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Synthesis and Anticancer Activity of Novel Urea and Thiourea Bearing Thiophene-2-carboxalate Derivatives

K. Ch. Gulipalli[a], P. Ravula[b], S. Bodige[a], S. Endoori[a], P. Koteswara Rao 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*
[b] *Department of Pharmaceutical Chemistry, Gurunanak Institutions Technical Campus, School of Pharmacy, Hyderabad, 501506 India*
[c] *Department of Pharmaceutical Chemistry, Gurukrupa Institute of Pharmacy, Maharashtra, 431129 India*
**e-mail: nareshvarma.klu@gmail.com*

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Abstract—A new series of urea and thiourea bearing thiophene-2-carboxalate derivatives has been designed against protein tyrosine phosphatase 1B (PTP1B) active site, synthesized and charecterized by ^{1}H and ^{13}C NMR, and mass spectra. The compounds have been evaluated for in vitro anticancer activity against different cancer cell lines using the MTT colorimetric assay and doxorubicin as a standard drug. Among the tested compounds, methyl 3-methoxy-4-{4-[3-(4-methoxyphenyl)thioureido]phenyl}thiophene-2-carboxylate demonstrates the highest inhibitory activity against MCF-7, K562, HepG2, MDA-MB-231, and HeLa cell lines. The new molecular structures and their interactions with PNP1B have been evaluated by docking studies.

Keywords: synthesis, thiophene-2-carboxalate, urea, thiourea, anticancer activity, molecular docking, protein tyrosine phosphatase inhibitor

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INTRODUCTION

Recently protein tyrosine phosphatase 1B (PTP1B) has been recognized as an attractive target for anticancer studies, especially for breast cancer [1–3]. Thiophene and its derivatives exhibit various biological activities including BACE1 inhibiting [4], anti-inflammatory [5], anti-HIV [6], and anti-cancer [7–10]. Urea and thiourea derivatives have received close attention as building blocks of molecules characterized as anticancer active due to their significant inhibitory action on protein tyrosine kinases (PTKs), Raf kinase, NADH oxidase, and DNA topoisomerase [11–13]. Thus, more small molecules bearing urea and thiourea building blocks need to be synthesized for developing new anticancer compounds. Recently we reported urea and thiourea derivatives of thieno[3,2-*d*]pyrimidine that displayed excellent to good anti cancer activity [14].

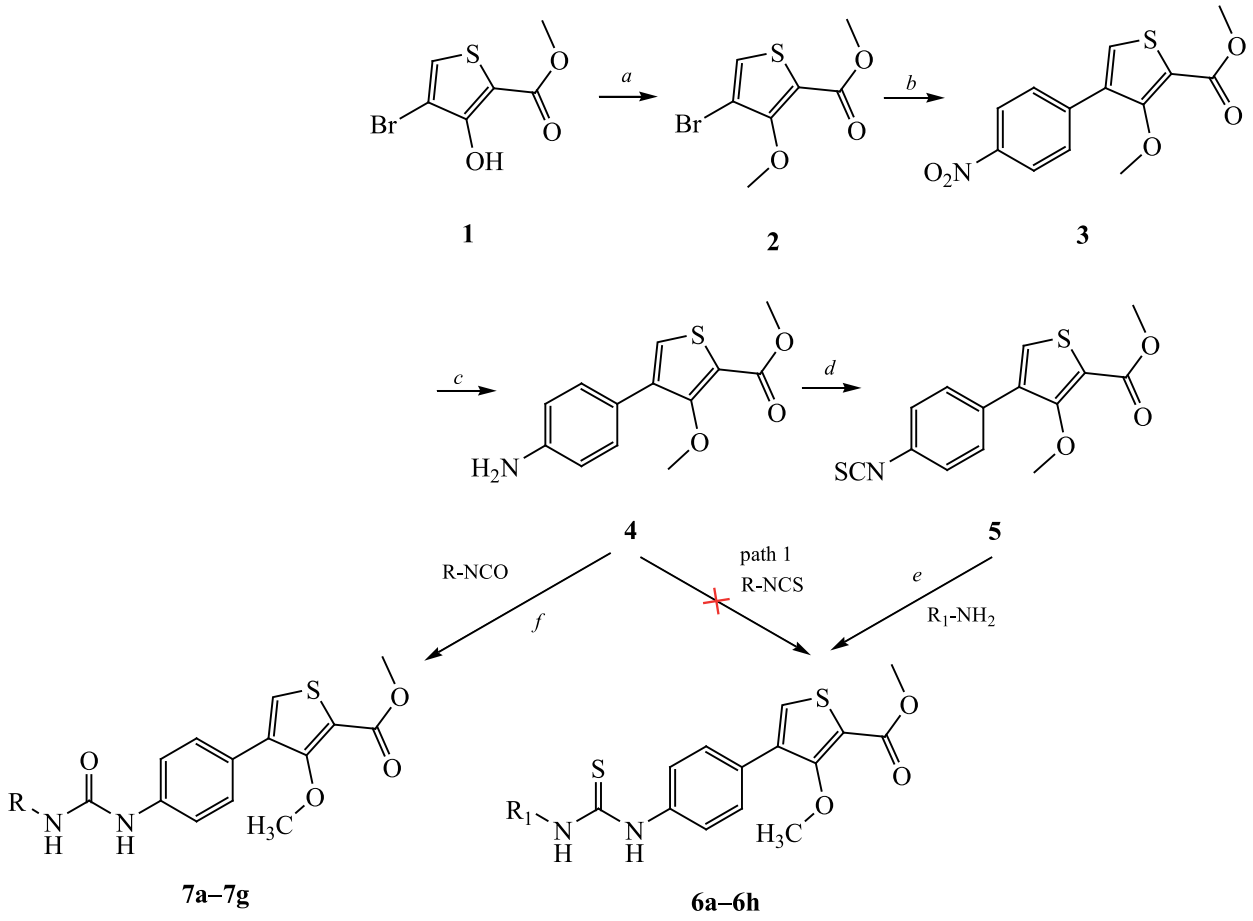
In the present study, we designed a series of urea and thiourea derivatives bearing thiophene moiety based on molecular docking against PTP1B, and evaluated those for in vitro cytotoxic activity against HeLa, MCF-7, K562, MDA-MB-231, HepG2, and HEK293 cell lines.

