table 2 that none of the compounds displayed significant toxic effect on HEK293 cells, indicating their specific toxicity towards the cancer cells and possible safety against normal cells.

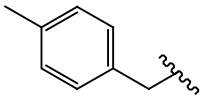
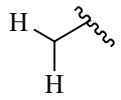
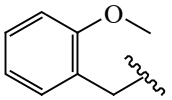
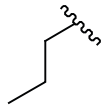
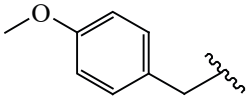
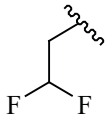
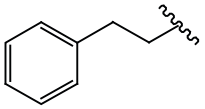
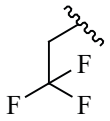
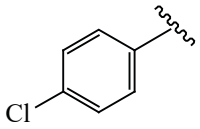
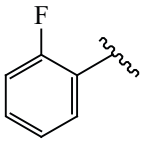
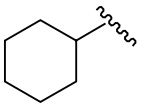
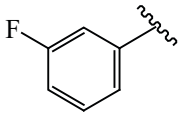
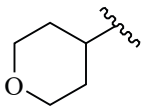
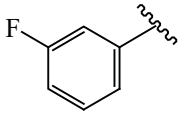
Molecular docking. In the present study, the synthesized compounds were docked into PTP1B (PDB code: 4y14) to explore their docking interactions at the active site. Docking studies revealed that the compounds **7a**–**7p** interacted with key amino acid residues of PTB1B such as ASP 48, TYR 46, ARG 24, and GLN 262 [13].

Compound **7m** showed effective interactions with ASP 48 of 27.0% binding at a distance of 1.38 Å, ARG 24 of 29.4% binding at 2.95 Å distance and ARG 24 of 10.9% binding at 3.48 Å distance through water. The compound was surrounded by PHE 182, ALA 27, GLN 262, TYR 46, and HIS 25. The extent of docking interactions of **7c** was also equally good, its naphthyl amide N–H group was likely to participate in polar interactions with ASP 48 of amino acid residues with 40.5% binding at a distance of 1.32 Å. The naphthyl amide C=O group interacted with GLN 262 of 42.9% binding at 2.12 Å distance through water. The propyl amide carbonyl group interacted with ALA 217 of 35.9% binding at a distance of 2.51 Å and with SER 216 of 32.9% binding at a distance of 2.72 Å. The samples of two- and three-dimensional structures of compounds **7m** and **7c** are showed in Figs. 3, 4.

EXPERIMENTAL

All chemicals were purchased from Sigma–Aldrich, Merck and Combi blocks, and used without further purification. Melting points were uncorrected and determined in open capillary tubes on a Guna Digital Melting Point apparatus. ^1H and ^{13}C NMR spectra were measured on a Bruker AMX 300 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C NMR using TMS as an internal standard and DMSO-d_6 as a solvent. Mass spectra were

Table 1. Protocol for the synthesis of the title compounds **7a**–**7p**

Comp. no.	R	Time, h	Yield, %	mp, °C	Comp. no.	R	Time, h	Yield, %	mp, °C
7a		12	81	131–133	**7i**		5 h	79	131–135
7b		12	83	127–129	**7j**		5 h	80	125–230
7c		12	76	120–124	**7k**		5 h	82	121–124
7d		12	71	131–136	**7l**		5 h	77	122–226
7e		12	73	135–139	**7m**		5 h	68	136–140
7f		12	71	126–129	**7n**		5 h	71	156–160
7g		12	81	135–240	**7o**		5 h	70	145–148
7h		12	72	199–205	**7p**		5 h	65	135-139

measured on an Agilent technologies mass spectrometer. Elemental analysis was performed on a Perkin-Elmer 240 CHN analyzer.

Methyl 4-bromo-3-methoxythiophene-2-carboxylate (2). To a suspension of 60% NaH (1.00 g, 25 mmol) in THF (15 mL), a solution of methyl 4-bromo-3-hydroxythiophene-2-carboxylate **1** (5.00 g, 21 mmol) in THF (60 mL) was added. The reaction mixture was stirred at 0°C for 30 min, and then MeI (9.00 g, 63.4 mmol) was added. The reaction mixture was stirred at room temperature for 16 h, quenched with cold water and extracted with ethyl acetate (3× 60 mL). The organic layer was washed with H_2O (60 mL), brine solution (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure to afford the product **2** as an off white solid. Yield 64%, mp 74–78°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 3.12 s (3H), 3.22 s (3H), 7.32 s (1H). ^{13}C NMR spectrum, δ, ppm: 165.1, 163.1, 133.9, 121.2, 112.7, 67.3, 57.3. MS: *m/z:* 252 [*M* + H]$^+$. Found, %: C 33.58; H 2.92. $C_7H_7BrO_3S$. Calculated, %: C 33.48; H 2.81.

