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A green method for the synthesis of isoxazolyl aryl thieno[2,3-*d*]pyrimidinones using reusable polyethylene glycol as simple solvent catalyst and evaluation of anti-microbial activity

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Novel series of isoxazolyl aryl thieno[2,3-*d*]pyrimidinones **4** have been synthesized from (*E*)-2-amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)-4-arylthiophene-3-carboxamide synthon **3**. Compound **3** is obtained by reaction of 2-(3-methyl-4-nitro-isoxazol-5-yl)-1-aryl-ethanone **1**, N_1-{3-methyl-5-[(*E*)-2-aryl-1-ethenyl]-4-isoxazolyl}-2-cyanoacetamide **2** and elemental sulfur in PEG-400. Isoxazolyl aryl thieno[2,3-*d*]pyrimidinones **4** have been obtained from compounds **3** by tandem *N*-acetylation and cyclodehydration with acetic anhydride. Compounds **3a-q** and **4a-q** have been characterized by IR, ^{1}H and ^{13}C NMR, and mass spectral data. The title compounds **4a-q** have been evaluated for their antimicrobial activity. Compounds **4b**, **4c**, **4d**, **4e**, **4j**, **4k**, **4l** and **4m** exhibit significant antimicrobial activity, compared to that of standard drugs.

Keywords: Green synthesis, polyethylene glycol (PEG), isoxazolylarylthieno[2,3-*d*]pyrimidinones, antimicrobial activity

Green synthetic routes are the main concern of the present century and current synthetic efforts are directed to achieve this goal. Certainly, there is emergency to replace toxic catalysts and volatile solvents with alternative green catalysts and solvents. Currently, polyethylene glycol-400 (PEG-400) is finding extensive use in organic synthesis as it is well known green solvent and catalyst. As we all know, PEG is a thermally stable, inexpensive, recoverable, and non-toxic hydrophilic polymer. Meanwhile, the high solubility of PEGs in water and several organic solvents including alcohol and acetone[1] instead of their insolubility in less polar solvents such as hexane makes them easy to recover and high-performance solvents for organic reactions[2–4]. Therefore, the use of an obviously benign and inexpensive solvent PEG-400 could yield significant green chemistry benefits.

Biological activity of substituted isoxazoles has made them a focus of medicinal chemistry over the years. Isoxazoles are potent analgesic, anti-inflammatory[5], antimicrobial[6], COX-2 inhibitory[7], antitubercular[8], anticonvulsant[9], and anticancer agents[10]. Heterocyclic derivatives, bearing thienopyrimidines scaffold exhibits a diversity of pharmacological effects such as kinase inhibition, antibacterial, antifungal, and immuno suppressive activity, and antidiabetic and anticancer activity[11–20]. Up to now, there are many different structures containing thienopyrimidine scores which have been synthesized. Despite the breadth of biological activities displayed by these agents, developing new nitrogen-containing heterocyclic derivatives as pharmaceuticals is still an important area of interest in the life sciences.

The promising bioactive diversity of isoxazole[21-27] and thienopyrimidine motif urges us to synthesize and biologically evaluate a series of novel structural variants of isoxazolyl aryl thieno[2,3-*d*]pyrimidinone **4** derivatives. Herein we envisaged a simple and efficient protocol for the synthesis of isoxazolyl aryl thieno[2,3-*d*]pyrimidinones **4** in high yields, using environmentally-friendly PEG-400 as a recyclable reaction medium and catalyst.

Results and Discussion

The key intermediate, (*E*)-2-amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)-4-arylthiophene-3-carboxamide **3** required for

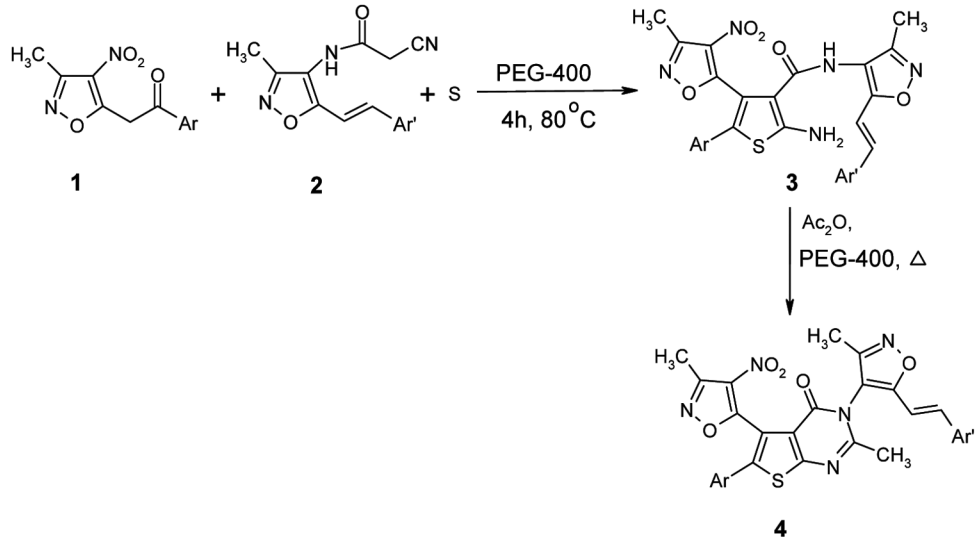
synthesis of target compounds was obtained by reacting2-(3-methyl-4-nitro-isoxazol-5-yl)-1-aryl-ethanone[26] **1**, N_1-{3-methyl-5-[(*E*)-2-aryl-1-ethenyl]-4-isoxazolyl}-2-cyanoacetamide[28] **2** and sulfur in PEG-400. Compounds **3** underwent tandem *N*-acetylation and cyclodehydration involving intramolecular cyclization to afford the title compounds *viz*., isoxazolyl aryl thieno[2,3-*d*]pyrimidinones **4** on heating with acetic anhydride for 5 h in PEG-400 (Scheme I). The structures of the newly synthesized compounds **3a-q** and **4a-q** have been established based on their spectral (IR, ^{1}H and ^{13}C NMR and MS) and analytical data.

The plausible mechanism involves, initially Knoevenagel condensation occurs between carbonyl **1** and activated nitrile **2** in presence of PEG-400. It is assumed that PEG-400 activates both carbonyl **1** and activated nitrile **2** to form ylidine intermediate **A**. PEG-400 polysulfide **B** has been formed *in situ* by the activation of elemental sulfur by PEG-400. Then this polysulfide **B** removes a proton from acidic α-methylene position creating a reactive nucleophilic polysulfide adduct **C**. The anion **C** makes a nucleophilic attack on triple bond of the cyano group to give cyclic imino product **D**, which on subsequent amino-imino tautomerization results in the formation of (*E*)-2-amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)-4-arylthiophene-3-carboxamide **3** (Scheme II).

With regard to sustainable chemistry issues, reagent recyclability is an important question. The separation of the products and the reaction medium were explored for the synthesis of product **4a** in PEG-400. We were pleased to find that the entire reaction medium could be successfully recycled for up to five runs with limited loss of activity (the yield decreased from 94% to 71% after 5 runs, Table I).