RESULTS AND DISCUSSION

As presented in Scheme 1, the key intermediate amine **4** was prepared from methyl 4-bromo-3-hydroxythiophene-2-carboxylate (**1**) using our previously reported synthetic approach [15]. The amine **4** reaction with 1,1′-thiocarbonyldiimidazole gave methyl 4-(4-isothiocyanatophenyl)-3-methoxythiophene-2-carboxylate (**5**), which reacted with different amines in a microwave reactor (P = 150 W, t = 85 °C) to afford the corresponding thiourea derivatives **6a**–**6h.** On the other hand, intermediate **4** reacted with different aryl isocyanates in a microwave reactor leading to the target compounds **7a**–**7g**.

Initially, we attempted to make thiourea derivatives by the reaction of amine **4** with the corresponding isothiocyanate (Scheme 2). *p*-Tolylisothiocyante was used as one of the precursors. The conventional reaction in different solvents (CH_2Cl_2 and THF) and bases (Et_3N and DIPEA) at different temperatures (Table 1) led to

Scheme 1. Synthesis of title compounds.



R = 3-CH_3Ph (**7a**), 4-CH_3Ph (**7b**), 3-FPh (**7c**), 4-FPh (**7d**), 4-BrPh (**7e**), 4-OCF_3Ph (**7f**), 3,5-di-FPh (**7g**); R_1 = 4-CH_3Ph (**6a**), 3-OCH_3Ph (**6b**), 4-OCH_3Ph (**6c**), 4-OCF_3Ph (**6d**), 3-FPh (**6e**), 4-F Ph (**6f**), 3,5-di-FPh (**6g**), 3-FPh (**6h**).

Reagents and conditions: (*a*) NaH (1.2 equiv), MeI (3.0 equiv), THF, 0°C, 16 h; (*b*) (4-nitrophenyl)boronic acid (1.2 equiv), K_2CO_3 (3.0 equiv), $Pd(PPh_3)_4$ (0.05 equiv), DME, 85°C; (*c*) Zn (3.0 equiv), NH_4Cl (3.0 equiv), EtOH, 85°C, 16 h; (*d*) 1,1-Thiocarbonyldiimidazole (1.2 equiv), THF, room temperature, 16 h; (*e*) R-NH_2 (1.2 equiv), THF, 85°C, μw, 20 min; (*f*) R-NCO (1.2 equiv), THF, 85°C, μw, 20 min.

formation of symmetrical thiourea **8** detected exclusively by LC-MS. The reaction without a base led to formation of the desired product with the yield ca 15% (Table 1, entry 7), but overall conversion was ca 30%. The following optimization of the reaction was extended to non-conventional approach that involved ultrasonic irradiation (entry 8, Table 1) and microwave irradiation (Table 1, entry 9). The MW initiated process resulted in 80% of conversion and 30% formation of the desired compound **6a,** and upon purification yield was lower than 19%.

For improving the process yield methyl 4-(4-isothiocyanatophenyl)-3-methoxythiophene-2-carboxylate (**5**) was introduced in the reaction with 4-methylaniline (Scheme 3) to give the desired thiourea derivatives **6a**–**6h** in excellent to good yields.

Following the same line, urea derivatives were synthesised by the reaction of amine compound **4** with *p*-tolyl isocyanate (Scheme 4) upon MW irradiation. The reaction proceeded readily with high yield of compounds **7a**–**7g**.

Table 1. Optimization of the reaction conditions for the synthesis of compound **6a** from amine **4**[a]

Entry no.	Solvent	Base	*T*, °C	Time, h	Conversion[b], %	Yield, %[b] (**6a** : **8**)
1	DCM	Et_3N	40	16.0	40	0 : 100
2	Chloroform	Et_3N	50	16.0	42	0 : 100
3	THF	Et_3N	25	16.0	50	0 : 100
4	THF	Et_3N	65	4.0	70	0 : 100
5	DCM	DIPEA	40	16.0	45	0 : 100
6	THF	DIPEA	65	4.0	65	0 : 100
7	THF	–	65	4.0	30	15 : 85
8	THF	–	65	2.0	35	20 : 80[c]
9	THF	–	80	0.5	80	30 : 70[d,e]

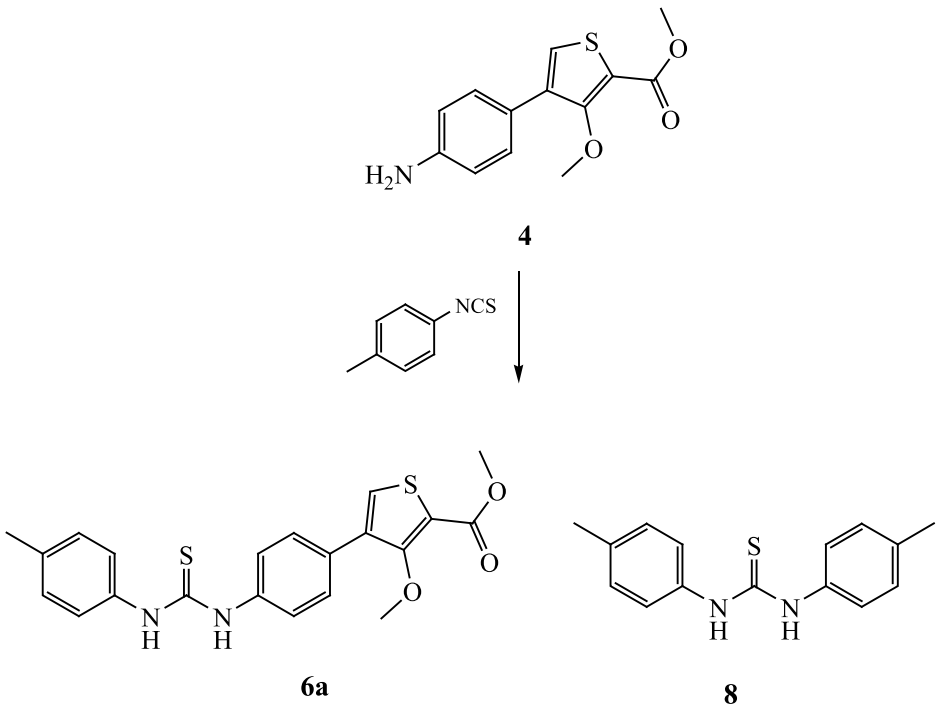
[a] All reactions were performed using amine **4** (1.00 mmol), *p*-tolylisothiocyanate (1.20 mmol), base (2.00 mmol).
[b] Conversion and yield estimated by LC-MS analysis.
[c] Ultrasonic irradiation.
[d] MW irradiation.
[e] Yield 19% upon purification.