Methyl 3-methoxy-4-(4-nitrophenyl)thiophene-2-carboxylate (3). To a solution of methyl 4-bromo-3-methoxythiophene-2-carboxylate **2** (3.40 g, 13.5 mmol) in dimethoxy ethane (60 mL), were added 4-nitro phenylboronic acid (2.78 g, 16.4 mmol), K_2CO_3 (2.87 g, 20.8 mmol), and H_2O (8 mL) at room temperature. The reaction mixture was degassed by purging Ar for 15 min, then $Pd(PPh_3)_4$ (800 mg, 0.7 mmol) was slowly added. The reaction mixture was stirred at 80°C under the atmosphere of Ar for 16 h, and the product was further extracted with ethyl

Table 2. *In vitro* anti cancer activity of the synthesized compounds

Compound	IC_{50}, μM					
	MCF-7	K562	HepG2	MDA-MB 231	HeLa	HEK293
7a	37.34	39.12	42.34	>50	>50	Not determined
7b	29.26	27.31	29.42	35.23	28.26	Not determined
7c	22.38	12.26	16.83	17.23	>50	Not determined
7d	19.34	25.59	Not determined	18.64	Not determined	43.35
7e	24.30	19.23	24.36	25.64	>50	Not determined
7f	19.57	28.63	25.18	36.56	>50	>50
7g	23.25	19.87	33.42	24.23	>50	Not determined
7h	33.63	28.15	>50	35.28	27.53	>50
7i	19.32	Not determined	29.89	19.22	25.36	Not determined
7j	14.57	28.58	26.27	17.37	33.82	48.23
7k	8.42	10.28	9.29	8.19	14.43	>50
7l	12.25	14.69	16.52	8.24	12.65	42.68
7m	2.25	5.83	6.43	5.36	22.36	Not determined
7n	12.35	10.36	12.73	16.18	15.32	Not determined
7o	15.53	8.23	22.53	28.23	17.33	>50
7p	36.27	26.22	42.78	34.43	29.28	Not determined
Doxorubicin	0.41	0.07	5.00	0.60	0.37	

acetate (3×100 mL). The organic layer was washed with H_2O (80 mL), brine solution (80 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The obtained crude product was purified by column chromatography (ethyl acetate–hexane) to afford com-pound **3** as an off white solid. Yield 85%, mp 135–140°C. ^{1}H NMR ($CDCl_3$) spectrum, δ, ppm: 3.88 s (3H), 3.92 s (3H), 7.61 s (1H), 7.79 d (2H, J = 9 Hz), 8.27 d (2H, J = 9 Hz). ^{13}C NMR spectrum, δ, ppm: 161.6, 160.3, 149.1, 137.4, 128.7, 126.1, 121.5, 116.7, 114.4, 62.1, 52.4. MS: *m/z:* 294 [M + H]$^+$. Found, %: C 54.01; H 3.89; N 4.86. $C_{13}H_{11}NO_5S$. Calculated, %: C 53.24; H 3.78; N 4.78.

Methyl 4-(4-aminophenyl)-3-methoxythiophene-2-carboxylate (4). To a solution of methyl 3-methoxy-4-(4-nitrophenyl) thiophene-2-carboxylate **3** (3.00 g, 10.2 mmol) in ethanol (30 mL) were added zinc powder (2.00 g, 30.6 mmol), NH_4Cl (1.60 g, 30.6 mmol) and catalytic amount of acetic acid at room temperature, and the reaction mixture was stirred at 80°C for 16 h. The obtained compound was extracted with ethyl acetate (3×70 mL). The combined organic layers were washed with H_2O (70 mL), brine solution (70 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (ethyl acetate–hexane) to afford compound **4** as an off white solid. Yield 76.5%, mp 115–120°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.76 s (3H), 3.80 s (3H), 5.26 br.s (2H), 6.61 d (2H, J = 8.4 Hz), 7.29 d (2H, J = 8.4 Hz), 7.71 s (1H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 160.9, 159.6, 148.4, 136.7, 128.0, 125.4, 120.8, 116.0, 113.7, 61.4, 51.7. MS: *m/z:* 264.1 [M + H]$^+$. Found, %: C 59.46; H 5.06; N 5.29. $C_{13}H_{13}NO_3S$. Calculated, %: C 59.30; H 4.98; N 5.32.

