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SYNTHESIS AND ANTIMICROBIAL SCREENING OF NOVEL ISOXAZOLYL THIAZOLO[5,4-*d*] PYRIMIDINE-2,5-(1*H*,4*H*)-DITHIONES

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Abstract

The synthesis of novel isoxazolyl thiazolo[5,4-*d*]pyrimidine-2,5-(1*H*,4*H*)-dithiones (**7a-h**) were achieved by the cyclocondensation of isoxazole amine (**1**) with chloro acetic acid (**2**) and carbon disulfide (**3**) in presence of piperidine followed by cyclization with aromatic aldehyde (**5**) and thiourea (**6**). All the compounds synthesized **4a-h** and 7**a-h** were characterized on the basis of their IR, ^{1}H NMR,^{13}C NMR and mass spectral data and screened for their antimicrobial activity.

Keywords: Isoxazolyl thiazolo[5,4-*d*]pyrimidine dithiones, Isoxazolyl-2-thioxothiazolidin-4-ones, Cyclocondensation, Antibacterial activity, Antifungal activity.

Introduction

Heterocyclic nucleus imparts an important role in medicinal chemistry and serves as a key template for the development of various therapeutic agents. Pyrimidine and fused pyrimidine derivatives are one of the most prominent structures found in nucleic acids including uracil, thymine, cytocine, adenine, and guanine,and are fundamental building blocks for deoxy ribonucliecacid (DNA), and ribonucleic acid (RNA).

Condensed pyrimidine derivatives have been reported as anti microbial[1], analgesic, anti-viral, anti-inflammatory[2], anti HIV[3], antitubercular[4], anti-tumour[5], anti-neoplastic[6], anti-malarial[7], diuretic[8], cardiovascular[9] agents and hypnotic drugs for the nervous system[10], calcium sensing receptors antagonists[11]. In addition it has been observed over the years that, thiazole nucleus posses different biological activities such as anti hypertensive[12], anti-Inflammatory[13], anti schizophrenic[14], antibacterial[15], anti-HIV[16], hypnotic[17], Anti-allergic[18], fibrinogen receptor antagonists with anti thrombotic activity[19], inhibitous of bacterial DNA gyrase B[20], activity and anti-inflammatory[21], bactericidal[22], and anti-viral activity as inhibitors of HIV-1 reverse transcriptase[23].

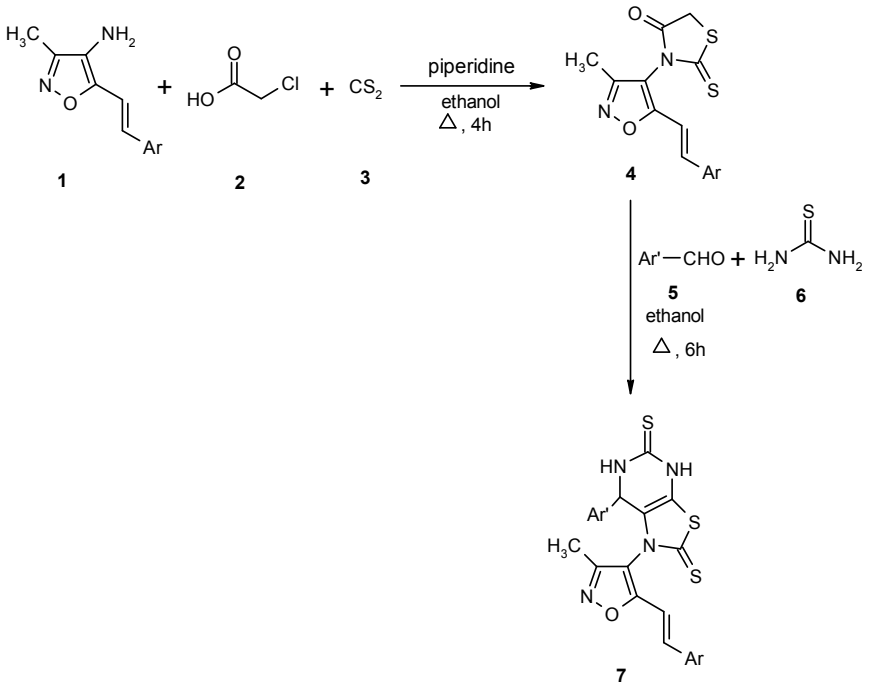
Similarly, isoxazole unit exhibits pharmacological properties such as anti-tumour[24], CNS activity[25], and analgesic[26], and anti microbial agents [27]. We thought it to be useful to construct a

system that combine these bio labile rings together in a single molecular framework to see the additive effect towards their biological activities. As a sequel to our project on the synthesis of isoxazolyl derivatives with potential biological activity[28], we, herein, wish to report the synthesis of novel isoxazolyl thiazolo[5,4-*d*]pyrimidine dithiones and their antimicrobial activity.