Anticancer activity. Anticancer activity of the synthesized compounds **6a**–**6h** and **7a**–**7g** was evaluated against MCF-7, K562,HepG2, MDA-MB-231, HeLa, and HEK293 cell lines using the MTT colorimetric assay and doxorubicin as a standard drug (Table 2). The products exhibited moderate to potent anticancer activity. Thiourea derivatives **6a**–**6h** were determined to be more active than the urea derivatives **7a**–**7g**.

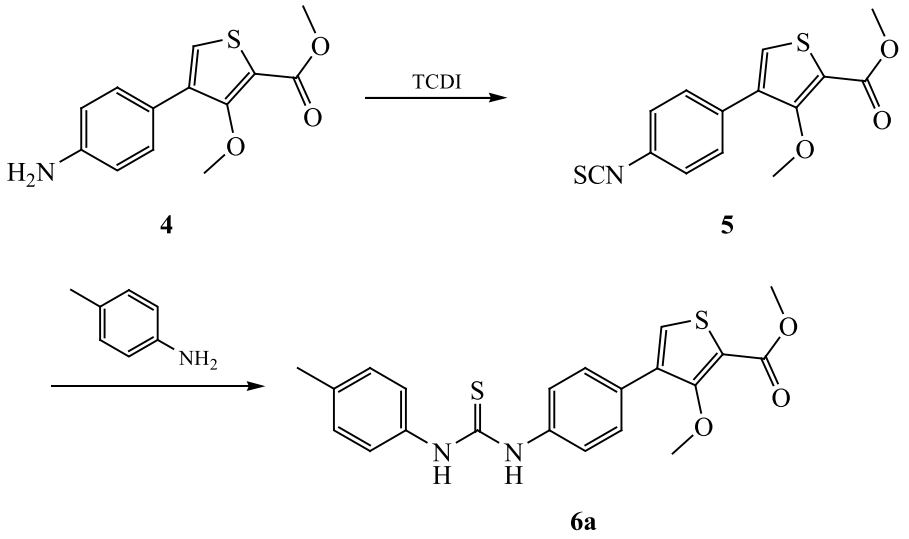
Among the tested compounds, the product **6c** with 4-methoxtphenyl ring demonstrated potent inhibitory activity, and replacement of the above substituent with 3-methoxyphenylhenyl in **6b** led to some decrease in activity. The product with 4-methylphenyl group **6a** was characterized by even lower anticancer activity. The product **6e** bearing 4-bromophenyl ring displayed more inhibitory activity against all cancer cell lines than the compound **6f** with 4-fluorophenyl ring.

Among the urea series **7a**–**7g**, 4-bromophenyl group containing compound **7e** demonstrated activity against

Scheme 2. Reaction of amine **4** with *p*-tolyl isothiocyante.

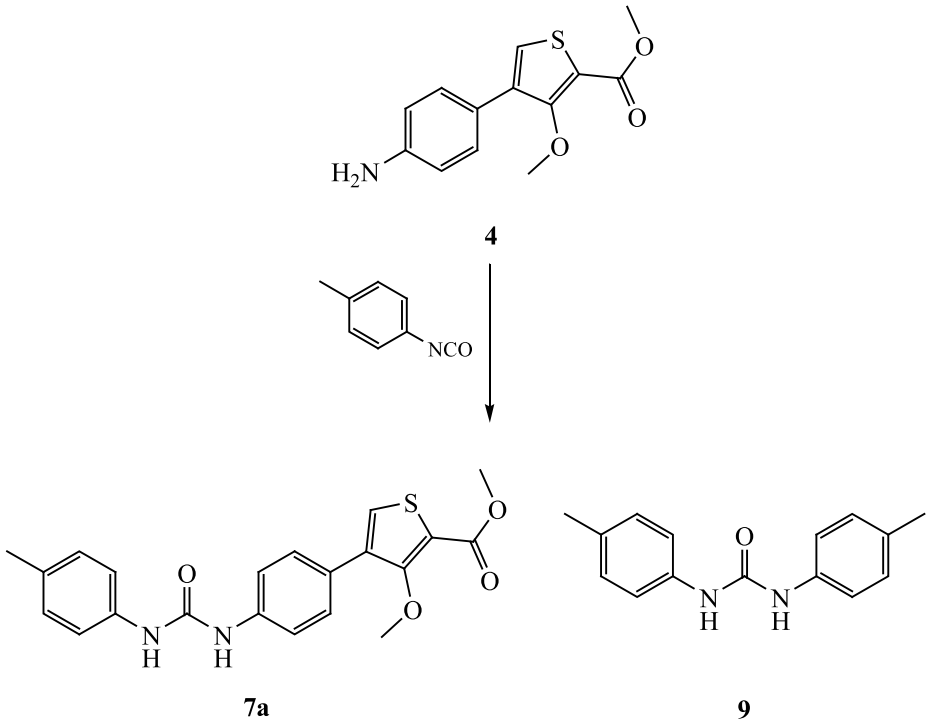


Scheme 3. Synthesis of thio urea derivatives.



4 **5** **6a**

Scheme 4. Reaction of amine **4** with *p*-tolyl isocyante.



4 **7a** **9**

MCF-7 and K562 cancer cell lines. All products exhibited no significant toxic effect.