Antimicrobial and Antifungal activity

The newly synthesized compounds **4a-q** were evaluated for their *in vitro* antibacterial activity against Gram-positive bacteria *viz*. *Bacillus*

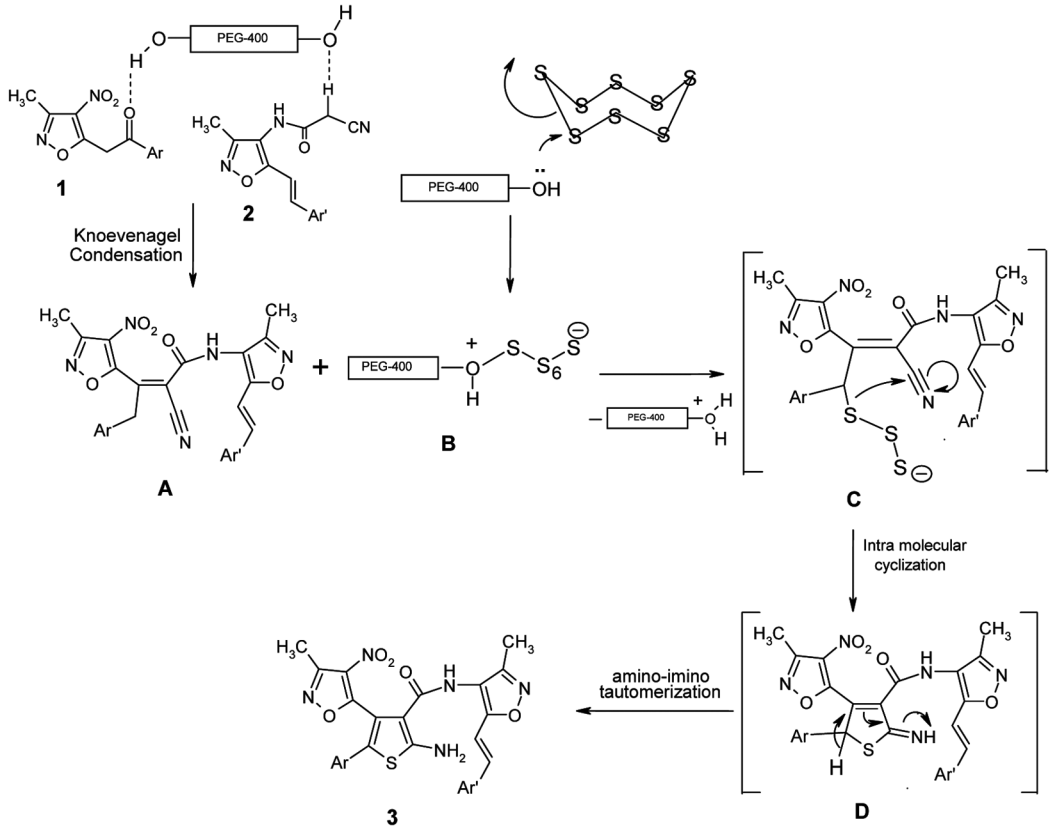


1 **2** **3** **4**

3/4

	Ar	Ar'		Ar	Ar'
a	C_6H_5	C_6H_5	**j**	2-ClC_6H_4	C_6H_5
b	C_6H_5	2-ClC_6H_4	**k**	4-ClC_6H_4	C_6H_5
c	C_6H_5	4-ClC_6H_4	**l**	2-BrC_6H_4	C_6H_5
d	C_6H_5	2-BrC_6H_4	**m**	4-BrC_6H_4	C_6H_5
e	C_6H_5	4-BrC_6H_4	**n**	2-OHC_6H_4	C_6H_5
f	C_6H_5	2-OHC_6H_4	**o**	4-$N(CH_3)_2C_6H_4$	C_6H_5
g	C_6H_5	4-$N(CH_3)_2C_6H_4$	**p**	2-$CH_3OC_6H_4$	C_6H_5
h	C_6H_5	2-$CH_3OC_6H_4$	**q**	2-$CH_3C_6H_4$	C_6H_5
i	C_6H_5	2-$CH_3C_6H_4$			

Scheme I — Synthesis of isoxazolyl aryl thieno[2,3-*d*]pyrimidinones



Scheme II — Plausible mechanism for the formation of (*E*)-2-amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)-4-arylthiophene-3-carboxamide

Table I — Recycling and reuse of PEG-400

Run	Yield (%)
1	94
2	89
3	84
4	78
5	71

sphaericus (MTCC 511), *Staphylococcus aureus* (MTCC 96), and *Bacillus subtilis* (MTCC 441), and Gram-negative bacteria *viz*. *Klebsiella aerogenes* (MTCC 39), *Pseudomonas aeruginosa* (MTCC 741), and *Chromobacterium violaceum* (MTCC 2656) at 100 mg/mL concentration. The *in vitro* antibacterial activity of the tested compounds was assessed by minimum inhibitory concentration (MIC) using broth dilution method[29]. Ciprofloxacin was used as standard drug for comparison. The results of antibacterial screening (Table II) reveal that the compounds **4b**, **4c**, **4d**, **4e**, **4j**, **4k**, **4l** and **4m** exhibited better activity when compared with that of standard Ciprofloxacin. In series **4**, compounds **4b**, **4c**, **4d**, **4e**, **4j**, **4k**, **4l** and **4m** possessing chloro and bromo groups as substituents on the benzene ring showed a better activity. Compound **4a** exhibited least activity because it has no substituent on benzene ring. However, the degree of inhibition varied both with the test compound and with the bacteria used in the present investigation. The compounds **4** having thieno[2,3-*d*]pyrimidinone ring showed maximum activity by inhibiting the growth of all the bacteria under investigation to a greater extent in comparison with the standard drug Ciprofloxacin, and compounds **4b**, **4c**, **4d**, **4e**, **4j**, **4k**, **4l** and **4m** can be exploited for the formulation of bactericides after detailed study. The title compounds **4a-q** were also evaluated for their antifungal activity against *Verticillium dahale*, *Alternaria solani*, *Fusarium oxysporum*, *Colletotrichum capsica*, *Rhizoctonia solani*, and *Pythium aphanidermatum* in acetone by agar cup bioassay method[30], using Clotrimazole as the standard drug. The antifungal activity results (Table III) indicate that compounds **4a-q** are significantly toxic towards all the fungi under investigation. In series **4**, compounds **4b**, **4c**, **4d**, **4e**, **4j**, **4k**, **4l** and **4m** exhibited high antifungal activity by

Table II — Antibacterial activity of isoxazolylarylthieno[2,3-*d*]pyrimidinones**4a-q**

Compd	Minimum inhibitory concentration in µg/mL (MIC)					
	Gram +ve bacteria			Gram −ve bacteria		
	B. sphaericus	*S. aureus*	*B. subtilis*	*K. aerogenes*	*P. aeruginosa*	*C. violaceum*
4a	20	21	18	20	21	19
4b	8	8	6	10	5	6
4c	10	9	9	10	10	5
4d	6	9	10	8	10	10
4e	8	10	11	10	8	10
4f	16	18	15	15	16	12
4g	21	21	17	21	20	19
4h	20	22	15	19	20	16
4i	17	24	18	19	21	14
4j	10	13	10	9	8	9
4k	8	8	8	9	10	9
4l	12	11	9	8	9	8
4m	8	8	10	8	10	10
4n	21	20	16	21	22	20
4o	23	19	20	19	18	16
4p	19	19	19	13	20	15
4q	17	24	17	16	17	16
Ciprofloxacin	24	28	20	22	25	20

Negative control (acetone) -No activity.