Results and Discussion

The synthesis of compounds **4** and **7** were accomplished by the synthetic sequence shown in scheme 1. The reaction of isoxazole amines **1**, chloro acetic acid **2** and carbondisulfide **3** in the presence of piperidine in ethanol furnished the corresponding novel 3-(3 methyl-5-styryl isaxazol-4-yl)thiazolidine-2,4-dithiones **4**. Cyclocondensation of compound **4** with aromatic aldehydes **5** and thiourea **6** in ethanol led to the formation of novel 6,7-dihydro-1-(3-methyl-5-styryl isoxazol-4-yl)-7 aryl-thiazolo[5,4-*d*] pyrimidine-2,5(1*H*,4*H*)-dithiones **7**. The structure of the products **4** and **7** have been established on the basis of IR, ^{1}H NMR, ^{13}C NMR and MS spectral data.

Compound **4** displayed a characteristic absorption band in the IR spectra around 1675 cm^{-1} due to C=O functional group. ^{1}H NMR spectra of **4** displayed a distinct singlet at δ 3.82 due to the methylene protons of thioxothiazolidinone ring, confirming the cycolcondensation. The mass spectrum of **4** confirmed the structure by exhibiting the molecular ion peak $[M]^+$ at *m/z* 316. Compound **7** displayed a characteristic absorption band in the IR spectra around 3341 and 3358 cm^{-1} due to the two NH functional groups, and did not exhibit absorption band due to C=O functional group present in its precursor **4**, confirming the cyclization. Similarly, the cyclization was supported by the ^{1}H NMR spectra of **7** that did not contain CH_2 proton signal, which is



4		7	
	Ar	**Ar**	**Ar'**
a,	C_6H_5	a, C_6H_5	C_6H_5
b,	2-ClC_6H_4	b, 2-ClC_6H_4	C_6H_5
c,	2-BrC_6H_4	c, 2-BrC_6H_4	C_6H_5
d,	2-OHC_6H_4	d, 2-OHC_6H_4	C_6H_5
e,	4-$CH_3OC_6H_4$	e, 4-$CH_3OC_6H_4$	C_6H_5
f,	4-$CH_3C_6H_4$	f, 4-$CH_3C_6H_4$	C_6H_5
g,	4-$N(CH_3)_2C_6H_4$	g, 4-$N(CH_3)_2C_6H_4$	C_6H_5
h,	3,4-$OCH_2OC_6H_4$	h, 3,4-$OCH_2OC_6H_4$	C_6H_5
		i, C_6H_5	2-ClC_6H_4
		j, C_6H_5	2-BrC_6H_4
		k, C_6H_5	4-$CH_3C_6H_4$
		l, C_6H_5	4-$CH_3OC_6H_4$

Scheme 1

present in its precursor **4**. The mass spectrum of the product 7 also agrees with the proposed structure which shows the molecular ion $[M]^+$ peak at *m/z* 462.

In order to study the scope of this reaction, different substituted 3-methyl 4-amino-5-styrylisoxazole, chloro acetic acid and carbon disulphide were utilized in this multi-component synthesis. The desired product was obtained in each case with moderate to good yield. Finally, the results indicate that this synthetic strategy permits the introduction of a diverse array of substituents on the 3-methyl-4-amino-5-styrylisoxazole and the approach proved to be of general applicability.

The IR spectra of 6,7 dihydro-1-(3-methyl-5-styrylisoxazol-4-yl)-7-aryl-thiazolo[5,4-*d*] pyrimidine-2,5(1*H*,4*H*)-dithiones **7** exhibited characteristic absorption bands at 3416 and 3350 cm^{-1} due to imino functional groups. The ^1H NMR spectra of 7 displayed two prominent signals as a doublet and singlet around δ 4.26 and 8.64 due to pyrimidine-CH and NH protons respectively conforming cyclization process. The mass spectrum of **7a**, showed a molecular ion $[M+]^+$ peak at m/z 462 supporting the product formation. The structures of compounds **7a-l** have been elucidated by elemental analyses, and spectral (IR, ^1H NMR, MS) data.