Methyl 4-[4-(2-naphthamido)phenyl]-3-methoxythiophene-2-carboxylate (5b). To a solution of 2-naphthoic acid (1.30 g, 7.6 mmol) in dichloromethane (20 mL) were added HATU (3.46 g, 9.10 mmol) and DIPEA (3.31 mL, 19.0 mmol) at room temperature and the mixture was stirred for 20 min. Then, methyl(4-aminophenyl)-3-methoxythiophene-2-carboxylate **4** (2.00 g, 7.6 mmol) was added and stirring was continued at room temperature for 4 h. The reaction mixture was diluted with water (40 mL) and extracted with dichloromethane (3×40 mL). The organic layer was washed with H_2O (50 mL), brine solution (50 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (ethyl acetate–hexane) to afford the title compound

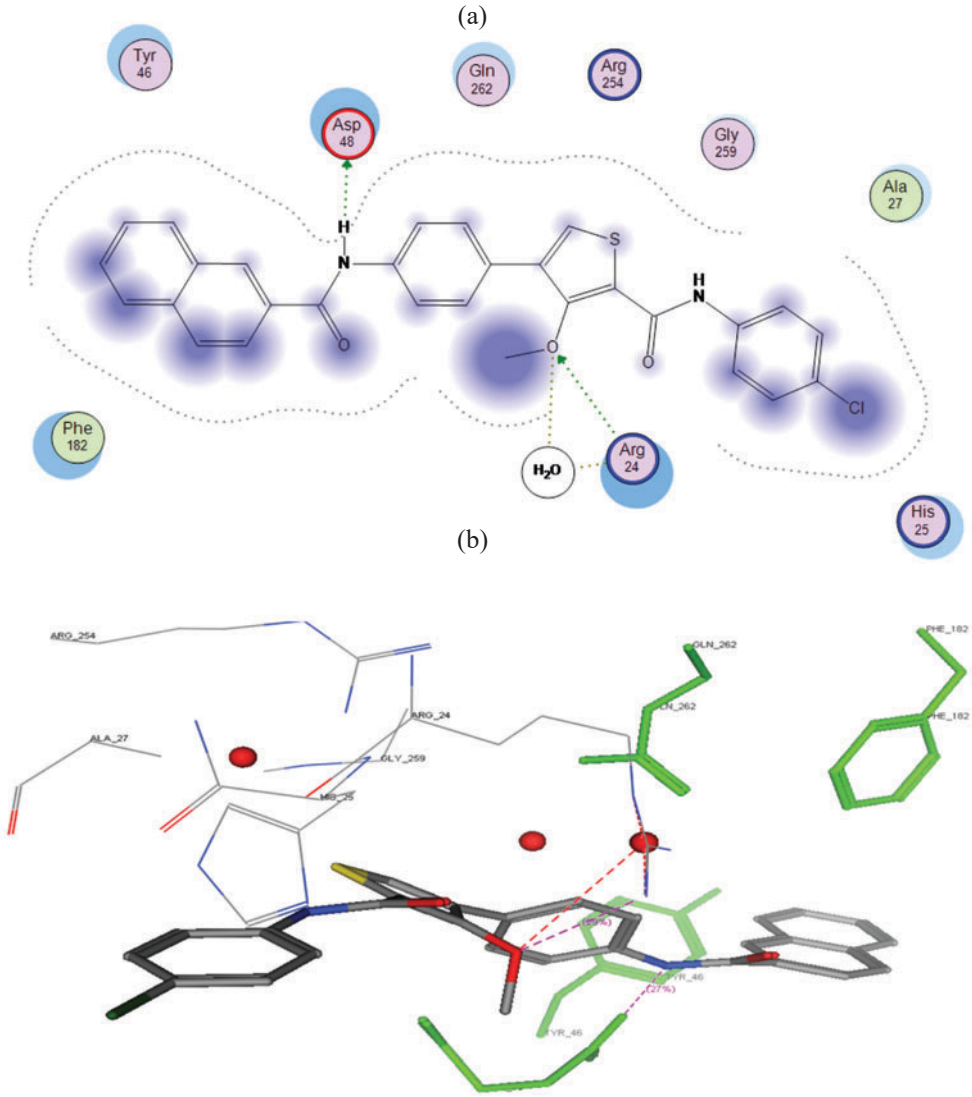


Fig. 3. (a) Two-dimensional representation of the interacting mode of 7m on PTP1B enzyme and (b) three-dimensional structural model of compound **7m** (purple) on PTP1B enzyme.

5b as an off white solid. Yield 80%, mp 122–129°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.82 s (6H), 7.65–7.63 m (5H), 7.78 d (1H, J = 6.0 Hz), 7.91 d (2H, J = 7.6 Hz), 7.98 s (1H), 8.03 s (1H), 8.09 d (1H, J = 8.6 Hz), 8.21 d (1H, J = 5.2 Hz), 10.71 s (1H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 167.3, 160.8, 159.6, 138.8, 159.5, 135.7, 134.6, 133.1, 130.1, 129.6, 128.7, 128.3, 127.8, 127.0, 126.3, 125.4, 125.0, 125.0, 119.7, 116.4, 61.8, 51.9. MS (MM): *m/z:* 418 $[M + H]^+$. Found, %: C 57.72; H 3.79; N 4.89. $C_{24}H_{19}NO_4S$. Calculated, %: C 57.69; H 3.85; N 4.84.

