Journal of Sulfur Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gsrp20

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Version of record first published: 10 Aug 2012.

To cite this article: Rajanarendar Eligeti , Ramakrishna Saini & Nagaraju Dharavath (2012): Design, synthesis, and antimicrobial activity of novel spiroisoxazolo[2,3-b][1,2,4]thiadiazole-2,2′-thiazolidine- 4′-ones and spiroisoxazolo[2,3-b][1,2,4]oxadiazole-2, 2′-thiazolidine-4′-ones, Journal of Sulfur Chemistry, 33:4, 427-438

To link to this article: http://dx.doi.org/10.1080/17415993.2012.703671

Journal of Sulfur Chemistry
Vol. 33, No. 4, August 2012, 427–438

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Design, synthesis, and antimicrobial activity of novel spiroisoxazolo[2,3-*b*][1,2,4]thiadiazole-2,2′-thiazolidine-4′-ones and spiroisoxazolo[2,3-*b*][1,2,4]oxadiazole-2,2′-thiazolidine-4′-ones

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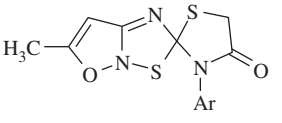
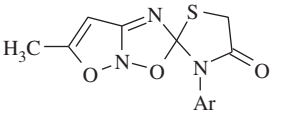
(*Received 2 May 2012; final version received 13 June 2012*)

A new synthetic strategy for the synthesis of novel 6-methyl-3′-aryl spiro[isoxazolo[2,3-*b*][1,2,4] thiadiazole-2,2′-thiazolidin]-4′-ones (**9a–9e**) and 6-methyl-3′-aryl spiro[isoxazolo[2,3-*b*][1,2,4]oxadiazole-2,2′-thiazolidin]-4′-ones (**12a–12e**) analogs is described. These compounds showed significant antimicrobial activity against all the standard strains.



9a–9e



12a–12e

Keywords: cyclocondensation reaction; spiro[isoxazolo[2,3-*b*][1,2,4]thiadiazole-2,2′-thiazolidin]-4′-ones; 6-methyl-3′-aryl spiro[isoxazolo[2,3-*b*][1,2,4]oxadiazole-2,2′-thiazolidin]-4′-ones; antibacterial activity; antifungal activity

1. Introduction

The increasing incidence of bacterial and fungal resistance to a large number of antimicrobial agents has prompted studies on the development of new potential antimicrobial compounds. The molecular manipulation of promising lead compounds is still a major line of approach to develop new drugs. It involves efforts to combine separate pharmacophoric groups of similar activity into one compound, thus making structural changes in the biological activity. So, the discovery of novel and potent antimicrobial agents is the best way to overcome microbial resistance and develop effective therapies (*1*).

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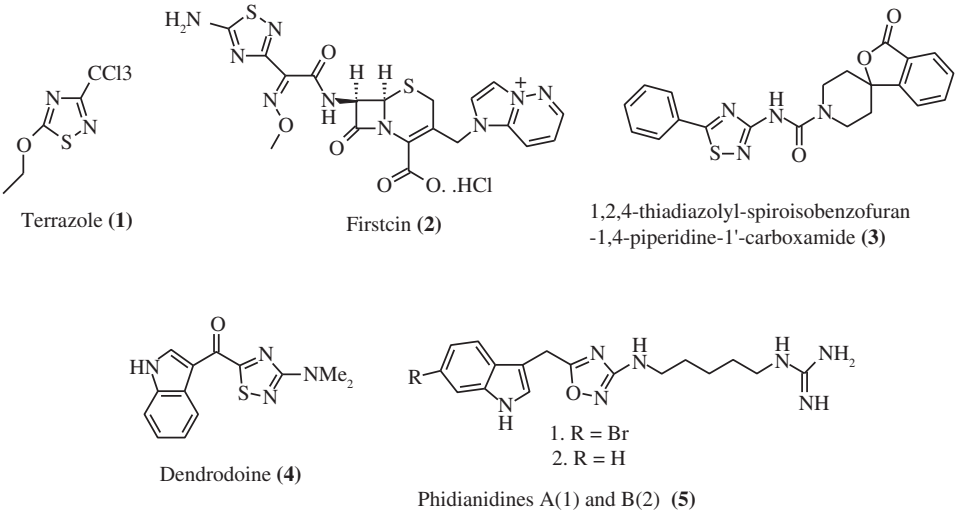
ISSN 1741-5993 print/ISSN 1741-6000 online

http://dx.doi.org/10.1080/17415993.2012.703671
http://www.tandfonline.com



Scheme 1. Examples of commercial [1,2,4]thiadiazole derivatives and naturally occurring [1,2,4]oxadiazoles with potential pharmacological activity.