Antibacterial activity

In vitro antimicrobial screening of the newly synthesized compounds **7a-l** were evaluated against two Gram-positive bacteria *viz., Bacillus subtilis* and *Streptococcus lactis,* two Gram-negative bacteria *viz., Escherichia coli* and *Pseudomonas aeuroginosa*, The *in vitro* antimicrobial activity of the tested compounds (Table 1) were evaluated by agar diffusion method [29]. *Nalidixic acid* is used as standard drug for comparison.

The bacterial isolates representing Gram-negative and Gram-positive bacteria were recovered on Nutrient and Mac Conkey agar. The selected compounds were tested *in vitro* using

the agar disk diffusion method taking Nalidixic acid as reference drug. The antibacterial potentialities of the tested compounds were estimated by placing pre-sterilized filter paper disks (5 mm in diameter) impregnated with 100 µg/disk using dimethylsulfoxide (DMSO) as solvent which showed no inhibition zones. The inhibition zones of the tested compounds were measured after 24-28 h incubation at 37^{o} C. The minimal inhibitory concentration (MIC) determination method of the biologically active compounds (Table 1) was applied using different concentrations per disk against Gram-negative and Gram-positive bacteria.

The results of *in vitro* anti microbial screening (Table 1) reveals that, all the tested compounds exhibited better activity compared to the reference drug. Compounds **7d** and **7e** exhibited much better activity towards gram positive bacteria, and exhibited excellent activity towards gram negative bacteria as compared to the reference drug Nalidixic acid. This may be due to presence of hydroxyl and methoxy groups as substituents on the benzene ring, besides the isoxazolyl thiazolo[5,4-*d*]pyrimidine dithione ring.

Table 1. Antibacterial activity of **7a-h**

Compound	Minimum inhibitory Concentration (MIC)[a.b]			
	Gram positive		Gram negative	
	B.subtilis	*B.lactis*	*E.coli*	*P.aeruginosa*
7a	20	22	19	26
7b	22	19	22	21
7c	24	20	25	24
7d	16	14	18	15
7e	15	17	13	13
7f	20	21	20	18
7g	21	14	23	20
7h	17	20	16	23
7i	23	16	21	25
7j	22	23	19	19
7k	25	21	22	26
7l	19	19	25	22
Nalidixic acid	30	25	28	30

[a]Negative control (DMSO) – No activity
[b]Concentration 100µg/disk

Antifungal activity

In vitro antimicrobial screening of the newly synthesized compounds **7a-l** were evaluated against two two fungal strains *viz; Aspergillus flavus* and *Trichoderma viridae*. The *in vitro* antimicrobial activity of the tested compounds (Table 2) were evaluated by agar diffusion method[30]. Fluconazole is used as standard drug for comparison.

The two fungal isolates A. *flavus* and *T. viridae* were isolated on Sabouraud dextrose agar (oxoid). They are isolated from clinical samples and identified to the species level according to different API systems (biomerilux). The selected compounds were tested *in vitro* using the agar

disk diffusion method taking Fluconazole as reference drug. The antifungal potentialities of the tested compounds were estimated by placing pre-sterilized filter paper disks (5 mm in diameter) impregnated with 100 µg/disk using dimethylsulfoxide (DMSO) as solvent which showed no inhibition zones. The inhibition zones of the tested compounds were measured after 24-28 h incubation at 28^{o} C after 5 days for fungi. The minimal inhibitory concentration (MIC) determination method of the biologically active compounds (Table 2) was applied using different concentrations per disk against fungi.

The results of *in vitro* antimicrobial screening (Table2) data reveals that most of the newly synthesized compounds exhibited good activity against fungi as compared with reference drug Fluconazole. Compounds **7d** and **7e** showed excellent activity and they inhibited the growth of fungi organisms to a remarkable extent with low MIC as that of standard drug.

Table 2**.** Antifungal activity of **7a-h**

Compound	Minimum inhibitory Concentration (MIC)[a.b]	
	A.flavus	*T.viridae*
7a	17	19
7b	20	18
7c	15	16
7d	11	10
7e	8	09
7f	16	15
7g	18	14
7h	12	18
7i	19	17
7j	15	15
7k	14	13
7l	20	16
Fluconazole	22	20

[a] Negative control (DMSO) – No activity
[b]Concentration 100µg/disk

In conclusion, we reported the multi-component (MCR-3) one-pot protocol for the synthesis of 6,7 dihydro-1-(3-methyl-5-styryl isoxazol-4-yl)-7 aryl thiazolo[5,4-*d*] pyrimidine-2,5(1*H*,4*H*)-dithiones, using commercially available materials. This synthesis benefits from a simple method of purification, which does not require chromatography. This ease of purification compliments the one-pot synthesis, making the technology practical, easy to perform and facile. The biological activity of the products will be published elsewhere. Moreover, fused pyrimidine ring derivatives are potent pharmacological agents, this study may motivate the researchers concerned in this field to explore the pharmacological activity of the compounds.