4-[4-(2-Naphthamido)phenyl]-3-methoxythiophene-2-carboxylic acid (6). To a solution of methyl 4-[4-(2-naphthamido)phenyl]-3-methoxythiophene-2-carboxylate (**5b**) (2.00 g, 4.79 mmol) in MeOH : THF (1 : 1, 20.0 mL) was added LiOH·H_2O (0.24 g, 5.75 mmol) in H_2O (2 mL) and the reaction mixture was stirred at room temperature for 16 h, then concentrated and the crude mixture was washed with EtOAc (1×10.0 mL). Aqueous layer pH was adjusted to 2 with 1 N HCl and extracted with EtOAC (2× 20.0 mL). The combined organic layers were washed with H_2O (10.0 mL), brine solution (10.0 mL), dried over Na_2SO_4, and concentrated to afford the title compound as white solid. Yield 88%, mp 185–187°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 13.01 br.s (1H), 10.57 s (1H), 8.62 s (1H), 8.12–8.10 m (1H), 8.06 br.s (2H), 8.04–8.01 m (1H), 7.95–7.94 m (1H), 7.92 br.s (2H), 7.66–7.63 m (4H), 3.83 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 161.9, 159.0, 138.7, 135.7, 134.2, 132.2, 132.0, 128.9, 128.03, 128.01,

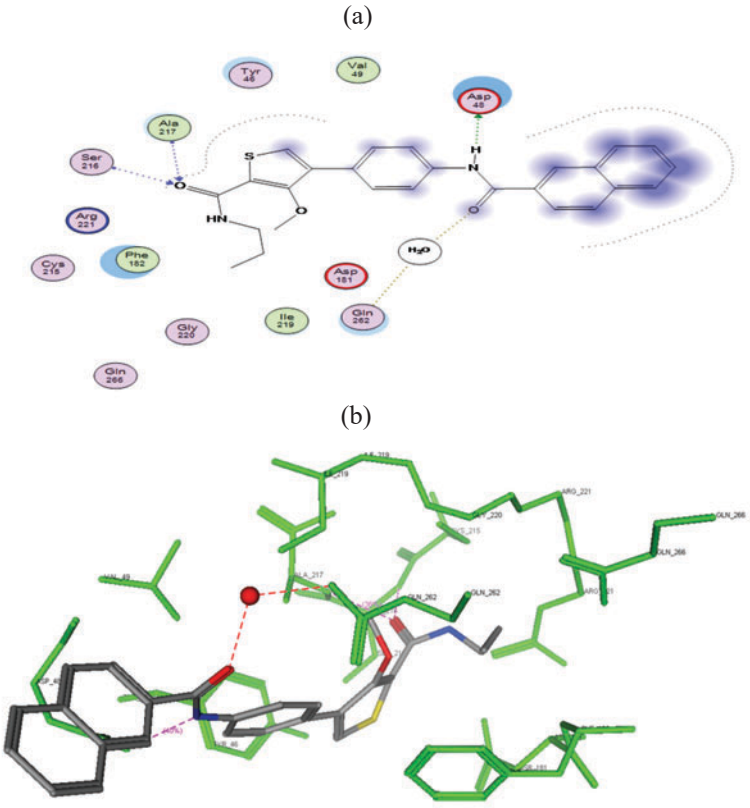


Fig. 4. (a) Two-dimensional representation of the interacting mode of **7c** on PTP1B enzyme and (b) three-dimensional structural model of compound **7c** (purple) on PTP1B enzyme.

127.8, 127.6, 126.9, 126.8, 124.4, 120.3, 118.1, 61.78. MS (MM): *m/z:* 404.1 $[M + H]^+$. Found, %: C 68.37; H 4.16; N 3.58. $C_{23}H_{17}NO_4S$. Calculated, %: C 68.47; H 4.25; N 3.47.

General procedure for the synthesis of title compounds (7a–7h). To a solution of compound **6** (0.24 mmol) in THF (5.00 mL) was added Et_3N (0.6 mmol) at room temperature and stirred for 15 min. Then, a solution of 50% T_3P in EtOAc (0.48 mmol) and an appropriate amine (0.27 mmol) were added and stirred at room temperature for 12 h. The reaction mixture was quenched with saturated $NaHCO_3$ solu-tion (10.0 mL) and extracted with EtOAC (2× 10.0 mL). The combined organic layers were washed with H_2O (5.00 mL), brine solution (5.00 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (methanol–dichloromethane) to afford the corresponding title compound **7a**–**7h**.

4-[4-(2-Naphthamido)phenyl]-3-methoxythiophene-2-carboxamide (7a). Off white solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.67 br.s (1H), 8.67 br.s (1H), 8.11–7.97 m (6H), 7.85 br.s (1H), 7.75–7.65 m (5H), 7.39 s (1H), 3.64 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.69, 165.62, 154.4, 152.2, 138.9, 138.6, 134.8, 134.3, 133.6, 132.1, 132.0, 129.2, 129.1, 128.9, 128.08, 128.01, 127.7, 127.6, 127.3, 126.8, 125.9, 124.7, 124.4, 122.4, 120.5, 120.3, 118.3, 61.36. MS (MM): *m/z:* 403.1 $[M+H]^+$. Found, %: C 68.74; H 4.41; N 6.88. $C_{23}H_{18}N_2O_3S$. Calculated, %: C 68.64; H 4.51; N 6.96.