Molecular docking. The target molecules were docked on the PTP1B active site for considering their binding interactions against PTP 1B. According to docking results, it was evident that the synthesized compounds **6a**–**6h** and **7a**–**7g** interacted with key amino acid residues of PTB1B such as ASP 48, TYR 46, ARG 24, and GLN 262 that are also known for their interactions with PTP1B inhibitors. The representative interactions are presented graphically for compound **6b** (Fig. 1). According to docking procedure carried out for **7b**, the urea NH group could participate in polar interactions with ASP 48 of amino acid residues with 40.0% binding at a distance of 1.59 Å, the other urea NH group interacted with ASP 48 of 61.1% binding at 1.69 Å. The carboxylate C=O group interacted with ARG 221 of 25.3% binding at a distance of 2.59 Å, and phenyl ring exhibited stacking

Table 2. In vitro anticancer activity of the synthesized compounds

Compound	IC_{50}, μM					
	MCF-7	K562	HepG2	MDA-MB 231	HeLa	HEK293
6a	8.84	9.12	13.34	> 50	14.23	Not determined
6b	5.26	7.30	9.43	5.33	8.29	Not determined
6c	2.16	3.27	6.23	1.35	9.63	Not determined
6d	8.32	10.32	Not determined	9.63	15.23	48.25
6e	10.28	9.24	15.33	12.54	18.63	Not determined
6f	12.56	14.23	18.12	16.56	22.35	> 50
6g	22.35	19.86	24.31	21.24	35.63	Not determined
6h	33.69	29.33	18.36	19.58	16.38	Not determined
7a	13.83	18.54	19.26	25.38	17.59	> 50
7b	19.82	> 50	22.19	29.12	25.36	Not determined
7c	24.17	22.58	23.21	37.34	34.81	48.23
7d	18.52	19.28	15.89	23.18	24.43	> 50
7e	14.23	11.69	17.62	16.34	32.55	48.58
7f	12.35	18.53	22.33	19.56	36.76	Not determined
7g	32.26	30.26	22.53	26.88	45.22	Not determined
Doxorubicin	0.41	0.07	5.00	0.60	0.37	

interactions with TYR 46. The compound molecule could be surrounded by ARG 47, GLN 267 and PHE 182.

EXPERIMENTAL

All chemicals were purchased from Sigma–Aldrich, Merck and Combi blocks and used without further purification. Melting points were uncorrected and determined in one end open capillary tubes on a Guna Digital Melting Point apparatus. Elemental analysis was performed on a Perkin-Elmer 240 CHN analyzer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AMX 300 spectrometer operating at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) using TMS as the internal reference and $CDCl_3$ or DMSO-d_6 as the solvents. Mass spectra were measured on an Agilent Technologies mass spectrometer.

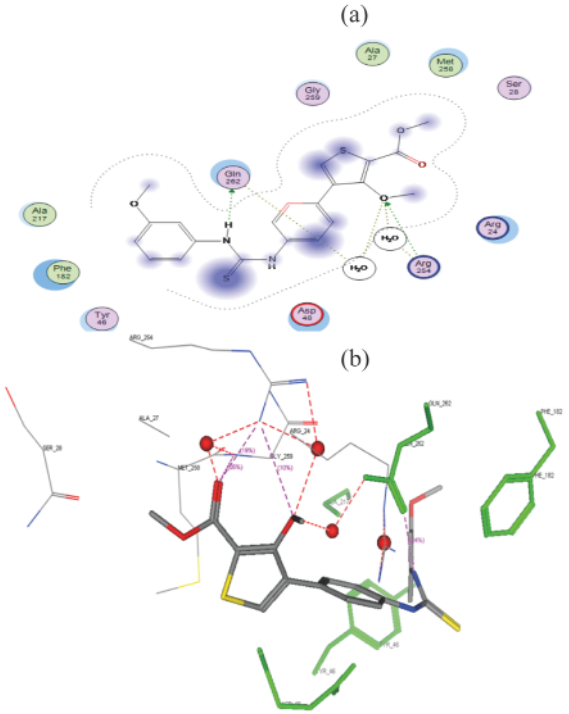


Fig. 1. (a) Two-dimensional representation of the interaction mode of compound **6b** with PTP1B enzyme and (b) three-dimensional structural model of **6b** on PTP1B enzyme.

Methyl 4-(4-isothiocyanophenyl)-3-methoxythiophene-2-carboxylate (5). To a solution of methyl 4-(4-aminophenyl)-3-methoxythiophene-2-carboxylate (2.50 g, 9.50 mmol) in CH_2Cl_2 (25 mL) was added 1,1′-thiocarbonyldiimidazole (2.03 g, 11.4 mmol) and the mixture was stirred at room temperature for 16 h. The solvent was evaporated, and the residue was purified by flash column chromatography (ethyl acetate–hexane) to afford pure compound **5** as an off-white solid. Yield 62%, mp 118–120°C. ^{1}H NMR spectrum, δ, ppm: 3.81 s (3H, $ArOCH_3$), 3.82 s (3H, $C{=}OOCH_3$), 7.51 d (2H, J = 8.8 Hz, H_{Ar}), 7.69 d (2H, J = 8.4 Hz, H_{Ar}), 8.09 s (1H, H_{Ar}). ^{13}C NMR spectrum, δ_C, ppm: 52.00, 62.07, 116.5, 126.2, 128.7, 129.0, 129.2, 133.8, 134.5, 159.4, 160.6. Found, %: C 55.15; H 3.75; N4.48. $C_{14}H_{11}NO_3S_2$. Calculated, %: C 55.06; H 3.63; N 4.59.

Synthesis of compounds 6a–6h. To a solution of methyl 4-(4-isothiocyanatophenyl)-3-methoxythiophene-

2-carboxylate (0.50 mmol) in THF (1.00 mL) was added the desired substituted aniline (0.60 mmol) at room temperature, and the mixture was subjected to MW irradiation upon stirring at 85°C and 150 W for 20 min. The reaction mixture was concentrated under reduced pressure. The obtained crude product was purified by column chromatography (ethyl acetate–hexane) to afford the corresponding pure compound.

Methyl 3-methoxy-4-[4-(3-*p*-tolylthioureido)-phenyl]thiophene-2-carboxylate (6a). Off white solid, yield 90%, mp 180–184°C. ^{1}H NMR spectrum, δ, ppm: 2.28 s (3H, $ArCH_3$), 3.81 s (3H, $ArOCH_3$), 3.82 s (3H, $C{=}OOCH_3$), 7.14 d (2H, J = 8.0 Hz, H_{Ar}), 7.35 d (2H, J = 8.4 Hz, H_{Ar}), 7.57 s (4H, H_{Ar}), 7.96 s (1H, H_{Ar}), 9.78 s (1H, C=SNH), 9.79 s (1H, C=SNH). ^{13}C NMR spectrum, δ_C, ppm: 20.50, 51.93, 61.94, 116.3, 123.2, 123.8, 127.4, 127.9, 128.9, 129.1, 133.7, 135.6, 136.7, 139.1, 159.6, 160.8, 179.4. Found, %: C 61.24; H 4.77; N6.68. $C_{21}H_{20}N_2O_3S_2$. Calculated, %: C61.14; H 4.89; N6.79. MS: *m/z*: 413.2 $[M + H]^+$.