Experimental Section

All the melting points were determined on a Cintex melting point apparatus and are uncorrected. Analytical TLC was performed on Merck precoated 60 F254 silica gel plates.

Visualization was done by exposing to iodine vapour. IR spectra (KBr pellet) were recorded on a PerkinElmer 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 tetramethyl silane as an internal standard. Mass spectral measurements were carried out by EI method on a Jeol JMC-300 spectrometer at 70 eV. Elemental analyses were performed on a Carlo Erba 106 and Perkin Elmer model 240 analyzers.

Synthesis of 3-(3-methyl-5-styryl isoxazol-4-yl)-2-thioxothiazolidin-4-ones (4a-h) general procedure 4a-h

A mixture of isoxazole amine (**1**) (1mmol), chloro acetic acid (**2**) (1mmol), and carbon disulfide (**3**) (1mmol) in ethanol (10 ml) were refluxed in presence of few drops of piperidine with stirring at 70^{o} C for 4h. After the completion of the reaction (monitored by TLC), the solvent was removed under pressure and added 30 ml of water to the residue, then extracted with ethyl acetate and the residue was purified by recrystallisation from methanol to produce **4a-h** in high yields.

Compound 4a: Brown solid (67%), mp 138-140^{o}C; IR: (KBr) cm^{-1} 1675 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.32 (s, 3H, isoxazole-CH_3), 3.82 (s, 2H, CH_2), 6.72 (d, 1H, CH=CH, J=12Hz), 6.81 (d, 1H, CH =CH, J=12Hz), 7.21- 7.43 (m, 5H, ArH); ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 11.65, 41.71, 106.35, 117.75, 124.49, 127.62, 128.12,128.38 131.81, 132.45, 137.82, 152.19, 160.01, 172.32, 189.73. EI-MS (70 eV) *m/z* : 316 $[M]^{+}$. Anal. Calcd for $C_{15}H_{12}N_2O_2S_2$: C, 56.93; H, 3.86; N, 8.85%. Found : C, 56.84; H, 3.79; N, 8.80%.

Compound 4b: Brown solid (63%), mp 145-146^{o}C; IR: (KBr) cm^{-1}1672 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.28 (s, 3H, isoxazole-CH_3), 3.92 (s, 2H, CH_2), 6.78 (d, 1H, CH=CH, J=12Hz), 6.83 (d, 1H, CH =CH, J=12Hz), 7.08-7.68 (m, 4H, ArH), ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.84, 37.25, 103.64, 126.46, 127.72,128.41 129.13, 132.21,133.62, 133.83, 136.68, 153.72, 161.12, 172.33, 190.65. EI-MS (70 eV) *m/z* : 350$[M]^{+}$. Anal. Calcd for $C_{15}H_{11}ClN_2O_2S_2$: C, 51.35; H, 3.16; N, 7.94%. Found: C, 51.27; H, 3.12; N, 7. 86%.

Compound 4c: Brown solid (69%), mp 144-145^{o}C; IR: (KBr) cm^{-1}1673 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.26 (s, 3H, isoxazole-CH_3), 4.01 (s, 2H, CH_2), 6.68 (d, 1H, CH=CH, J=12Hz), 6.78 (d, 1H, CH=CH, J=12Hz), 7.31-7.64 (m, 4H, ArH); ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.94,. 38.61, 103.74, 118.95 126.89, 128.32,128.67 129.35, 133.12,130.28 136.79. 154.21, 162.23, 173.32, 189.53. EI-MS (70 eV) *m/z*: 394 $[M]^{+}$. Anal. Calcd for $C_{15}H_{11}BrN_2O_2S_2$: C, 44.69; H, 2.86; N, 7.29%. Found : C, 44.51; H, 2.81; N, 7.22%.

Compound 4d: Brown solid (72%), mp 154-155^{o}C; IR: (KBr) cm^{-1} 1678(CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.32 (s, 3H, isoxazole-CH_3), 4.21 (s, 2H, CH_2), 6.53 (d, 1H, CH=CH, J=12Hz), 6.83 (d, 1H, CH=CH, J=12Hz), 7.14-7.54 (m, 4H, ArH); ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.91, 38.76, 102.65,120.63, 124.25 126.60, 128.43,128.73 129.61, 132.15, 135.45, 154.12, 162.25, 172.81, 189.21. EI-MS (70 eV) *m/z* : 332 $[M]^{+}$. Anal. Calcd for $C_{15}H_{12}N_2O_3S_2$: C, 54.20; H, 3.66; N, 8.44. Found : C, 54.15; H, 3.61; N, 13.20.