4-[4-(2-Naphthamido)phenyl]-3-methoxy-*N*-methylthiophene-2-carboxamide (7b). Off white solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.58 br.s (1H), 8.61 s (1H), 8.12–8.09 m (6H), 8.06–8.02 m (3H), 7.96–7.93 m (2H), 7.86–7.84 m (1H), 7.80 br.s (1H), 7.66–7.62 m (4H), 3.61 s (3H), 2.85 d (3H, J = 4.4 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 160.9, 153.9, 138.8, 134.7, 134.2, 132.1, 132.0, 129.1, 128.9, 128.02, 128.00, 127.8, 127.6, 127.3, 126.8, 125.3, 124.4, 124.2, 120.3, 61.37, 26.20. MS (MM): *m/z:* 417.0 $[M + H]^+$. Found, %: C 69.33; H 4.74; N 6.61. $C_{24}H_{20}N_2O_3S$. Calculated, %: C 69.21; H 4.84; N 6.73.

4-[4-(2-Naphthamido)phenyl]-3-methoxy-*N*-propylthiophene-2-carboxamide (7c). White solid. ^{1}H NMR spectrum, (DMSO-d_6) δ, ppm: 10.59 br.s (1H),

8.60 s (1H), 8.12–8.02 m (5H), 7.93 br.s (1H), 7.81 s (1H), 7.65–7.62 m (5H), 3.63 s (3H), 3.31–3.26 m (2H), 1.59–1.54 m (2H), 0.91 t (3H, J = 7.6 Hz). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 160.3, 153.8, 138.8, 134.6, 134.2, 132.1, 132.0, 129.0, 128.9, 128.0, 127.9, 127.8, 127.7, 127.6, 127.3, 126.8, 125.3, 124.4, 120.3, 61.34, 42.96, 22.49, 11.30. MS (MM): *m/z:* 445.1 [M + H]$^+$. Found, %: C 69.33; H 4.74; N 6.61. $C_{24}H_{20}N_2O_3S$. Calculated, %: C 69.21; H 4.84; N 6.73.

4-[4-(2-Naphthamido)phenyl]-*N*-(2,2-difluoroethyl)-3-methoxythiophene-2-carboxamide (7d). Off white solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.62 br.s (1H), 8.64 s (1H), 8.20–8.17 m (1H), 8.13–8.11 m (3H), 8.03–7.94 m (3H), 7.89 s (1H), 7.74–7.66 m (4H), 6.36–6.07 m (1H), 3.84–3.76 m (2 H), 3.65 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.7, 160.9, 154.7, 138.9, 134.8, 134.3, 132.2, 132.0, 128.94, 128.91, 128.0, 127.7, 127.6, 127.4, 126.8, 126.4, 124.4, 120.4, 120.2, 117.0, 114.6, 112.3, 61.51, 41.14. MS (MM): *m/z:* 467.0 [M + H]$^+$. Found, %: C 64.25; H 4.41; N 6.11. $C_{25}H_{20}F_2N_2O_3S$. Calculated, %: C 64.37; H 4.32; N 6.01.

4-[4-(2-Naphthamido)phenyl]-*N*-(2,2,2-trifluoroethyl)-3-methoxythiophene-2-carboxamide (7e). White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.58 br.s (1H), 8.61 s (1H), 8.12–8.02 m (5H), 7.97–7.95 m (2H), 7.66–7.62 m (5H), 4.20–4.11 m (2 H), 3.64 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 160.9, 155.0, 138.9, 134.8, 134.2, 132.1, 132.0, 128.9, 128.0, 127.8, 127.7, 127.6, 127.4, 126.8, 124.4, 123.4, 122.7, 122.5, 120.3, 61.56, 60.42. MS (MM): *m/z:* 485.1 [M + H]$^+$. Found, %: C 61.88; H 3.86; N 5.88. $C_{25}H_{19}F_3N_2O_3S$. Calculated, %: C 61.98; H 3.95; N 5.78.

4-[4-(2-Naphthamido)phenyl]-*N*-(1-cyanocyclopropyl)-3-methoxythiophene-2-carboxamide (7f). White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.57 br.s (1H), 8.68 s (1H), 8.12–8.01 m (5H), 7.93–7.91 m (2H), 7.66–7.61 m (5H), 3.62 s (3H), 1.60–1.56 m (2H),1.37–1.32 m (2H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 170.9, 165.7, 166.8, 160.6, 144.1, 140.0, 139.5, 137.2, 134.1, 134.0, 133.2, 133.0, 132.9, 132.7, 132.1, 132.0, 129.6, 127.7, 127.5, 126.1, 125.6, 125.4, 66.80, 65.67, 25.56, 21.70. MS (MM): *m/z:* 468.1 [M + H]$^+$. Found, %: C 69.47; H 4.44; N 8.89. $C_{27}H_{21}N_3O_3S$. Calculated, %: C 69.36; H 4.53; N 8.99.