Table III — Antifungal activity of isoxazolylarylthieno[2,3-*d*]pyrimidinones**4a-q**

Compd	Minimum inhibitory concentration in µg/mL (MIC)					
	V. dahale	*A. solani*	*F. oxysporum*	*C. capsici*	*R. solani*	*P. aphanidermatum*
4a	24	18	22	19	23	27
4b	9	9	11	7	9	6
4c	10	8	8	5	8	10
4d	6	10	9	6	8	10
4e	8	10	13	12	10	8
4f	19	18	22	18	24	24
4g	18	20	21	19	23	26
4h	21	19	18	21	25	26
4i	20	20	19	23	23	28
4j	9	9	10	7	6	5
4k	8	9	9	8	9	10
4l	11	8	10	10	8	8
4m	7	10	9	9	10	12
4n	24	22	28	16	21	26
4o	27	19	24	17	20	22
4p	21	20	20	15	24	21
4q	22	21	19	19	23	23
Clotrimazole	26	22	28	20	25	30

Negative control (acetone) -No activity.

inhibiting the growth of fungi to a remarkable extent, which may be due to the presence of chloro and bromo substituents on the benzene ring. However, the degree of spore germination inhibition varied with the test compound as well as with the fungi under investigation. The antifungal activity of the compounds **4a-q** has shown better activity, when compared with the standard drug clotrimazole. It is noteworthy that compounds **4b**, **4c**, **4d**, **4e**, **4j**, **4k**, **4l** and **4m** may be exploited for control of wilt diseases of different crops as fungicides after detailed study.

Experimental Section

All the melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical TLC was performed on Merck precoated 60 F_{254} silica gel plates. Visualization was done by exposure to iodine vapour. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in ppm

(δ) with tetramethylsilane as internal standard. ESI mass spectra were recorded on a Agilent LC-MSD mass spectrometer. Elemental analyses were performed on a Carlo Erba 106 and Perkin-Elmer model 240 analysers.

PEG-400 promoted synthesis of (*E*)-2-amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)-4-arylthiophene-3-carboxamides, 3a-q

To 2-(3-methyl-4-nitro-isoxazol-5-yl)-1-aryl-ethanone **1** (1 mmol) in PEG-400 (10 mL), N_1-{3-methyl-5-[(*E*)-2-aryl-1-ethenyl]-4-isoxazolyl}-2-cyanoacetamide**2** (1 mmol) and sulfur (1 mmol)were added and the contents are refluxed with stirring at 80°C for 4 h. After completion of the reaction, as was indicated by TLC, ether (10 mL) was added, and the reaction mixture was stirred for 2 min, and allowed to settle for 5 min. Cooling in an acetone dry ice-bath caused solidification of solvent medium. This allowed us to decant the ether layer. The sequence was repeated twice with 10 mL portion of ether, the combined ether layers were concentrated under reduced pressure, and the resulting crude product was purified by silica gel column chromatography. The product was eluted with ethyl acetate and hexane (2:1) to afford the pure product **3**.

(*E*)-2-Amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)-4-phenylthiophene-3-carboxamide, 3a: Yield 94%; yellow solid. mp 123–125°C. IR (KBr) cm^{-1}: 3300 (CONH), 3210 (NH_2), 1664 (CO), 1570, 1634 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 6.51 (d, 1H, ═CH, *J* = 12 Hz), 6.67 (d, 1H, ═CH, *J* = 12 Hz), 6.91–7.43 (m, 10H, Ar-H), 8.03 (s, 1H, CONH, D_2O exchangeable), 8.76 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.56, 12.10, 100.23, 100. 58, 121.14, 122.40, 124.80, 126.28, 127.13, 127.50, 127.68, 128.32, 128.73, 129.28, 129.59, 130.80, 131.15, 131.73, 131.80, 134.76, 137.67, 149.95, 154.02, 158.63, 158.91, 163.90, 168.41. MS: *m/z* 528 $(M + H)^+$. Anal. Calcd for $C_{27}H_{21}N_5O_5S$: C, 61.47; H, 4.01; N, 13.28; S, 6.08. Found: C, 61.43; H, 4.00; N, 13.26; S, 6.05%.

(*E*)-2-Amino-*N*-(5-(2-chlorostyryl)-3-methylisoxazol-4-yl)-5-(3-methyl-4-nitroisoxazol-5-yl)-4-phenylthiophene-3-carboxamide, 3b:Yield 96%; yellow solid. mp 141–142°C. IR (KBr) cm^{-1}: 3310 (CONH), 3215 (NH_2), 1672 (CO), 1568, 1636 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.26 (s, 6H, 2isoxazole-CH_3), 6.48 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.02–7.51 (m, 9H, Ar-H), 7.94 (s, 1H, CONH, D_2O exchangeable), 8.88 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.66, 12.41, 100.34, 100. 46, 121.18, 122.52, 124.78, 126.32, 127.14, 127.60, 127.79, 128.47, 128.78, 129.37, 129.61, 130.86, 131.34, 131.75, 131.92, 134.52, 137.68, 149.62, 154.55, 158.42, 158.58, 163.82, 168.56. MS: *m/z* 562 $(M + H)^+$. Anal. Calcd for $C_{27}H_{20}ClN_5O_5S$: C, 57.70; H, 3.59; N, 12.46; S, 5.71. Found: C, 57.75; H, 3.56; N, 12.47; S, 5.74%.

(*E*)-2-Amino-*N*-(5-(4-chlorostyryl)-3-methylisoxazol-4-yl)-5-(3-methyl-4-nitroisoxazol-5-yl)-4-phenylthiophene-3-carboxamide, 3c: Yield 95%; yellow solid. mp 147–149°C. IR (KBr) cm^{-1}: 3324 (CONH), 3220 (NH_2), 1655 (CO), 1555, 1642 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.72 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.45 (m, 9H, Ar-H), 8.00 (s, 1H, CONH, D_2O exchangeable), 8.54 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.62, 12.58, 100.42, 100. 67,121.24, 122.54, 124.68, 126.21, 127.26, 127.43, 127.71, 128.43, 128.68, 129.32, 129.61,130.75, 131.23, 131.56, 131.87, 134.53, 137.78, 149.38, 154.11, 158.49, 158.88, 163.84, 168.57. MS: *m/z* 562 $(M + H)^+$. Anal. Calcd for $C_{27}H_{20}ClN_5O_5S$: C, 57.70; H, 3.59; N, 12.46; S, 5.71. Found: C, 57.72; H, 3.57; N, 12.44; S, 5.72%.

(*E*)-2-Amino-*N*-(5-(2-bromostyryl)-3-methylisoxazol-4-yl)-5-(3-methyl-4-nitroisoxazol-5-yl)-4-phenylthiophene-3-carboxamide, 3d: Yield 91%; yellow solid. mp 174–176°C. IR (KBr) cm^{-1}: 3305 (CONH), 3220 (NH_2), 1675 (CO), 1575, 1630 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.72 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.56 (m, 9H, Ar-H), 8.00 (s, 1H, CONH, D_2O exchangeable), 8.45 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.75, 12.44, 100.48, 100. 61, 121.28, 122.32, 124.45, 126.31, 127.26, 127.65, 127.79, 128.42, 128.81, 129.30, 129.71, 130.56, 131.45, 131.56, 131.79, 134.66, 137.73, 149.56, 154.12, 158.51, 158.78, 163.48, 168.57. MS: *m/z* 606 $(M + H)^+$. Anal. Calcd for $C_{27}H_{20}BrN_5O_5S$: C, 53.47; H, 3.32; N, 11.55; S, 5.29. Found: C, 53.49; H, 3.30; N, 11.51; S, 5.32%.