Compound 4e: Brown solid (68%), mp 159-160^{o}C; IR: (KBr) cm^{-1} 1671 (CO); ^{1}HNMR (300 MHz, $CDCl_3$) δ ppm: 2.26 (s, 3H, isoxazole-CH_3), 3.57 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 6.47 (d, 1H, CH=CH,J=12Hz), 6.78 (d, 1H, CH=CH,J=12Hz), 7.01-7.68 (m, 4H, ArH), ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.39, 40.53, , 103.91, 118.21, 124.68, 125.91, 127.64, 128.44,128.76 132.21, 135.68, 155.28,160.29, 163.43, 174.35, 190.63. EI-MS (70 eV) *m/z* : 346

$[M]^+$. Anal. Calcd for $C_{16}H_{14}N_2O_3S_2$: C, 55.47; H, 4.07; N, 8.09%. Found : C, 55.41; H, 4.02; N, 8.05%.

Compound 4f: Brown solid (64%), mp 143-145^oC; IR: (KBr) cm^{-1} 1680 (CO); ^{1}HNMR (300 MHz, $CDCl_3$) δ ppm: 2.23 (s, 3H, isoxazole-CH_3), 4.39 (s, 2H, CH=CH), 6.71 (d, 1H, ArH), 6.84 (d, 1H, CH=CH), 7.10 (d, 2H, ArH), 7.24 (d, 2H, ArH); ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 11.69, 28.54, 32.21, 40.53, 103.91, 125.91, 127.64, 128.45,128.85,138.98, 132.81, 135.96, 156.28, 164.43, 175.35, 191.63. EI-MS (70 eV) *m/z* : 330 $[M]^+$. Anal. Calcd for $C_{16}H_{14}N_2O_2S_2$: C, 58.16; H, 4.27; N, 8.48%. Found : C, 58.12; H, 4.23; N, 8.41%.

Compound 4g: Brown solid (62%), mp 142-143^oC; IR: (KBr) cm^{-1} 1672 (CO); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.43 (s, 3H, isoxazole-CH_3), 3.01 (s, 6H, $N(CH_3)_2$), 4.49 (s, 2H, CH_2), 6.15 (d, 1H, CH=CH, J=12Hz), 6.84 (d, 1H, CH=CH, J=12Hz), 7.31 (d, 2H, ArH),7.48 (d, 2H, ArH); ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 11.93, 28.56 40.21, 40.53, 104.41, 117.56,117.89 127.21,128.61,128.79, 132.44, 134.81, 136.96, 151.28, 164.23, 175.85, 191.39. EI-MS (70 eV) *m/z* : 359 $[M]^+$. Anal. Calcd for $C_{17}H_{17}N_3O_2S_2$: C, 56.80; H, 4.76; N, 11.68%. Found : C, 56.74; H, 4.71; N, 11.63%.

Compound 4h: Brown solid (64%), mp 148-150^oC; IR: (KBr) cm^{-1} 1669 (CO) ; ^{1}HNMR (300 MHz, $CDCl_3$) δ ppm: 2.30 (s, 3H, isoxazole-CH_3), 4.29 (s, 2H, CH2), 5.28 (s, 3H, OCH_2O), 6.25 (d, 1H, HC=CH, J=12Hz), 6.54 (d, 1H, CH=CH, J=12Hz), 7.30 (s, 2H, ArH), 7.46 (d, 2H, ArH); ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 11.98, 42.21, 103.91, 105.63, 115.45, 122.47, 123.24, 125.34, 134.81, 150.34, 151.21, 154.57, 158.28, 164.23, 174.25, 192.13. EI-MS (70 eV) *m/z* : 360 $[M]^+$. Anal. Calcd for $C_{16}H_{12}N_2O_4S_2$: C, 53.42; H, 3.34; N, 7.70 %. Found : C, 53.29; H, 3.30; N, 7.66%

Synthesis of 6,7-dihydro-1-(3-methyl-5-styrylisoxazol-4-yl)-7-aryl-thiazolo[5,4-*d*] pyrimidine-2,5(1*H*,4*H*)-dithiones (7a-l)

3-(3-Methyl-5-styrylisoxazole-4-yl)thiazolidine-2,4-dithiones (**4**) (1mmol), freshly distilled aromatic aldehyde (**5**) (1mmol) and thiourea (**6**) (1mmol) were taken in ethanol (10 mL) and the contents were heated at 45 ^oC with stirring for 6h. After the completion of the reaction (monitored by TLC), the reaction mixture was cooled and poured in to ice cold water. The separated solid was filtered and recrystallized from ethyl acetate.