[1,2,4]Thiadiazoles have been widely claimed to be useful insecticidal, herbicidal, and fungicidal agents (*2*). In recent years, many biologically active [1,2,4]thiadaizoles, exhibiting interesting medicinal properties for the potential treatment of human diseases have been disclosed (*3–9*). For example, 5-ethoxy-3-(trichloromethyl)-[1,2,4]thiadiazole **1** is a soil fungicide marketed as *etridiazole* or *terrazole* (*10*). The injectable cephalosporin antibiotic cefozopran hydrochloride known as Firstcin **2**, possess a thiadiazole group (*11*). The [1,2,4]thiadiazole system is present in potential pharmaceuticals (*12*) and cytotoxic natural products **3**,**4** (*13*). New bioactive [1,2,4]oxadiazoles phidianidines A(1) and B(2) **5** isolated from marine organisms (*14*) are found to exhibit *in vitro* cytotoxicity on various tumor and non-tumor mammalian cells (*15*) (Scheme 1). However, the role and importance of the thiadiazole moiety in all of these compounds, with regard to the mechanism of the biological response of the targeted enzymes are not clearly understood at the molecular level.

Isoxazole derivatives have been reported with diverse structural features and versatile biological properties such as antitumor (*16*), CNS–active (*17*), analgesic (*18*), antimicrobial (*19*), and muscle relaxant (*20*) activity for the treatment of hyper cholsteremia and hyperlipidemia (*21*), as organic electrolytes for non-aqueous batteries (*22*), in photographic emulsions (*23*), as synthetic intermediates (*24*), and as chemotherapeutic agents (*25*).

New hybrid moieties secured by linking isoxazoles with [1,2,4]thiadiazole-2,2′-thiazolidinone and [1,2,4]oxadiazole-2,2′-thiazolidinone promise to offer fascinating scaffolds of fundamental interest to both sulfur and medicinal chemistry. Design of synthetic methods for the efficient preparation of these tri-heterocyclic compounds, however, is necessary. Hence, we embarked on the synthesis and bioassay of the compounds having isoxazolo-[1,2,4]thiadiazole-2,2′-thiazolidinone, and [1,2,4]oxadiazole-2,2′-thiazolidinone moieties embedded in a fused molecular framework to improve specificity and efficacy of these scaffolds against microorganisms. As a sequel to our work on the synthesis of fuse disoxazoles (*26*), we report herein the synthesis and biological evaluation of novel 6-methyl-3′-aryl[spiro[2,3-*b*][1,2,4]thiadiazole-2,2′-thiazolidin]-4′-ones (**9**) and 6-methyl-3′-aryl[spiro[2,3-*b*][1,2,4]oxadiazole-2,2′-thiazolidin]-4′-ones (**12**).