(*E*)-2-Amino-*N*-(5-(4-bromostyryl)-3-methylisoxazol-4-yl)-5-(3-methyl-4-nitroisoxazol-5-yl)-4-phenylthiophene-3-carboxamide, 3e: Yield 93%; yellow solid. mp 169–171°C. IR (KBr) cm^{-1}: 3315 (CONH), 3215 (NH_2), 1670 (CO), 1565, 1630 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 6H, 2isoxazole-CH_3), 6.58 (d, 1H, ═CH, *J* = 12 Hz), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 7.02–7.48 (m, 9H, Ar-H), 7.80 (s, 1H, CONH, D_2O exchangeable), 8.60 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.78, 12.23, 100.41, 100. 32,121.22, 122.51, 124.78, 126.35, 127.22, 127.48, 127.73, 128.24, 128.68, 129.30, 129.61,130.45, 131.23, 131.54, 131.77, 134.53, 137.32, 149.81, 154.11, 158.43, 158.56, 163.78,168.52. MS: *m/z* 606 $(M + H)^+$. Anal. Calcd for $C_{27}H_{20}BrN_5O_5S$: C, 53.47; H, 3.32; N, 11.55; S, 5.29. Found: C, 53.51; H, 3.27; N, 11.54; S, 5.31%.

(*E*)-2-Amino-*N*-(5-(2-hydroxystyryl)-3-methylisoxazol-4-yl)-5-(3-methyl-4-nitroisoxazol-5-yl)-4-phenylthiophene-3-carboxamide, 3f: Yield 90%; yellow solid. mp 153–155°C. IR (KBr) cm^{-1}: 3315 (OH), 3310 (CONH), 3215 (NH_2), 1664 (CO), 1575, 1630 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 6H, 2isoxazole-CH_3), 5.00 (s, 1H, OH, D_2O exchangeable), 6.50 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.02–7.52 (m, 9H, Ar-H), 8.00 (s, 1H, CONH, D_2O exchangeable), 8.66 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.65, 12.27, 100.41, 100. 63, 121.23, 122.48, 124.76, 126.33, 127.26,127.55, 127.73, 128.21, 128.72, 129.30, 129.66, 130.67, 131.24, 131.58, 131.77, 134.50,137.22, 149.61, 154.17, 158.57, 158.84, 163.59, 168.30. MS: *m/z* 544 $(M + H)^+$. Anal. Calcd for $C_{27}H_{21}N_5O_6S$: C, 59.66; H, 3.89; N, 12.88; S, 5.90. Found: C, 59.65; H, 3.92; N, 12.86; S, 5.87%.

(*E*)-2-Amino-*N*-(5-(4-(dimethylamino)styryl)-3-methylisoxazol-4-yl)-5-(3-methyl-4-nitroisoxazol-5-yl)-4-phenylthiophene-3-carboxamide, 3g: Yield 92%; yellow solid. mp 166–168°C. IR (KBr) cm^{-1}: 3300 (CONH), 3225 (NH_2), 1660 (CO), 1565, 1635 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 6H, 2isoxazole-CH_3), 2.68 (s, 6H, $N(CH_3)_2$), 6.50 (d, 1H, ═CH, *J* = 12 Hz), 6.62 (d, 1H, ═CH, *J* = 12 Hz), 6.95–7.51 (m, 9H, Ar-H), 7.85 (s, 1H, CONH, D_2O exchangeable), 8.51 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.29, 12.33, 42.68, 100.45, 100. 41, 121.23, 122.58, 124.72, 126.39, 127.22,127.61, 127.79, 128.24, 128.68, 129.33, 129.62, 130.70, 131.24, 131.55, 131.74, 134.64,137.58, 149.66, 154.11, 158.54, 158.72, 163.66, 168.54. MS: *m/z* 571 $(M + H)^+$. Anal. Calcd for $C_{29}H_{26}N_6O_5S$: C, 61.04; H, 4.59; N, 14.73; S, 5.62. Found: C, 61.08; H, 4.58; N, 14.61; S, 5.59%.

(*E*)-2-Amino-*N*-(5-(2-methoxystyryl)-3-methylisoxazol-4-yl)-5-(3-methyl-4-nitroisoxazol-5-yl)-4-phenylthiophene-3-carboxamide, 3h: Yield 91%; yellow solid. mp 157–159°C. IR (KBr) cm^{-1}: 3305 (CONH), 3225 (NH_2), 1665 (CO), 1565, 1630 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.26 (s, 6H, 2isoxazole-CH_3), 3.68 (s, 3H, OCH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.62 (d, 1H, ═CH, *J* = 12 Hz), 7.10–7.48 (m, 9H, Ar-H), 8.02 (s, 1H, CONH, D_2O exchangeable), 8.45 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.46,12.43, 62.05, 100.21, 100. 41, 121.18, 122.46, 124.84, 126.34, 127.11, 127.55, 127.61, 128.41,128.70, 129.24, 129.53, 130.78, 131.10, 131.61, 131.77, 134.72, 137.60, 149.46, 154.12, 158.43, 158.71, 163.94, 168.56. MS: *m/z* 558 $(M + H)^+$. Anal. Calcd for $C_{28}H_{23}N_5O_6S$: C, 60.31; H, 4.16; N, 12.56; S, 5.75. Found: C, 60.36; H, 4.15; N, 12.57; S, 5.78%.

(*E*)-2-Amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-(2-methylstyryl)isoxazol-4-yl)-4-phenylthiophene-3-carboxamide, 3i: Yield 88%; yellow solid. mp 163–165°C. IR (KBr) cm^{-1}: 3315 (CONH), 3215 (NH_2), 1660 (CO), 1565, 1625 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 2.52 (s, 3H, CH_3), 6.54 (d, 1H, ═CH, *J* = 12 Hz), 6.64 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.51 (m, 9H, Ar-H), 8.14 (s, 1H, CONH, D_2O exchangeable), 8.53 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.44,12.61, 26.25, 100.12, 100. 42, 121.33, 122.57, 124.81, 126.22, 127.10, 127.56, 127.67, 128.31,128.53, 129.34, 129.67, 130.85, 131.25, 131.53, 131.68, 134.70, 137.47, 149.90, 154.11, 158.52, 158.61, 163.64, 168.55. MS: *m/z* 542 $(M + H)^+$. Anal. Calcd for $C_{28}H_{23}N_5O_5S$: C, 62.10; H, 4.28; N, 12.93; S, 5.92. Found: C, 62.06; H, 4.27; N, 12.97; S, 5.97%.