Compound 7a: Brown solid (61%), mp 153-154^oC; IR: (KBr) cm^{-1} 3341 (NH), 3358 (NH),^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.25 (s, 3H, isoxazole-CH_3), 4.45 (d, 1H, pyrimidine ring-CH), 6.53 (d, 1H, CH=CH, J=12Hz), 6.85 (d, 1H, CH=CH, J=12Hz), 7.45-7.68 (m, 10H, ArH), 8.63 (s, 1H, NH, D_2O exchangeable), 9.45 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (75MHz, $CDCl_3$) δ (ppm): 11.86, 60.72, 102.47, 109.38, 124.44, 125.87, 126.88, 128.88, 128.92, 129.82, 131.23, 133.88, 133.72, 134.85, 135.73, 136.89, 141.43, 144.88, 149.79, 154.84, 161.27, 183.48, 192.58. EI-MS (70 eV) *m/z* : 462 $[M]^+$. *Anal.* Calcd for $C_{23}H_{18}N_4OS_3$: C, 59.73; H, 3.92; N, 12.11%. Found : C, 59.68; H, 3.87; N, 12.05%.

Compound 7b: Brown solid (73%), mp157-159^oC; IR: (KBr) cm^{-1} 3364 (NH), 3416 (NH), ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.36 (s, 3H, isoxazole-CH_3), 4.42 (d, 1H, pyrimidine ring -CH), 6.42 (d, 1H, CH=CH, J=12Hz), 6.93 (d, 1H, CH=CH, J=12Hz), 7.56-7.78 (m, 10H, ArH), 8.52 (s, 1H, NH, D_2O exchangeable), 9.31 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.18, 61.35, 104.24, 109.86, 125.17, 126.35, 126.98, 128.87, 128.56, 129.82, 130.67, 132.96, 133.76, 134.18, 135.53, 137.58, 140.84, 145.65, 150.19, 155.58, 163.43, 184.32,

193.24. EI-MS (70 eV) *m/z* : 496 $[M]^+$. Anal. Calcd for $C_{23}H_{17}N_4OClS_3$: C, 55.58; H, 3.46; N, 11.27%. Found : C, 55.51; H, 3.42; N, 11.20%.

Compound 7c: Brown solid (70%), mp 150-152^oC; IR:3337 (NH), 3368 (NH), ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.34 (s, 3H, isoxazole-CH_3), 4.50 (d, 1H, pyrimidine ring -CH), 6.65 (d, 1H, CH=CH, J=12Hz), 6.89 (d, 1H, CH=CH, J=12Hz), 7.45-7.58 (m, 10H, ArH), 8.64 (s, 1H, NH, D_2O exchangeable), 9.24 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.03, 60.82, 103.16, 109.67, 124.78, 125.68, 127.85, 128.89, 128.53, 129.98, 130.56, 132.86, 133.42, 134.96, 135.68, 136.79, 140.48, 143.88, 148.79, 154.83, 160.63, 182.64, 192.78. EI-MS (70 eV) *m/z* : 540 $[M]^+$. Anal. Calcd for $C_{23}H_{17}BrN_4OS_3$: C, 51.13; H, 3.16; N, 10.35%. Found : C, 51.02; H, 3.11; N, 10.30%.

Compound 7d: Brown solid (72%), mp 155-156^oC; IR: (KBr) cm^{-1} 3358 (NH), 3369 (NH), ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.31 (s, 3H, isoxazole-CH_3), 4.47 (d, 1H, pyrimidine ring -CH), 6.57 (d, 1H, CH=CH, J=12Hz), 6.91 (d, 1H, CH=CH, J=12Hz), 7.45-7.68 (m, 10H, ArH), 8.55 (s, 1H, NH, D_2O exchangeable), 9.32 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 11.95, 61.88, 103.32, 110.18, 124.65, 125.87, 126.85, 128.55, 128.82, 129.91, 131.25, 132.77, 133.83, 134.92, 136.11, 136.82, 141.32, 144.68, 148.67, 155.84, 162.53, 183.34, 192.44. EI-MS (70 eV) *m/z* : 478 $[M]^+$. *Anal.* Calcd for $C_{23}H_{18}N_4O_2S_3$: C, 57.73; H, 3.79; N, 11.78%. Found : C, 57.67; H, 3.72; N, 11.73%.