4-[4-(2-Naphthamido)phenyl]-*N*-cyclohexyl-3-methoxythiophene-2-carboxamide (7g). Off white solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.57 br.s (1H), 8.61 s (1H), 8.11–8.01 m (4H), 7.96–7.94 m (2H), 7.83 br.s (1H), 7.67–7.62 m (4H), 7.60–7.58 m (1H), 3.81 br.s (1H), 3.63 s (3H), 1.85 br.s (2H), 1.69 br.s (2H), 1.58–1.55 m (1H), 1.42–1.35 m (4 H),1.23–1.21 m (1H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 159.4, 153.7, 138.8, 134.6, 134.2, 132.1, 132.0, 128.9, 128.0, 127.9, 127.8, 127.6, 127.3, 126.8, 125.3, 124.4, 120.3, 61.23, 47.33, 32.15, 24.29, 20.61. MS (MM): *m/z:* 485.0 [M + H]$^+$. Found, %: C 71.97; H 5.73; N 5.69. $C_{29}H_{28}N_2O_3S$. Calculated, %: C 71.87; H 5.82; N 5.78.

4-[4-(2-Naphthamido)phenyl]-*N*-(tetrahydro-2*H*-pyran-4-yl)-3-methoxythiophene-2-carboxamide (7h). White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.57 br.s (1H), 8.61 s (1H), 8.12–8.09 m (1H), 8.06–8.02 m (3H), 7.96–7.93 m (2H), 7.84 br.s (1H), 7.71–7.68 m (1H), 7.65–7.63 m (4H), 4.06–4.02 m (1H), 3.87–3.85 m (2H), 3.65 s (3H), 3.45–3.40 m (2H), 1.83–1.80 m (2H), 1.67–1.57 m (2H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 159.6, 153.9, 138.8, 134.6, 134.2, 132.1, 132.0, 128.9, 128.0, 127.8, 127.6, 127.4, 126.8, 125.4, 124.4, 120.3, 65.82, 61.27, 45.10, 32.98. MS (MM): *m/z:* 487.0 [M + H]$^+$. Found, %: C 69.21; H 5.28; N 5.66. $C_{28}H_{26}N_2O_4S$. Calculated, %: C 69.11; H 5.39; N 5.76.

General procedure for the synthesis of title compounds 7i–7p. To a solution of compound **6** (0.24 mmol) in DMF (5.00 mL) were added HATU (0.29 mmol) and DIPEA (0.49 mmol) at room temperature and stirred for 15 min. Then, the appro-priate amine (0.27 mmol) was added, and the mixture was stirred at room temperature for 5 h. Upon completion of the process the reaction mixture was diluted with water (10.0 mL) and extracted with EtOAC (2×10.0 mL). The combined organic layers were washed with H_2O (5.00 mL), brine solution (5.00 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (methanol–dichloromethane) to afford the correspond-ing title compound **7i–7p**.

4-[4-(2-Naphthamido)phenyl]-*N*-(4-methylbenzyl)-3-methoxythiophene-2-carboxamide (7i). White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.60 br.s (1H), 8.60 s (1H), 8.38–8.35 m (1H), 8.12–8.09 m (1H), 8.07–8.02 m (3H), 7.94 br.s (3H), 7.67–7.62 m (4H), 7.26–7.24 m (2H), 7.16–7.14 m (2H), 4.50 d (2H, J = 6.0 Hz), 3.69 s (3H), 2.28 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 162.4, 154.1, 138.7, 136.3, 135.8, 134.7, 134.2, 132.08, 132.01, 129.0, 128.9, 128.8, 128.0, 127.9, 127.8, 127.6, 127.3, 127.1, 126.8, 125.8, 124.3, 124.0, 120.5, 61.43, 42.15, 20.60. MS (MM): *m/z:* 507.0 [M +

H]$^+$. Found, %: C 73.59; H 5.06; N 5.64. $C_{31}H_{26}N_2O_3S$. Calculated, %: C 73.49; H 5.17; N 5.53.

***N*-(2-Methoxybenzyl)-4-[4-(2-naphthamido)phenyl]-3-methoxythiophene-2-carboxamide (7j).** White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.62 br.s (1H), 8.60 s (1H), 8.28–8.25 m (1H), 8.12–7.92 m (6H), 7.89–7.84 m (1H), 7.67–7.64 m (4H), 7.31–7.24 m (2H), 7.05–6.92 m (2H), 4.52 d (2H, *J* = 6.0 Hz), 3.87 s (3H), 3.72 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.79, 165.71, 160.3, 156.8, 154.0, 138.7, 134.6, 134.2, 132.07, 132.02, 129.0, 128.9, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 127.3, 126.8, 126.3, 125.9, 124.3, 124.1, 120.5, 120.3, 120.2, 110.5, 61.38, 55.35, 38.33. MS (MM): *m/z:* 523.0 [*M* + H]$^+$. Found, %: C 71.34; H 5.12; N 5.24. $C_{31}H_{26}N_2O_4S$. Calculated, %: C 71.24; H 5.01; N 5.36.