(*E*)-2-Amino-4-(2-chlorophenyl)-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl) thiophene-3-carboxamide, 3j: Yield 93%; yellow solid. mp 149–151°C. IR (KBr) cm^{-1}: 3300 (CONH), 3215 (NH_2), 1672 (CO), 1568, 1636 (NO_2); ^{1}H NMR

(300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 6.52 (d, 1H, ═CH, *J* = 12 Hz), 6.66 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.45 (m, 9H, Ar-H), 7.98 (s, 1H, CONH, D_2O exchangeable), 8.60 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.26, 12.40, 100.33, 100. 68, 121.24, 122.38, 124.44, 126.25, 127.24, 127.62, 127.88, 128.20, 128.55, 129.31, 129.62, 130.65, 131.22, 131.54, 131.68, 134.54, 137.60, 149.87, 154.16, 158.56, 158.79, 163.82, 168.34. MS: *m/z* 562 $(M + H)^+$. Anal. Calcd for $C_{27}H_{20}ClN_5O_5S$: C, 57.70; H, 3.59; N, 12.46; S, 5.71. Found: C, 57.72; H, 3.63; N, 12.43; S, 5.73%.

(*E*)-2-Amino-4-(4-chlorophenyl)-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl) thiophene-3-carboxamide, 3k: Yield 93%; yellow solid. mp 149–151°C. IR (KBr) cm^{-1}: 3315 (CONH), 3210 (NH_2), 1675 (CO), 1565, 1640 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.66 (d, 1H, ═CH, *J* = 12 Hz), 7.10–7.55 (m, 9H, Ar-H), 8.03 (s, 1H, CONH, D_2O exchangeable), 8.42 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.26, 12.47, 100.20,100. 47, 121.23, 122.56, 124.71, 126.30, 127.23, 127.48, 127.74, 128.44, 128.69, 129.30,129.61, 130.66, 131.25, 131.58, 131.79, 134.70, 137.57, 149.75, 154.32, 158.53, 158.71,163.84, 168.55. MS: *m/z* 562 $(M + H)^+$. Anal. Calcd for $C_{27}H_{20}ClN_5O_5S$: C, 57.70; H, 3.59; N, 12.46; S, 5.71. Found: C, 57.74; H, 3.58; N, 12.49; S, 5.74%.

(*E*)-2-Amino-4-(2-bromophenyl)-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl) thiophene-3-carboxamide, 3l: Yield 93%; yellow solid. mp 179–181°C. IR (KBr) cm^{-1}: 3312 (CONH), 3210 (NH_2), 1655 (CO), 1565, 1630 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.26 (s, 6H, 2isoxazole-CH_3), 6.54 (d, 1H, ═CH, *J* = 12 Hz), 6.70 (d, 1H, ═CH, *J* = 12 Hz), 6.95–7.42 (m, 9H, Ar-H), 8.02 (s, 1H, CONH, D_2O exchangeable), 8.40 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.26, 12.49, 100.43, 100. 69,121.24, 122.49, 124.88, 126.58, 127.33, 127.70, 127.98, 128.42, 128.63, 129.18, 129.29,130.63, 131.22, 131.57, 131.79, 134.62, 137.51, 149.40, 154.11, 158.28, 158.61, 163.78, 168.33. MS: *m/z* 606 $(M + H)^+$. Anal. Calcd for $C_{27}H_{20}BrN_5O_5S$: C, 53.47; H, 3.32; N, 11.55; S, 5.29. Found: C, 53.50; H, 3.31; N, 11.58; S, 5.25%.

(*E*)-2-Amino-4-(4-bromophenyl)-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl) thiophene-3-carboxamide, 3m: Yield 90%; yellow solid. mp 171–173 °C. IR (KBr) cm^{-1}: 3305 (CONH), 3215 (NH_2), 1675 (CO), 1560, 1635 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.202 (s, 6H, 2isoxazole-CH_3), 6.62 (d, 1H, ═CH, *J* = 12 Hz), 6.70 (d, 1H, ═CH, *J* = 12 Hz), 7.05–7.55 (m, 9H, Ar-H), 8.05 (s, 1H, CONH, D_2O exchangeable), 8.60 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.36, 12.60, 100.33, 100.68, 121.10, 122.47, 124.66, 126.36, 127.30, 127.59, 127.77, 128.24, 128.56, 129.38, 129.69, 130.56, 131.26, 131.64, 131.77, 134.72, 137.60, 149.75, 154.09, 158.53, 158.79, 163.42, 168.56. MS: *m/z* 606 $(M + H)^+$. Anal. Calcd for $C_{27}H_{20}BrN_5O_5S$: C, 53.47; H, 3.32; N, 11.55; S, 5.29. Found: C, 53.42; H, 3.31; N, 11.57; S, 5.30%.

(*E*)-2-Amino-4-(2-hydroxyphenyl)-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)thiophene-3-carboxamide, 3n: Yield 92%; yellow solid. mp 161–163°C. IR (KBr) cm^{-1}: 3320 (OH), 3300 (CONH), 3215 (NH_2), 1675 (CO), 1560, 1642 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.28 (s, 6H, 2isoxazole-CH_3), 486 (s, 1H, OH, D_2O exchangeable), 6.55 (d, 1H, ═CH, *J* = 12 Hz), 6.628 (d, 1H, ═CH, *J* = 12 Hz), 7.02–7.50 (m, 9H, Ar-H), 7.85 (s, 1H, CONH, D_2O exchangeable), 8.05 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.47, 12.48, 100.36, 100. 62, 121.11, 122.32, 124.78, 126.18,127.22, 127.36, 127.55, 128.45, 128.70, 129.35, 129.68, 130.66, 131.22, 131.75, 131.92, 134.66, 137.48, 149.65, 154.13, 158.50, 158.69, 163.71, 168.33. MS: *m/z* 544 $(M + H)^+$. Anal. Calcd for $C_{27}H_{21}N_5O_6S$: C, 59.66; H, 3.89; N, 12.88; S, 5.90. Found: C, 59.68; H, 3.93; N, 12.83; S, 5.94%.

(*E*)-2-Amino-4-(4-(dimethylamino)phenyl)-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)thiophene-3-carboxamide, 3o: Yield 94%; yellow solid. mp 177–179 °C. IR (KBr) cm^{-1}: 3310 (CONH), 3210 (NH_2), 1675 (CO), 1560, 1645 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 2.62 (s, 6H, $N(CH_3)_2$), 6.58 (d, 1H, ═CH, *J* = 12 Hz), 6.66 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.42 (m, 9H, Ar-H), 7.50 (s, 1H, CONH, D_2O exchangeable), 8.55 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.34, 12.37, 42.68, 100.43, 100. 64, 121.23, 122.56, 124.78, 126.33, 127.46, 127.63, 127.77, 128.11, 128.58, 129.36, 129.71, 130.66, 131.10, 131.58, 131.76, 134.53, 137.43, 149.74, 154.08, 158.42, 158.62, 163.80, 168.35. MS: *m/z* 571 $(M + H)^+$. Anal.

Calcdfor $C_{29}H_{26}N_6O_5S$: C, 61.04; H, 4.59; N, 14.73; S, 5.62. Found: C, 61.00; H, 4.56; N, 14.79; S, 5.66%.