Methyl 3-methoxy-4-{4-[3-(3-methoxyphenyl)-thioureido]phenyl}thiophene-2-carboxylate (6b). Light yellow solid, yield 81%, mp 175–179°C. ^{1}H NMR spectrum, δ, ppm: 3.74 s (3H, $C{=}OOCH_3$), 3.82 s (6H, $2OCH_3$), 6.71 dd (1H, J = 8.0 Hz, J = 2.0 Hz, H_{Ar}), 7.03 d (1H, J = 8.4 Hz, H_{Ar}), 7.18 s (1H, H_{Ar}), 7.24 t (1H, J = 8.0 Hz, H_{Ar}), 7.57 s (4H, H_{Ar}), 7.97 s (1H, H_{Ar}), 9.89 s (1H, C=SNH), 9.90 s (1H, C=SNH). ^{13}C NMR spectrum, δ_C, ppm: 51.04, 51.93, 61.94, 109.1, 109.7, 115.4, 116.2, 123.3, 127.4, 127.9, 129.2, 135.6, 139.0, 140.4, 159.2, 159.6, 160.7, 179.1. Found, %: C 58.96; H 4.79; N6.43. $C_{21}H_{20}N_2O_4S_2$. Calculated, %: C58.86; H 4.70; N6.54. MS: *m/z*: 429.1 $[M + H]^+$.

Methyl 3-methoxy-4-{4-[3-(4-methoxyphenyl)-thioureido]phenyl}thiophene-2-carboxylate (6c). Yellow solid, yield 85%, mp 140–144°C. ^{1}H NMR spectrum, δ, ppm: 3.78 s (3H, $C{=}OOCH_3$), 3.81 s (6H, $2OCH_3$), 7.14 d (2H, J = 8.4 Hz, H_{Ar}), 7.34 d (2H, J = 8.4 Hz, H_{Ar}), 7.56 s (4H, H_{Ar}), 7.96 s (1H, H_{Ar}), 9.79 s (1H, C=SNH), 9.80 s (1H, C=SNH). ^{13}C NMR spectrum, δ_C, ppm: 51.89, 60.14, 61.91, 116.2, 123.2, 123.8, 127.4, 127.8, 128.8, 129.1, 133.7, 135.6, 136.7, 139.1, 159.6, 160.7, 179.4. Found, %: C 58.96; H 4.79; N6.43. $C_{21}H_{20}N_2O_4S_2$. Calculated, %: C 58.86; H 4.70; N6.54. MS: *m/z*: 429.1 $[M + H]^+$.

Methyl 3-methoxy-4-{4-[3-(4-(trifluoromethoxy)-phenyl)thioureido]phenyl}thiophene-2-carboxylate (6d). Brown solid, yield 78%, mp 174–178°C. ^{1}H NMR spectrum, δ, ppm: 3.82 (6H, $2OCH_3$), 7.14 d (2H, J = 8.4 Hz, H_{Ar}), 7.35 d (2H, J = 8.0 Hz, H_{Ar}), 7.57 s (4H, H_{Ar}), 7.97 s (1H, H_{Ar}), 9.80 s (1H, C=SNH), 9.81 s (1H, C=SNH). ^{13}C NMR spectrum, δ_C, ppm: 51.91, 61.84, 116.3, 118.3, 119.4, 121.7, 127.1, 127.3, 135.8, 138.9, 139.1, 142.6, 152.4, 159.6, 160.8. Found, %: C 52.38; H 3.45; N5.70. $C_{21}H_{17}F_3N_2O_4S_2$. Calculated, %: C 52.27; H 3.55; N5.81. MS: *m/z*: 483.1 $[M + H]^+$.

Methyl 4-{4-[3-(3-fluorophenyl)thioureido]-phenyl}-3-methoxythiophene-2-carboxylate (6e). Orange solid, yield 84%, mp 184–189°C. ^{1}H NMR spectrum, δ, ppm: 3.82 s (3H, $ArOCH_3$), 3.83 s (3H, $C{=}OOCH_3$), 6.93–6.97 m (1H, H_{Ar}), 7.26–7.28 m (1H, H_{Ar}), 7.33–7.39 m (1H, H_{Ar}), 7.54–7.60 s (5H, H_{Ar}), 7.98 s (1H, H_{Ar}), 10.0 br. s (2H, C=SNH). ^{13}C NMR spectrum, δ_C, ppm: 51.94, 61.97, 109.8, 110.0, 110.6, 110.8, 116.3, 118.9, 123.4, 127.5, 128.0, 129.5, 129.9, 130.0, 135.6, 138.8, 141.2, 141.3, 159.6, 160.8, 162.9, 179.3. Found, %: C 57.58; H, 4.21; N, 6.62. $C_{20}H_{17}FN_2O_3S_2$. Calculated, %: C 57.68; H, 4.11; N, 6.73. MS: *m/z*: 417.0 $[M + H]^+$.

Methyl 4-{4-[3-(4-fluorophenyl)thioureido]-phenyl}-3-methoxythiophene-2-carboxylate (6f). Brown solid, yield 83%, mp 176–180°C. ^{1}H NMR spectrum, δ, ppm: 3.82 s (3H, $ArOCH_3$), 7.18 t (2H, J = 8.8 Hz, H_{Ar}), 7.47–7.51 m (2H, H_{Ar}), 7.58 s (4H, H_{Ar}), 7.97 s (1H, H_{Ar}), 3.83 s (3H, $C{=}OOCH_3$), 9.24 s (1H, C=SNH), 9.86 s (1H, C=SNH). ^{13}C NMR spectrum, δ_C, ppm: 51.91, 61.93, 114.9, 115.1, 116.2, 123.3, 126.1, 127.5, 127.9, 129.3, 135.6, 135.7, 138.9, 157.9, 159.6, 160.3, 160.7, 179.7. Found, %: C 57.78; H 4.19; N 6.62. $C_{20}H_{17}FN_2O_3S_2$. Calculated, %: C 57.68; H 4.11; N6.73. MS: *m/z*: 417.0 $[M + H]^+$.