4-[4-(2-Naphthamido)phenyl]-*N*-(4-methoxybenzyl)-3-methoxythiophene-2-carboxamide (7k). Off-white solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.57 br.s (1H), 8.60 s (1H), 8.31–8.30 m (1H), 8.11–8.09 m (1H), 8.06–8.01 m (3H), 7.94 d (2H, *J* = 8.8 Hz), 7.84 br.s (1H), 7.66–7.62 m (4H), 7.29 d (2H, *J* = 8.8 Hz), 6.91 d (2H, *J* = 8.4 Hz), 4.45 d (2H, *J* = 6.0 Hz), 3.73 s (3H), 3.60 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 166.09, 162.7, 154.5, 139.0, 136.6, 136.2, 135.0, 134.5, 132.4, 132.3, 129.3, 129.2, 129.1, 128.3, 128.2, 128.1, 127.9, 127.7, 127.5, 127.2, 126.1, 124.6, 124.4, 120.8, 61.76, 55.37, 42.47, 20.92. MS (MM): *m/z:* 523.0 [*M* + H]$^+$. Found, %: C 71.35; H 5.11; N 5.25. $C_{31}H_{26}N_2O_4S$. Calculated, %: C 71.24; H 5.01; N 5.36.

4-[4-(2-Naphthamido)phenyl]-3-methoxy-*N*-phenethylthiophene-2-carboxamide (7l). Off-white solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.59 br.s (1H), 8.62 s (1H), 8.12–8.10 m (1H), 8.07–8.02 m (3H), 7.96 d (2H, *J* = 8.4 Hz), 7.86–7.83 m (1H), 7.81 br.s (1H), 7.68–7.61 m (4H), 7.34–7.28 m (4H), 7.24–7.20 m (1H), 3.62–3.57 m (2H), 3.47 s (3H), 2.88 br.s (2H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 162.2, 153.9, 139.2, 138.8, 134.6, 134.2, 132.2, 132.0, 129.0,128.9, 128.6, 128.4, 128.0, 127.8, 127.6, 127.3, 126.8, 126.1, 125.6, 124.4, 124.2, 120.4, 61.18, 40.16, 34.99. MS (MM): *m/z:* 507.0 [*M* + H]$^+$. Found, %: C 73.59; H 5.26; N 5.63. $C_{31}H_{26}N_2O_3S$. Calculated, %: C 73.49; H 5.17; N 5.53.

4-[4-(2-Naphthamido)phenyl]-*N*-(4-chlorophenyl)-3-methoxythiophene-2-carboxamide (7m). White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.61 br.s (1H), 9.84 s (1H), 8.62 s (1H), 8.12–8.10 m (1H), 8.07 br.s (2H), 8.04–8.02 m (1H), 7.99–7.95 m (3H), 7.79 d (2H, *J* = 8.8 Hz), 7.69–7.62 m (4H), 7.44 d (2H, *J* = 8.8 Hz), 3.75 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 162.2, 154.6, 138.9, 137.1, 134.7, 134.2, 132.1, 132.0,128.9, 128.8, 128.6, 128.4, 128.0, 127.8, 127.6, 127.5, 126.5, 124.4, 123.2, 121.8, 120.4, 115.1, 61.70. MS (MM): *m/z:* 513.0 [*M* + H]$^+$. Found, %: C 67.82; H 4.23; N 5.35. $C_{29}H_{21}ClN_2O_3S$. Calculated, %: C 67.90; H 4.13; N 5.46.

4-[4-(2-Naphthamido)phenyl]-*N*-(2-fluorophenyl)-3-methoxythiophene-2-carboxamide (7n). White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.61 br.s (1H), 9.82 s (1H), 8.62 s (1H), 8.29–8.25 m (1H), 8.12–8.10 m (1H), 8.07 br.s (2H), 8.04–8.02 m (1H), 8.00–7.90 m (3H), 7.69–7.62 m (4H), 7.38–7.33 m (1H), 7.27–7.16 m (2H), 3.74 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 158.7, 154.5, 153.8, 151.4, 139.1, 134.8, 134.2, 132.1, 132.0, 128.9, 128.6, 128.0, 127.8, 127.6, 127.5, 126.8, 126.1, 126.0, 124.8, 124.7, 124.4, 123.6, 122.1, 120.4, 115.3, 115.1, 61.67. MS (MM): *m/z:* 496.9 [*M* + H]$^+$. Found, %: C 70.25; H 4.16; N 5.53. $C_{29}H_{21}FN_2O_3S$. Calculated, %: C 70.15; H 4.26; N 5.64.