(*E*)-2-Amino-4-(2-methoxyphenyl)-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)thiophene-3-carboxamide, 3p: Yield 94%; yellow solid. mp 163–165°C. IR (KBr) cm^{-1}: 3300 (CONH), 3215 (NH_2), 1665 (CO), 1570, 1625 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 3.52 (s, 3H, OCH_3), 6.58 (d, 1H, ═CH, *J* = 12 Hz), 6.64 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.50 (m, 9H, Ar-H), 7.80 (s, 1H, CONH, D_2O exchangeable), 8.40 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.78, 12.63, 62.05, 100.39, 100. 62, 121.24, 122.48, 124.87, 126.24, 127.23, 127.56, 127.78,128.22, 128.83, 129.58, 129.69, 130.40, 131.16, 131.63, 131.84, 134.46, 137.37, 149.90, 154.11, 158.68, 158.61, 163.66, 168.58. MS: *m/z* 558 $(M + H)^+$. Anal. Calcd for $C_{28}H_{23}N_5O_6S$: C, 60.31; H, 4.16; N, 12.56; S, 5.75. Found: C, 60.34; H, 4.18; N, 12.52; S, 5.71%.

(*E*)-2-Amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-5-styrylisoxazol-4-yl)-4-(*o*-tolyl) thiophene-3-carboxamide, 3q: Yield 90%; yellow solid. mp 168–170°C. IR (KBr) cm^{-1}: 3320 (CONH), 3210 (NH_2), 1665 (CO), 1560, 1630 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 2.48 (s, 3H, CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.10–7.42 (m, 9H, Ar-H), 8.00 (s, 1H, CONH, D_2O exchangeable), 8.65 (bs, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.42, 12.38, 26.25, 100.46,100. 69, 121.31, 122.46, 124.58, 126.34, 127.26, 127.59, 127.67, 128.38, 128.53, 129.16, 129.45, 130.76, 131.26, 131.55, 131.76, 134.58, 137.66, 149.63, 154.12, 158.35, 158.67, 163.88, 168.56. MS: *m/z* 542 $(M + H)^+$. Anal. Calcd for $C_{28}H_{23}N_5O_5S$: C, 62.10; H, 4.28; N, 12.93; S, 5.92. Found: C, 62.12; H, 4.31; N, 12.96; S, 5.87%.

General procedure for PEG-400 promoted synthesis of new isoxazolylarylthieno[2,3-*d*]pyrimidinones, 4a-q

To (*E*)-2-amino-5-(3-methyl-4-nitroisoxazol-5-yl)-*N*-(3-methyl-4-styrylisoxazol-5-yl)-4-arylthiophene-3-carboxamides **3** (1 mmol) in PEG-400 (10 mL), acetic anhydride (5 mL) was added and the contents are refluxed with for 5 h. After completion of the reaction, as was indicated by TLC, ether (10 mL) was added, and the reaction mixture was stirred for 2 min, and allowed to settle for 5 min. Cooling in an acetone dry ice-bath caused solidification of solvent medium. This allowed us to decant the ether layer. The sequence was repeated twice with 10 mL portion of ether, the combined ether layers were concentrated under reduced pressure, and the resulting crude product was recrystallized from ethanol to give compound **4**.

(*E*)-2-Methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl)-5-phenylthieno [2,3-*d*]pyrimidin-4(3*H*)-one, 4a: Yield 81%; yellow solid. mp 258–260°C. IR (KBr) cm^{-1}: 1665 (CO), 1565, 1650 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 2.40 (s, 3H, pyrimidine-CH_3),6.48 (d, 1H, ═CH, *J* = 12 Hz), 6.62 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.45 (m, 10H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.66, 12.15, 22.26, 100.13, 100.44, 121.24, 122.51, 124.68, 126.22, 127.22, 127.46, 127.61, 128.36, 128.70, 129.21, 129.60, 130.64, 131.34, 131.70, 131.89, 134.55, 137.62, 149.80, 154.14, 158.55, 158.90, 162.68, 163.54, 168.22. MS: *m/z* 552 $(M + H)^+$. Anal. Calcd for $C_{29}H_{21}N_5O_5S$: C, 63.15; H, 3.84; N, 12.70; S, 5.81. Found: C, 63.12; H, 3.88; N, 12.75; S, 5.78%.

(*E*)-3-(5-(2-Chlorostyryl)-3-methylisoxazol-4-yl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4b: Yield 83%; yellow solid. mp 312–314°C. IR (KBr) cm^{-1}: 1680 (CO), 1560, 1645 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 6H, 2isoxazole-CH_3), 2.48 (s, 3H, pyrimidine-CH_3), 6.56 (d, 1H, ═CH, *J* = 12 Hz), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.90–7.26 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.56, 12.36,22.48, 100.26, 100.54, 121.21, 122.62, 124.71, 126.35, 127.21, 127.55, 127.73, 128.40, 128.68, 129.25, 129.67, 130.24, 131.54, 131.76, 131.99, 134.50, 137.42, 149.60, 154.24, 158.35,158.60, 162.62, 163.51, 168.53. MS: *m/z* 586 $(M + H)^+$. Anal. Calcd for $C_{29}H_{20}ClN_5O_5S$: C, 59.44; H, 3.44; N, 11.95; S, 5.47. Found: C, 59.38; H, 3.47; N, 11.97;S, 5.51%.

(*E*)-3-(5-(4-Chlorostyryl)-3-methylisoxazol-4-yl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4c: Yield 81%; yellow solid. mp 301–303°C. IR (KBr) cm^{-1}: 1675 (CO), 1560, 1648 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 2.42 (s, 3H, pyrimidine-CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.38 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.47,

12.34,22.28, 100.26, 100.36, 121.26, 122.45, 124.52, 126.28, 127.34, 127.55, 127.69, 128.21, 128.58,129.34, 129.45, 130.71, 131.28, 131.76, 131.90, 134.46, 137.67, 149.54, 154.23, 158.44,158.67, 162.25, 163.14, 168.38. MS: *m/z* 586 $(M + H)^+$. Anal. Calcd for $C_{29}H_{20}ClN_5O_5S$: C, 59.44; H, 3.44; N, 11.95; S, 5.47. Found: C, 59.47; H, 3.48; N, 11.93; S, 5.49%.

(*E*)-3-(5-(2-Bromostyryl)-3-methylisoxazol-4-yl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4d: Yield 84%; yellow solid. mp 354–356°C. IR (KBr) cm^{-1}: 1660 (CO), 1545, 1640 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.26 (s, 6H, 2isoxazole-CH_3), 2.50 (s, 3H, pyrimidine-CH_3), 6.50 (d, 1H, ═CH, *J* = 12 Hz), 6.64 (d, 1H, ═CH, *J* = 12 Hz), 6.98–7.40 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.45, 12.38,22.44, 100.25, 100.67, 121.32, 122.45, 124.56, 126.35, 127.26, 127.51, 127.75, 128.42, 128.58,129.20, 129.42, 130.51, 131.44, 131.78, 131.94, 134.41, 137.34, 149.65, 154.23, 158.42,158.76, 162.53, 163.33, 168.43. MS: *m/z* 630 $(M + H)^+$. Anal. Calcd for $C_{29}H_{20}BrN_5O_5S$: C, 55.25; H, 3.20; N, 11.11; S, 5.09. Found: C, 55.29; H, 3.24; N, 11.07; S, 5.11%.