Methyl 4-{4-[3-(3,5-difluorophenyl)thioureido]-phenyl}-3-methoxythiophene-2-carboxylate (6g). Orange solid, yield 87%, mp 168–171°C. ^{1}H NMR spectrum, δ, ppm: 3.79 s (3H, $ArOCH_3$), 3.81 s (3H, $C{=}OOCH_3$), 7.03–7.07 m (1H, H_{Ar}), 7.28–7.34 m (1H, H_{Ar}), 7.50–7.56 m (4H, H_{Ar}), 7.92 s (1H, H_{Ar}), 8.06–8.12 m (1H, H_{Ar}), 8.54 s (1H, C=SNH), 9.12 s (1H, C=SNH). ^{13}C NMR spectrum, δ_C, ppm: 51.87, 61.79, 103.7, 110.9, 111.1, 116.2, 118.0, 121.9, 123.9, 124.0, 127.1, 127.3, 127.9, 135.7, 139.0, 152.1, 153.4, 159.6, 160.8. Found, %: C 55.19; H 3.82; N6.35. $C_{20}H_{16}F_2N_2O_3S_2$. Calculated, %: C 55.29; H 3.71; N6.45. MS: *m/z*: 435.2 $[M + H]^+$.

Methyl 4-{4-[3-(5-fluoropyridin-3-yl)thioureido]-phenyl}-3-methoxythiophene-2-carboxylate (6h). Off white solid, yield 76%, mp 135–139°C. ^{1}H NMR spectrum, δ, ppm: 3.82 s (3H, $ArOCH_3$), 3.83 s (3H,

C=OOCH$_3$), 7.55–7.62 m (4H, H$_{Ar}$), 7.99 s (1H, H$_{Ar}$), 8.07–8.11 m (1H, H$_{Ar}$), 8.33 d (1H, J = 2.4 Hz, H$_{Ar}$), 8.49 s (1H, H$_{Ar}$), 10.1 s (1H, C=SNH), 10.2 s (1H, C=SNH). ^{13}C NMR spectrum, δ_C, ppm: 51.94, 62.00, 116.3, 117.5, 117.7, 123.6, 127.6, 128.1, 129.8, 132.5, 132.7, 135.5, 137.6, 138.4, 141.0, 156.9, 159.5, 159.6, 160.7, 179.9. Found, %: C 54.75; H 3.76; N10.17. $C_{19}H_{16}FN_3O_3S_2$. Calculated, %: C 54.66; H 3.86; N10.07. MS: *m/z*: 418.0 [M + H]$^+$.

Synthesis of compounds 7a–7g. To a solution of methyl 4-(4-aminophenyl)-3-methoxythiophene-2-carboxylate (0.38 mmol) in THF (1.00 mL), was added a desired isocyanate (0.45mmol) at room temperature, and the mixture was subjected to MW irradiation upon stirring at 85°C and 150 W for 20 min. The reaction mixture was concentrated under reduced pressure. The obtained crude product was purified by column chromatography (ethyl acetate–hexane) to afford the corresponding pure product.

Methyl 3-methoxy-4-[4-(3-*m*-tolylureido)phenyl]-thiophene-2-carboxylate (7a). Off white solid, yield 71%, mp 182–186°C. ^{1}H NMR spectrum, δ, ppm: 2.29 s (3H, ArCH$_3$), 3.81 s (3H, ArOCH$_3$), 3.82 s (3H, C=OOCH$_3$), 6.70 dd (1H, J = 8.4 Hz, J = 2.4 Hz, H$_{Ar}$), 7.03 d (1H, J = 8.4 Hz, H$_{Ar}$), 7.18 s (1H, H$_{Ar}$), 7.23 t (1H, J = 8.0 Hz, H$_{Ar}$), 7.56 s (4H, H$_{Ar}$), 7.97 s (1H, H$_{Ar}$), 9.88 s (1H, C=ONH), 9.89 s (1H, C=ONH). ^{13}C NMR spectrum, δ_C, ppm: 20.51, 55.02, 61.92, 109.0, 109.7, 115.4, 116.2, 123.3, 123.8, 127.4, 127.9, 129.1, 129.2, 135.6, 139.0, 140.4, 159.2, 159.6, 160.7, 179.1. Found, %: C 63.72; H 5.17; N7.17. $C_{21}H_{20}N_2O_4S$. Calculated, %: C 63.62; H 5.08; N 7.07. MS: *m/z*: 397.1 [M + H]$^+$.

Methyl 3-methoxy-4-[4-(3-*p*-tolylureido)phenyl]-thiophene-2-carboxylate (7b). Orange solid, yield 76%, mp 174–178°C. ^{1}H NMR spectrum, δ, ppm: 2.28 s (3H, ArCH$_3$), 3.80 s (3H, ArOCH$_3$), 3.81 s (3H, C=OOCH$_3$), 7.28–7.30 m (3H, H$_{Ar}$), 7.53–7.57 m (5H, H$_{Ar}$), 7.92 s (1H, H$_{Ar}$), 8.38 s (1H, C=ONH), 8.91 s (1H, C=ONH). ^{13}C NMR spectrum, δ_C, ppm: 20.50, 51.93, 61.95, 116.3, 123.2, 123.8, 123.8, 127.4, 127.9, 128.9, 129.1, 133.7, 135.6, 136.7, 139.1, 159.6, 160.8, 179.4. Found, %: C 63.73; H 5.18; N7.18. $C_{21}H_{20}N_2O_4S$. Calculated, %: C 63.62; H 5.08; N 7.07. MS: *m/z*: 397.1 [M + H]$^+$.

Methyl 4-{4-[3-(3-fluorophenyl)ureido]phenyl}-3-methoxythiophene-2-carboxylate (7c). Off white solid, yield 85%, mp 181–185°C. ^{1}H NMR spectrum, δ, ppm: 3.82 s (3H, ArOCH$_3$), 3.83 s (3H, C=OOCH$_3$), 6.92–6.97 m (1H, H$_{Ar}$), 7.27 d (2H, J = 8.4 Hz, H$_{Ar}$), 7.33–7.39 m (1H, H$_{Ar}$), 7.53–7.60 m (5H, H$_{Ar}$), 7.98 s (1H, H$_{Ar}$), 10.0 br. s (2H, C=ONH). ^{13}C NMR spectrum, δ_C, ppm: 51.87, 61.90, 109.7, 109.9, 110.5, 110.7, 116.2, 118.8, 123.3, 127.4, 127.9, 129.4, 129.8, 129.9, 135.5, 138.7, 141.1, 141.2, 159.5, 160.7, 162.9, 179.2. Found, %: C 59.89; H 4.17; N7.10. $C_{20}H_{17}FN_2O_4S$. Calculated, %: C 59.99; H 4.28; N 7.00. MS: *m/z*: 401.1 [M + H]$^+$.