4-[4-(2-Naphthamido)phenyl]-*N*-(3-fluorophenyl)-3-methoxythiophene-2-carboxamide (7o). White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.62 br.s (1H), 9.91 s (1H), 8.64 s (1H), 8.13 br.s (1H), 8.04–7.93 m (5H), 7.78–7.41 m (8H), 7.00–6.95 m (1H), 3.76 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.6, 159.3, 154.7, 139.0, 134.8, 134.3, 134.2, 132.2, 132.0, 130.4, 130.3, 128.9, 128.8, 128.0, 127.6, 127.5, 126.8, 126.7, 124.4, 123.1, 120.4, 120.2, 61.75. MS (MM): *m/z:* 496.9 [*M* + H]$^+$. Found, %: C 70.26; H 4.15; N 5.54. $C_{29}H_{21}FN_2O_3S$. Calculated, %: C 70.15; H 4.26; N 5.64.

4-[4-(2-Naphthamido)phenyl]-3-methoxy-*N*-(pyridin-3-yl)thiophene-2-carboxamide (7p). White solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.65 br.s (1H), 9.91 s (1H), 8.94 s (1H), 8.37–8.36 m (1H), 8.23–8.20 m (1H), 8.13–8.09 m (2H), 8.03–8.01 m (3H), 7.97 br.s (1H), 7.74–7.65 m (6H), 7.44–7.41 m (1H), 3.77 s (3H). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 165.7, 165.6, 163.2, 162.2, 159.5, 152.2, 144.8, 142.0, 139.0, 138.5, 134.9, 134.8, 134.2, 133.6, 132.1, 132.0, 129.2, 128.9, 128.8, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 126.8, 124.4, 123.5, 123.0, 122.4, 120.4, 118.3, 61.74. MS (MM): *m/z:* 480.1 [*M* + H]$^+$. Found, %: C 70.22; H 4.31; N 8.66. $C_{28}H_{21}N_3O_3S$. Calculated, %: C 70.13; H 4.41; N 8.76.

In vitro evaluation of anticancer activity. MCF-7, MDA-MB-231, K562, HepG2, HeLa, and HEK293 were procured from National Centre for Cell Sciences, Pune, India. All tumour cells were grown in DMEM

media supplemented with 10% heat inactivated foetal bovine serum (FBS), 100 mg/mL streptomycin, 100 IU/mL penicillin, and 2 mM-glutamine. All cell lines were maintained under humidified atmosphere with 5% CO_2 at 37°C. Cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells (5×10^3 cells/well) were inoculated in 96-well culture plate and cultured with or without compounds at different concentrations (10, 5, 1, 0.5, 0.1, and 0.01 μM for dose response study) in duplicates for 24 h in a final volume of 200 μl. Afterwards, the medium was removed and 20 μL of MTT (5 mg/mL in PBS) were added. After 3 h of incubation at 37°C, 100 μL of DMSO were added to each well, and plates were agitated for 1 min. The absorbance was measured at 570 nm with a multi-well plate reader (Victor3, Perkin Emler). Percent inhibition of proliferation was calculated as a fraction of control.

Molecular docking. Docking was performed on Windows 2002, MOE 2008.10 version. Protein tyrosine phosphatase 1B was retrieved from the protein data bank (PDB code: 4y14), and the enzyme was visualized using sequence option, and further co-factors were deleted. The partial charge of the protein was adjusted with the help of force field method AMBER 99. Later, the protein was subjected to 3D protonation at cut off 12.0, and further hydrogen was added according to standard geometry and the receptor was energy minimized using force field MMFF94x at 0.01 kJ/mol gradients. The ligand structures were written by using a builder module, and adjusting the partial charges using Hamilton MMFF94 force field method and subsequently 3D protonation and hydrogen addition was performed according to standard geometry. Ligands were energy minimized at cut off 12 using force field MMFF94x at 0.01 kJ/mol gradient and 6.0 Å grid was generated on the active site of the enzyme. Docking was performed using the option simulation followed by dock on selected active site amino acids using sequence option, and eventually docked using setting options such as receptor and solvent, alpha triangle, selected residues, affinity dG, force field refinement, and best 30 poses. After obtaining docking results, the best pose was retained. The resultant best pose score values in the series were used for analysis of docking and interaction.

CONCLUSIONS

A novel series of thiophene-2-carboxamide derivatives is synthesized and the molecular structures confirmed by ^1H and ^{13}C NMR, and mass spectra. The synthesized compounds are evaluated for their *in vitro* cytotoxic activity by MTT assay. The compound **7m** containing the 4-Cl-phenyl substituent exhibits the potent inhibitory activity against MCF-7, K562, HepG2, and MDA-MB-231 cell lines. The molecular docking study performed for the synthesized compounds against PTP1B indicates their possible binding interactions at the active site. The accumulated data indicate that the tested compounds may lead to potent anticancer agents.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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