Compound 7e : Brown solid (74%), mp 140-142^oC; IR: 3372 (NH), 3448 (NH),^{1}HNMR (300 MHz, $CDCl_3$) δ ppm: 2.25 (s, 3H, isoxazole-CH_3), 4.37 (d, 1H, pyrimidine ring -CH), 6.58 (d, 1H, CH=CH, J=12Hz), 6.84 (d,1H, CH=CH, J=12Hz), 7.46-7.84 (m, 10H, ArH), 8.56 (s, 1H, NH, D_2O exchangeable), 9.69 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.48, 61.58, 103.54, 109.24, 124.65, 125.98, 126.68, 128.49, 128.78, 130.18, 131.24, 132.89, 133.78, 133.89, 136.21, 136.86, 141.46, 144.48, 150.26, 156.86, 163.24, 184.23, 194.28. EI-MS (70 eV) *m/z* : 492 $[M]^+$. *Anal.* Calcd for $C_{24}H_{20}N_4O_2S_3$: C, 58.51; H, 4.09; N, 11.37%. Found : C, 58.66 H, 4.03; N, 11.30%.

Compound 7f: Brown solid (62%), mp 156-158^oC; IR: (KBr) cm^{-1} 3384 (NH), 3457 (NH),^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.31(s, 3H, isoxazole-CH_3), 4.23 (d, 1H, pyrimidine ring -CH), 6.68 (d, 1H, CH=CH, J=12Hz), 6.89 (d, 1H, CH=CH, J=12Hz), 7.36-7.76 (m, 10H, ArH), 8.44 (s, 1H, NH, D_2O exchangeable), 9.68 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 11.74, 60.85, 103.32, 109.36, 124.46, 125.75, 126.61, 128.92, 128.34, 130.11, 130.63, 132.75, 133.46, 133.92, 135.42, 136.78, 141.23, 145.37, 149.28, 155.42, 162.53, 183.48, 193.56. EI-MS (70 eV) *m/z* : 476 $[M]^+$. *Anal.* Calcd for $C_{24}H_{20}N_4OS_3$: C, 60.33; H, 3.76; N, 13.08%. Found : C, 60.22; H, 3.71; N, 13.13%.

Compound 7g: Brown solid (63%), mp 154-155^oC; IR: (KBr) cm^{-1} 3372 (NH), 3407 (NH), ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.38 (s, 3H, isoxazole-CH_3), 3.35 (s, 6H, $N(CH_3)_2$), 4.39 (d, 1H, pyrimidine ring -CH), 6.69 (d, 1H, CH=CH, J=12 Hz), 6.97 (d, 1H, CH=CH, J=12 Hz), 7.25-7.65 (m, 10H, ArH), 8.86 (s, 1H, NH, D_2O exchangeable), 9.65 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.13, 63.85, 103.69, 109.86, 124.68, 126.87, 127.52, 129.83, 129.92, 130.44, 131.75, 134.82, 135.66, 136.55, 136.82, 137.79, 141.56, 145.68, 149.66, 156.46, 163.28, 183.24, 192.85. EI-MS (70 eV) *m/z* : 505$[M]^+$. *Anal.* Calcd for $C_{25}H_{23}N_5OS_3$: C, 59.33; H, 4.58; N, 13.85%. Found : C, 59.28; H, 4.53; N, 13.81%.

Compound 7h: Brown solid (60%), mp 150-151^oC; IR: (KBr) cm^{-1} 3348 (NH), 3377 (NH),^{1}HNMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 3H, isoxazole-CH_3), 4.39 (d, 1H, pyrimidine ring -CH), 6.61 (d, 1H, CH=CH, J=12MHz), 6.82 (d,1H, CH=CH, J=12 MHZ), 7.45-7.68 (m,

10H, ArH), 8.52 (s, 1H, NH, D_2O exchangeable), 9.12 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 11.83, 60.65, 102.26, 109.08, 124.25, 125.65, 126.65, 128.86, 128.12, 129.91, 130.25, 132.89, 133.62, 133.95, 135.61, 136.69, 140.42, 143.98, 148.59, 154.63, 160.23, 182.39, 191.48. EI-MS (70 eV) *m/z* : 506 $[M]^+$. *Anal.* Calcd for $C_{24}H_{18}N_4O_3S_3$: C, 56.93; H, 3.58; N, 11.08%. Found : C, 56.87; H, 3.54; N, 11.02%.