Methyl 4-{4-[3-(4-fluorophenyl)ureido]phenyl}-3-methoxythiophene-2-carboxylate (7d). Brown solid, yield 71%, mp 171–175°C. ^{1}H NMR spectrum, δ, ppm: 3.81 s (6H, 2OCH$_3$), 7.29 s (2H, H$_{Ar}$), 7.54 s (6H, H$_{Ar}$), 7.92 s (1H, H$_{Ar}$), 8.83 s (1H, C=ONH), 8.91 s (1H, C=ONH). ^{13}C NMR spectrum, δ_C, ppm: 51.90, 61.83, 116.3, 118.3, 119.4, 121.7, 127.1, 127.3, 127.9, 135.8, 138.9, 139.1, 142.6, 152.4, 159.6, 160.8. Found, %: C 59.88; H 4.18; N7.11. $C_{20}H_{17}FN_2O_4S$. Calculated, %: C 59.99; H 4.28; N7.00. MS: *m/z*: 401.1 [M + H]$^+$.

Methyl 4-{4-[3-(4-bromophenyl)ureido]phenyl}-3-methoxythiophene-2-carboxylate (7e). Brown solid, yield 77%, mp 153–156°C. ^{1}H NMR spectrum, δ, ppm: 3.80 s (3H, ArOCH$_3$), 3.81 s (3H, C=OOCH$_3$), 7.15 d (1H, J = 8.0 Hz, H$_{Ar}$), 7.24 t (1H, J = 8.0 Hz, H$_{Ar}$), 7.32 d (1H, J = 8.0 Hz, H$_{Ar}$), 7.54 s (4H, H$_{Ar}$), 7.87 s (1H, H$_{Ar}$), 7.92 s (1H, H$_{Ar}$), 8.87 s (1H, C=ONH), 8.92 s (1H, C=ONH). ^{13}C NMR spectrum, δ_C, ppm: 51.89, 61.82, 116.3, 117.0, 117.2, 118.4, 120.4, 120.6, 121.7, 124.3, 124.6, 127.1, 127.4, 127.9, 130.6, 135.8, 139.0, 141.3, 152.2, 159.6, 160.8. Found, %: C 52.17; H, 3.79; N, 6.16. $C_{20}H_{17}BrN_2O_4S$. Calculated, %: C 52.07; H, 3.71; N, 6.07. MS: *m/z*: 462.9 [M + H]$^+$.

Methyl 3-methoxy-4-{4-[3-(4-(trifluoromethoxy)-phenyl)ureido]phenyl}thiophene-2-carboxylate (7f). Off white solid, yield 68%, mp 162–166°C. ^{1}H NMR spectrum, δ, ppm: 3.80 s (3H, ArOCH$_3$), 3.82 s (3H, C=OOCH$_3$), 7.29 d (2H, J = 8.4 Hz, H$_{Ar}$), 7.54–7.59 m (6H, H$_{Ar}$), 7.92 s (1H, H$_{Ar}$), 8.85 s (1H, C=ONH), 8.93 s (1H, NHC=ONH). ^{13}C NMR spectrum, δ_C, ppm: 51.87, 61.80, 116.3, 118.2, 119.4, 121.6, 127.1, 127.3, 127.9, 135.8, 138.9, 139.1, 142.6, 152.3, 159.6, 160.8. Found, %: C 54.18; H 3.55; N6.12. $C_{21}H_{17}F_3N_2O_5S$. Calculated, %: C 54.08; H 3.67; N 6.0. MS: *m/z*: 467.1 [M + H]$^+$.

Methyl 4-{4-[3-(3,5-difluorophenyl)ureido]phenyl}-3-methoxythiophene-2-carboxylate (7g). Brown solid, yield 79%, mp 168–172°C. ^{1}H NMR spectrum, δ, ppm: 3.80 s (3H, ArOCH$_3$), 3.81 s (3H, C=OOCH$_3$), 7.03–7.08 m (1H, H$_{Ar}$), 7.28–7.34 m (1H, H$_{Ar}$), 7.51–7.56 m (4H, H$_{Ar}$), 7.92 s (1H, H$_{Ar}$), 8.07–8.13 m (1H, H$_{Ar}$), 8.54 s (1H, C=ONH), 9.13 s (1H, C=ONH). ^{13}C NMR spectrum, δ_C, ppm: 51.89, 61.82, 116.3, 118.0, 122.0, 123.9, 124.0,

127.1, 127.3, 127.9, 135.7, 139.0, 152.2, 159.6, 160.8. Found, %: C 57.31; H 3.74; N 6.79. $C_{20}H_{16}F_2N_2O_4S$. Calculated, %: C 57.41; H 3.85; N 6.70. MS: *m/z*: 418.1 $[M + H]^+$.

Anticancer activity. Synthesized molecules were tested for anticancer activity by the MTT colorimetric assay against human cancer cell lines including breast cancer (MDA-MB-231), cervix carcinoma (HeLa), breast adeno carcinoma (MCF-7), hepato cellular carcinoma (HepG2), chronic myeloid leukemia (K562), and hepato cellular carcinoma (HEK293). 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 cells were maintained at temperature of 37°C under humidified atmosphere. Approximately 5×10^3 cells were allowed to adhere for 24 h in 96-well culture plate. Compounds dissolved in the DMSO (10 to 100 μg/mL) were added to the culture wells in triplicate to the 96 well plate and incubated under 5% CO_2 atmosphere at 37°C for 48 h. Afterwards, the cell culture was stained with 20 μL of MTT (5 mg/mL in PBS) for 3 h, and then DMSO (100 μL) was added to each 96-well plate. Absorbance of the test solution was measured at 540 nm using Benesphera plate reader. The results were expressed in IC_{50} and calculated by using origin software [16, 17].