(*E*)-3-(5-(2-Bromostyryl)-3-methylisoxazol-4-yl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4e: Yield 85%; yellow solid. mp 360–362°C. IR (KBr) cm^{-1}: 1682 (CO), 1550, 1635 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 2.40 (s, 3H, pyrimidine-CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.20–7.48 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.47, 12.39,22.31, 100.28, 100.46, 121.33, 122.48, 124.52, 126.24, 127.36, 127.58, 127.76, 128.22, 128.57,129.29, 129.53, 130.41, 131.30, 131.57, 131.81, 134.56, 137.55, 149.71, 154.22, 158.58,158.73, 162.57, 163.50, 168.38. MS: *m/z* 630 $(M + H)^+$. Anal. Calcd for $C_{29}H_{20}BrN_5O_5S$: C, 55.25; H, 3.20; N, 11.11; S, 5.09. Found: C, 55.27; H, 3.16; N, 11.14; S, 5.06%.

(*E*)-3-(5-(2-Hydroxystyryl)-3-methylisoxazol-4-yl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4f: Yield 82%; yellow solid. mp 245–247°C. IR (KBr) cm^{-1}: 3310 (OH), 1685 (CO), 1545, 1640 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 2.46 (s, 3H, pyrimidine-CH_3), 5.20 (s, 1H, OH, D_2O exchangeable), 6.54 (d, 1H, ═CH, *J* = 12 Hz), 6.62 (d, 1H, ═CH, *J* = 12 Hz), 7.04–7.30 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 1147, 12.56, 22.32, 100.26, 100.51, 121.33, 122.50,124.47, 126.39, 127.19, 127.55, 127.71, 128.41, 128.77, 129.19, 129.58, 130.52, 131.26,131.64, 131.76, 134.45, 137.55, 149.67, 154.24, 158.58, 158.84, 162.55, 163.48, 168.37. MS: *m/z* 568 $(M + H)^+$. Anal. Calcd for $C_{29}H_{21}N_5O_6S$: C, 61.37; H, 3.73; N, 12.34; S, 5.65. Found: C, 61.41; H, 3.71; N, 12.37; S, 5.69%.

(*E*)-3-(5-(4-(Dimethylamino)styryl)-3-methylisoxazol-4-yl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4g: Yield 79%; yellow solid. mp 377–379°C. IR (KBr) cm^{-1}: 1680 (CO), 1565, 1645 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.26 (s, 6H, 2isoxazole-CH_3), 2.42 (s, 3H, pyrimidine-CH_3), 2.62 (s, 6H, $N(CH_3)_2$), 6.58 (d, 1H, ═CH, *J* = 12 Hz), 6.62 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.38 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.51, 12.31, 22.46, 42.68, 100.44, 100. 69, 121.27, 122.38,124.58, 126.21, 127.3, 127.57, 127.60, 128.34, 128.53, 129.38, 129.69, 130.85, 131.25,131.53, 131.71, 134.55, 137.42, 149.73, 154.11, 158.51, 158.88, 162.42, 163.77, 168.56. MS: *m/z* 595 $(M + H)^+$. Anal. Calcd for $C_{31}H_{26}N_6O_5S$: C, 62.61; H, 4.41; N, 14.13; S, 5.39. Found: C, 62.63; H, 4.37; N, 14.19; S, 5.38%.

(*E*)-3-(5-(2-Methoxystyryl)-3-methylisoxazol-4-yl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4h: Yield 81%; yellow solid. mp 345–347°C. IR (KBr) cm^{-1}: 1675 (CO), 1565, 1650 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 6H, 2isoxazole-CH_3), 2.48 (s, 3H, pyrimidine-CH_3), 3.62 (s, 3H, OCH_3), 6.52 (d, 1H, ═CH, *J* = 12 Hz), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 7.02–7.42 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.39, 12.28, 22.53, 58.32, 100.29, 100. 44, 121.21, 122.32, 124.56, 126.09, 127.10,127.42, 127.55, 128.12, 128.58, 129.33, 129.61, 130.78, 131.26, 131.56, 131.73, 134.51,137.69, 149.62, 154.22, 158.43, 158.81, 162.42, 163.56, 168.58. MS: *m/z* 582 $(M + H)^+$. Anal. Calcd for $C_{30}H_{23}N_5O_6S$: C, 61.95; H, 3.99; N, 12.04; S, 5.51. Found: C, 61.92; H, 4.03; N, 12.00; S, 5.49%.

(*E*)-2-Methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-(2-methylstyryl)isoxazol-4-yl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4i: Yield 80%; yellow solid. mp 331–333°C. IR (KBr) cm^{-1}:

1680 (CO), 1565, 1645 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.28 (s, 6H, 2isoxazole-CH_3), 2.42 (s, 3H, pyrimidine-CH_3), 2.60 (s, 3H, Ar-CH_3), 6.62 (d, 1H, ═CH, *J* = 12 Hz), 6.70 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.32 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.48, 12.62, 22.59, 26.48, 100.13, 100. 40, 121.28, 122.48, 124.62, 126.31, 127.22,127.41, 127.59, 128.26, 128.54, 129.31, 129.61, 130.74, 131.22, 131.54, 131.74, 134.44,137.51, 149.49, 154.11, 158.21, 158.49, 162.22, 163.74, 168.62. MS: *m/z* 566 $(M + H)^+$. Anal. Calcd for $C_{30}H_{23}N_5O_5S$: C, 63.71; H, 4.10; N, 12.38; S, 5.67. Found: C, 63.74; H, 4.13; N, 12.42; S, 5.62%.

(*E*)-5-(2-Chlorophenyl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4j: Yield 83%; yellow solid. mp 312–314°C. IR (KBr) cm^{-1}: 1670 (CO), 1560, 1655 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.28 (s, 6H, 2isoxazole-CH_3), 2.40 (s, 3H, pyrimidine-CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.28 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.42,12.35, 22.24, 100.43, 100.64, 121.14, 122.54, 124.58, 126.32, 127.28, 127.36, 127.60, 128.46,128.60, 129.11, 129.50, 130.61, 131.30, 131.75, 131.84, 134.51, 137.60, 149.60, 154.24,158.52, 158.78, 162.58, 163.44, 168.11. MS: *m/z* 586 $(M+H)^+$. Anal. Calcd for $C_{29}H_{20}ClN_5O_5S$: C, 59.44; H, 3.44; N, 11.95; S, 5.47. Found: C, 59.46; H, 3.49; N, 12.00; S, 5.45%.

(*E*)-5-(4-Chlorophenyl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl) thieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4k: Yield 81%; yellow solid. mp 327–329°C. IR (KBr) cm^{-1}: 1685 (CO), 1558, 1640 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 2.52 (s, 3H, pyrimidine-CH_3), 6.50 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.05–7.32 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.42,12.56, 22.28, 100.24, 100.38, 121.44, 122.58, 124.47, 126.25, 127.32, 127.51, 127.56, 128.45,128.68, 129.11, 129.51, 130.56, 131.22, 131.57, 131.64, 134.38, 137.59, 149.66, 154.24,158.32, 158.57, 162.51, 163.22, 168.11. MS: *m/z* 586 $(M+H)^+$. Anal. Calcd for $C_{29}H_{20}ClN_5O_5S$: C, 59.44; H, 3.44; N, 11.95; S, 5.47. Found: C, 59.49; H, 3.43; N, 11.96; S, 5.47%.