Compound 7i: Brown solid (64%), mp 158-159°C; IR: (KBr) cm^{-1} 3348 (NH), 3367 (NH),^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.20 (s, 3H, isoxazole-CH_3), 4.39 (d, 1H, pyrimidine ring -CH), 6.61 (d, 1H, CH=CH, J=12Hz), 6.82 (d, 1H, CH=CH, J=12Hz), 7.45-7.68 (m, 10H, ArH), 8.52 (s, 1H, NH, D_2O exchangeable), 9.12 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.33, 62.15, 103.36, 110.18, 124.55, 125.86, 126.98, 128.36, 128.94, 129.98, 131.26, 132.78, 133.88, 134.22, 136.11, 137.29, 141.52, 144.48, 149.65, 153.63, 160.53, 181.42, 190.62. EI-MS (70 eV) *m/z* : 496 $[M]^+$. *Anal.* Calcd for $C_{23}H_{17}ClN_4OS_3$: C, 55.58; H, 3.46; N, 11.28%. Found : C, 55.50; H, 3.41; N, 11.20%.

Compound 7j: Brown solid (64%), mp 163-165°C; IR: (KBr) cm^{-1} 3368 (NH), 3452 (NH), ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.18 (s, 3H, isoxazole-CH_3), 4.26 (d, 1H, pyrimidine ring -CH), 6.46 (d, 1H, CH=CH, J=12Hz), 6.76 (d, 1H, CH=CH, J=12Hz), 7.36-7.78 (m, 10H, ArH), 8.64 (s, 1H, NH, D_2O exchangeable), 9.23 (s,1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.34, 61.68, 103.46, 110.28, 124.34, 125.78, 126.86, 128.96 129.12, 129.91, 130.46, 132.68, 133.78, 133.98, 135.84, 136.84, 142.24, 144.18, 149.29, 155.68, 160.64, 183.42, 193.36. EI-MS (70 eV) *m/z* : 540$[M]^+$. *Anal.* Calcd for $C_{23}H_{17}BrN_4OS_3$: C, 51.13; H, 3.16; N, 10.35%. Found : C, 51.05; H, 3.12; N, 10.30%.

Compound 7k: Brownsolid (71%), mp156-157°C; IR (KBr) cm^{-1} 3362 (NH), 3457 (NH), ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.28 (s, 3H, isoxazole-CH_3), 2.68 (s, 3H, Ar-CH_3), 4.29 (d, 1H, pyrimidine ring-CH), 6.49 (d, 1H, CH=CH, J=12Hz), 6.75 (d, 1H, CH=CH, J=12Hz), 7.34-7.78 (m, 10H, ArH), 8.56 (s, 1H, NH, D_2O exchangeable), 9.46 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 11.93, 61.25, 103.31, 110.18, 124.42, 125.58, 126.86, 128.67, 128.86, 129.94, 130.58, 132.53, 132.68, 134.25, 136.43, 136.84, 141.34, 144.63, 149.19, 155.33, 161.47, 183.29, 193.36. EI-MS (70 eV) *m/z* : 464 $[M]^+$. *Anal.* Calcd for $C_{24}H_{20}N_4OS_3$: C, 60.33; H, 3.76; N, 12.48%. Found : C, 60.21; H, 3.72; N, 12.32%.

Compound 7l: Brown solid (62%), mp 159-160°C; IR: (KBr) cm^{-1} 3652 (NH), 3439 (NH), ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 2.42 (s, 3H, isoxazole-CH_3), 3.59 (s, 3H, OCH_3), 4.46 (d, 1H, pyrimidine ring-CH), 6.56 (d, 1H, CH=CH, J=12Hz), 6.74 (d, 1H, CH=CH, J=12Hz), 7.36-7.72 (m, 10H, ArH), 8.46 (s, 1H, NH, D_2O exchangeable), 9.58 (s, 1H, NH, D_2O exchangeable): ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 12.64, 61.46, 103.36, 109.27, 124.36, 125.78, 126.86, 128.24, 128.68, 129.96, 131.43, 132.82, 133.95, 134.15, 135.78, 136.48, 140.84, 144.26, 149.64, 155.86, 162.46, 183.48, 192.68. EI-MS (70 eV) *m/z* :492 $[M]^+$. *Anal.* Calcd for $C_{24}H_{20}N_4O_2S_3$: C, 58.53; H, 4.06; N, 11.38. Found : C, 58.48; H, 4.02; N, 11.33%.

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