Molecular docking. Molecular docking for all the compounds was carried out on MOE 2008.10 software by using windows 2002 operating system. PTP 1B enzyme was taken from the PDB and by utilizing sequence choice the enzyme was imagined and also additional cofactors were removed. The partial charge of the protein was balanced with the assistance of force field technique AMBER 99. At that point, the protein was exposed through 3D protonation at cut off 12.0, and then hydrogen was introduced by standard geometry and energy was minimized. The ligand structures were composed by utilizing a developer module, and modifying the partial charges, and therefore 3D protonation and hydrogen addition was performed by standard geometry. Ligands energy was minimized at cut off 12 and 6.0 Å grid was produced on the active site of the enzyme. Docking was carried out on amino acids of selective site, and finally docked by selecting solvent and receptor. Out of the resultant 30 postures for each structure, best pose was selected to comprehend molecular interactions [18].

CONCLUSIONS

A new series of urea and thiourea bearing thiophene-2-carboxamide derivatives has been designed, synthesized and evaluated for in vitro anticancer activity against different cancer cell lines using the MTT colorimetric assay. Among the testeded compounds, methyl 3-methoxy-4-{4-[3-(4-methoxyphenyl)thioureido]phenyl}-thiophene-2-carboxylate **(6c)** demonstrates the highest inhibitory activity against MCF-7, K562, HepG2, MDA-MB-231, and HeLa cell lines. The tested compounds show key interactions as those of known PTP1B inhibitors in docking studies.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

1. Tonks, N.K. and Muthuswamy, S.K., *Cancer Cell.,* 2007, vol. 11, p. 214. https://doi.org/10.1016/j.ccr.2007.02.022
2. Bjorge, J.D., Pang, A., and Fujita, D.J., *J. Biol.Chem.,* 2000, vol. 275, p. 41439. https://doi.org/10.1074/jbc.M004852200
3. Julien, S.G., Dubé, N., Read, M., Penney, J., Paquet, M., Han, Y., Kennedy, B.P., Muller, W.J., and Tremblay, M.L., *Nat. Genet.,* 2007, vol. 39, p. 338. https://doi.org/10.1038/ng1963
4. Fobare, W.F., Solvibile, W.R., Robichaud, A.J., Malamas, M.S., Manas, E., Turner, J., Hu, Y., Wagner, E., Chopra, R., Cowling, R., Jin, G., and Bard, J., *Bioorg. Med. Chem. Lett.,* 2007, vol. 17, p. 5353. https://doi.org/10.1016/j.bmcl.2007.08.010
5. Rajender Kumar, P., Raju, S., Satish Goud, P., Sailaja, M.,.Sarma, M.R., Om Reddy, G., Prem Kumar, M., Krishna Reddy, V.V.R.M., Suresh, T., and Hegde, P., *Bioorganic Med. Chem.,* 2004, vol. 12, p. 1221. https://doi.org/10.1016/j.bmc.2003.11.003
6. Bonini, C., Chiummiento, L., De Bonis, M., Funicello, M.M., Lupattelli, P., Suanno, G., Berti, F., and Campaner, P., *Tetrahedron.,* 2005, vol. 61, p. 6580. https://doi.org/10.1016/j.tet.2005.04.048
7. Battaglia, E., Bagrel, D., Migianu, E., Brault, L., Néguesque, A., and Kirsch, G., *Eur. J. Med. Chem.,* 2005, vol. 40, p. 757.

https://doi.org/10.1016/j.ejmech.2005.02.010

8. Buduma, K., Chinde, S., Dommati, A.K., Sharma, P., Shukla, A., Srinivas, K.V.N.S., Arigari, N.K., Khan, F., Tiwari, A.K., Grover, P., and Jonnala, K.K., *Bioorg. Med. Chem. Lett.,* 2016, vol. 26, p. 1633. https://doi.org/10.1016/j.bmcl.2016.01.073
9. Romagnoli, R., Baraldi, P.G., Lopez-Cara, C., Salvador, M.K., Preti, D., Tabrizi, M.A., Balzarini, J., Nussbaumer, P., Bassetto, M., Brancale, A., Fu, X.H., Gao, Y., Li, J., Zhang, S.Z., Hamel, E., Bortolozzi, R., Basso, G., and Viola, G., *Bioorg. Med. Chem.,* 2013, vol. 22, p. 5097. https://doi.org/10.1016/j.bmc.2013.12.030
10. Sztanke, M., Rzymowska, J., and Sztanke, K., *Bioorg. Med. Chem.,* 2015, vol. 23, p. 3448. https://doi.org/ 10.1016/j.bmc.2015.04.037
11. Saeed, S., Rashid, N., Jones, P.G., Ali, M., and Hussain, R., *Eur. J. Med. Chem.,* 2010, vol. 45, p. 1323. https://doi.org/10.1016/j.ejmech.2009.12.016
12. Li, H.Q., Lv, P.C., Yan, T., and Zhu, H.L., *Anticancer Agents. Med. Chem.,* 2009, vol. 9, p. 471. https://doi.org/ 10.2174/1871520610909040471
13. Lv, P.C., Li, H.Q., Sun, J., Zhou, Y., and Zhu, H.L., *Bioorg. Med. Chem.,* 2010, vol. 18, p. 4606. https://doi.org/10.1016/j.bmc.2010.05.034
14. Bodige, S., Ravula, P., Gulipalli, K.C., Endoori, S., Cherukumalli, P.K.R., Seelam, N., and Chandra, J.N.N.S., *Anticancer Agents Med. Chem.,* 2018, vol. 18, p. 891. https://doi.org/10.2174/1871520618666180209151018
15. Gulipalli, K.C., Bodige, S., Ravula, P., Endoori, S., Vanaja, G.R., Suresh Babu, G., Narendra Sharath Chandra, J.N., and Seelam, N., *Bioorg. Med. Chem. Lett.,* 2017, vol. 27, p. 3558. https://doi.org/ 10.1016/j.bmcl.2017.05.047
16. Ravula, P., Bodige, S., Gulipalli, K.C., Vamaraju, H.B., Narendra Sharath Chandra, J.N., and Paturi, M., *J. Heterocycl. Chem.,* 2018, vol. 55, p. 1313. https://doi.org/ 10.1002/jhet.3163
17. Ravula, P. Vamaraju, H.B., Paturi, M., and Sharath Chandra, J.N.N., *Arch. Pharm. (Weinheim).,* 2018, vol. 351, p. 1. https://doi.org/ 10.1002/ardp.201700234
18. Parameshwar, R., Harinadha Babu, V., Manichandrika, P., Narendra Sharath Chandra, J.N., and Swetha, K., *EXCLI J.,* 2016, vol.15, p. 187. https://doi.org/ 10.17179/excli2016-103