(*E*)-5-(2-Bromophenyl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4l: Yield 80%; yellow solid. mp 360–362°C. IR (KBr) cm^{-1}: 1668 (CO), 1545, 1650 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 2.40 (s, 3H, pyrimidine-CH_3), 6.42 (d, 1H, ═CH, *J* = 12 Hz), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.94–7.21 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.46,12.30, 22.32, 100.26, 100.53, 121.18, 122.31, 124.35, 126.42, 127.51, 127.69, 127.77, 128.23,128.57, 129.11, 129.48, 130.54, 131.21, 131.56, 131.57, 134.48, 137.51, 149.73, 154.21,158.48, 158.70, 162.44, 163.31, 168.26. MS: *m/z* 630 $(M+H)^+$. Anal. Calcd for $C_{29}H_{20}BrN_5O_5S$: C, 55.25; H, 3.20; N, 11.11; S, 5.09. Found: C, 55.26; H, 3.17; N, 11.12; S, 5.12%.

(*E*)-5-(4-Bromophenyl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl) thieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4m: Yield 81%; yellow solid. mp 359–361°C. IR (KBr) cm^{-1}: 1670 (CO), 1550, 1645 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 2.42 (s, 3H, pyrimidine-CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.72 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.32 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.36,12.45, 22.29, 100.23, 100.64, 121.21, 122.47, 124.58, 126.31, 127.33, 127.51, 127.69, 128.30,128.42, 129.11, 129.57, 130.55, 131.21, 131.58, 131.67, 134.32, 137.44, 149.61, 154.24,158.38, 158.66, 162.58, 163.50, 168.37. MS: *m/z* 630 $(M+H)^+$. Anal. Calcd for $C_{29}H_{20}BrN_5O_5S$: C, 55.25; H, 3.20; N, 11.11; S, 5.09. Found: C, 55.22; H, 3.22; N, 11.09; S, 5.04%.

(*E*)-5-(2-Hydroxyphenyl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4n: Yield 80%; yellow solid. mp 257–259°C. IR (KBr) cm^{-1}: 3325 (OH), 1680 (CO), 1545, 1650 (NO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.24 (s, 6H, 2isoxazole-CH_3), 2.44 (s, 3H, pyrimidine-CH_3), 5.32 (s, 1H, OH, D_2O exchangeable), 6.58 (d, 1H, ═CH, *J* = 12 Hz), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.28 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.34, 12.42, 22.34, 100.43, 100.67, 121.34,122.44, 124.51, 126.20, 127.34, 127.58, 127.77, 128.26, 128.45, 129.11, 129.64, 130.60,131.43, 131.75, 131.84, 134.51, 137.55, 149.42, 154.24, 158.26, 158.64, 162.44, 163.38,168.40. MS: *m/z* 568 $(M + H)^+$. Anal. Calcd for $C_{29}H_{21}N_5O_6S$: C, 61.37; H, 3.73; N, 12.34; S, 5.65. Found: C, 61.39; H, 3.76; N, 12.39; S, 5.67%.

(*E*)-5-(4-(Dimethylamino)phenyl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4o: Yield 80%; yellow solid. mp 385–387°C. IR

(KBr) cm^{-1}: 1675 (CO), 1560, 1655 (NO_2); ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 6H, 2isoxazole-CH_3), 2.48 (s, 3H, pyrimidine-CH_3), 2.68 (s, 6H, $N(CH_3)_2$), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.05–7.30 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.44, 12.29, 22.43, 42.67, 100.33, 100. 52, 121.24, 122.44, 124.57, 126.34, 127.23, 127.46, 127.71, 128.22, 128.51, 129.32, 129.64, 130.73, 131.24,131.53, 131.67, 134.38, 137.52, 14961, 154.11, 158.42, 158.63, 162.28, 163.70, 168.32. MS: *m/z* 595 $(M + H)^+$. Anal. Calcd for $C_{31}H_{26}N_6O_5S$: C, 62.61; H, 4.41; N, 14.13; S, 5.39. Found: C, 62.58; H, 4.39; N, 14.16; S, 5.35%.

(*E*)-5-(2-Methoxyphenyl)-2-methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one, 4p: Yield 79%; yellow solid. mp 341–343°C. IR (KBr) cm^{-1}: 1680 (CO), 1555, 1650 (NO_2); ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 2.52 (s, 3H, pyrimidine-CH_3), 3.68 (s, 3H, OCH_3), 6.50 (d, 1H, ═CH, *J* = 12 Hz), 6.68 (d, 1H, ═CH, *J* = 12 Hz), 7.00–7.28 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.46, 12.52, 22.56, 58.24, 100.33, 100. 61, 121.24, 122.36, 124.76, 126.35, 127.23,127.40, 127.62, 128.42, 128.56, 129.36, 129.61, 130.57, 131.23, 131.53, 131.74, 134.46,137.61, 149.75, 154.12, 158.33, 158.61, 162.32, 163.46, 168.55. MS: *m/z* 582 $(M + H)^+$. Anal. Calcd for $C_{30}H_{23}N_5O_6S$: C, 61.95; H, 3.99; N, 12.04; S, 5.51. Found: C, 61.98; H, 4.01; N, 12.01; S, 5.54%.

(*E*)-2-Methyl-6-(3-methyl-4-nitroisoxazol-5-yl)-3-(3-methyl-5-styrylisoxazol-4-yl)-5-(*o*-tolyl)thieno [2,3-*d*]pyrimidin-4(3*H*)-one, 4q: Yield 79%; yellow solid. mp 348–350°C. IR (KBr) cm^{-1}: 1685 (CO), 1560, 1655 (NO_2); ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.22 (s, 6H, 2isoxazole-CH_3), 2.46 (s, 3H, pyrimidine-CH_3), 2.68 (s, 3H, Ar-CH_3), 6.60 (d, 1H, ═CH, *J* = 12 Hz), 6.72 (d, 1H, ═CH, *J* = 12 Hz), 7.20–7.48 (m, 9H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. 11.45, 12.32, 22.61, 26.57, 100.35, 100. 59, 121.24, 122.50, 124.86, 126.38, 127.23, 127.60,127.78, 128.22, 128.63, 129.18, 129.60, 130.60, 131.25, 131.43, 131.60, 134.56, 137.67,149.68, 154.24, 158.56, 158.71, 162.44, 163.84, 168.48. MS: *m/z* 566 $(M + H)^+$. Anal. Calcd for $C_{30}H_{23}N_5O_5S$: C, 63.71; H, 4.10; N, 12.38; S, 5.67. Found: C, 63.67; H, 4.07; N, 12.35; S, 5.71%.

Conclusions

In conclusion, we report the convenient and efficient process for the synthesis of isoxazolylarylthieno[2,3-*d*]pyrimidinones**4** derivatives by use of PEG-400 as a recyclable medium and catalyst without the addition of any additive or organic co‐solvent and using inexpensive and commercially available materials with potential medicinal properties.. Present methodology offers very attractive features such as simple experimental procedure, higher yields and economic viability, and will have wide scope in organic synthesis. The newly synthesized novel isoxazolylarylthieno[2,3-*d*]pyrimidinones **4a-q** evaluated for their antimicrobial activity. It has been found that the derivatives **4b**, **4c**, **4d**, **4e**, **4j**, **4k**, **4l** and **4m** exhibited good antimicrobial activity compared to the reference drugs.

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