2. Chemistry

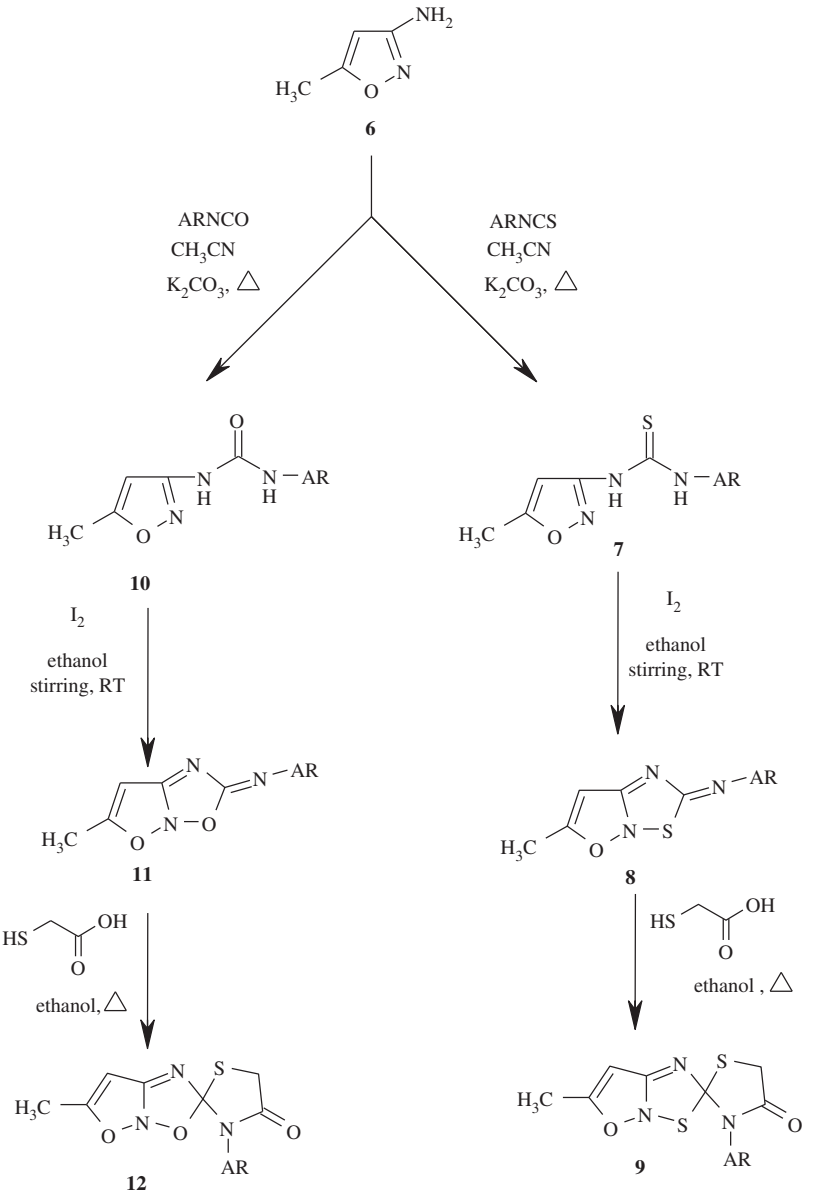
The synthesis of the compounds **7–12** was accomplished by the synthetic sequence shown in Scheme 2. The reaction of commercially available 3-amino-5-methyisoxazole **6** with aryl isothiocyanate and aryl isocyanate in the presence of K_2CO_3 in CH_3CN afforded the key intermediates, viz. 1-(5-methyl-3-isoxazolyl)-3-arylthioureas **7** (*27*) and 1-(5-methyl-3-isoxazolyl)-3-arylureas **10** (*28*), respectively. Compounds **7** and **10** upon refluxing in ethanol in the presence of I_2 led to the formation of the cyclized products (*Z*)-*N*-(6-methyl-2*H*-isoxazolo [2,3-*b*][1,2,4]thiadiazol-2-ylidene)-anilines **8** and (*Z*)-*N*-(6-methyl-2*H*-isoxazolo[2,3-*b*][1,2,4]oxadiazol-2-ylidene)-anilines **11**, respectively. Cyclocondensation of **8** and **11** with mercaptoacetic acid in ethanol led to the formation of novel 6-methyl-3′-aryl[spiro[2,3-*b*][1,2,4]thiadiazole-2,2′-thiazolidin]-4′-ones (**9**) and 6-methyl-3′-aryl[spiro[2,3-*b*][1,2,4]oxadiazole-2,2′-thiazolidin]-4′-ones (**12**), respectively. The structures of the products **7–12** have been elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. Elemental analyses are satisfactory and confirm elemental composition and purity of the newly synthesized compounds **7–12**.

Compound **8** displayed a characteristic absorption band in the IR spectra around 1615 cm^{-1} due to the C=N functional group. The absence of NH functional group absorption bands in the IR of **8** clearly confirms the cyclization. In the ^{1}H NMR spectra of **8**, the absence of NH proton signals, which are present in its precursor **7**, also establishes the cyclization. The mass spectrum of **8** agrees well with the cyclized structure, which shows the molecular ion peak, $[M]^+$, at m/z 231. The ^{1}H NMR spectrum of **9** displayed a distinct signal at δ 4.11 due to the methylene protons of the thiazolidinone ring confirming the formation of the spirothiazolidinone ring. The mass spectrum of **9** confirmed the structure by exhibiting the molecular ion peak $[M]^+$ at m/z 305.

Compound **11** displayed a characteristic absorption band in the IR spectra around 1617 cm^{-1} due to the C=N functional group and did not exhibit absorption bands due to NH and CO functional groups present in its precursor **10**, confirming the cyclization. Similarly, the cyclization was supported by the ^{1}H NMR spectrum of **11** that did not contain the NH proton signals that are present in its precursor **10**. The mass spectrum of the product **11** also agrees with the cyclized structure that shows the molecular ion $[M]^+$ peak at m/z 215. The ^{1}H NMR spectrum of **12** displayed a characteristic signal at δ 4.23 due to the methylene protons of the thiazolidinone ring confirming the formation of the spirothiazolidinone ring.

3. Antibacterial activity

Antibacterial activities of **9a–9e** and **12a–12e** in acetone were performed by the broth dilution method using nutrient agar against Gram-negative bacteria *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Chormobacteium violaceum*, and Gram-positive bacteria *Bacillus subtilis*, *Bacillus sphaericus*, and *Staphylococcus aureus* at 100 μg/ml concentration. The minimum inhibitory concentration (MIC) was done by the broth dilution method (*29*). Ciprofloxacin was used as a standard for comparison. The ready-made nutrient broth medium (HiMedia, 24 g) was suspended in distilled water (100 ml) and heated until it dissolved completely. The medium and test tubes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound is dissolved in acetone at a concentration of 100 μg/ml and is added to the first test tube, which is serially diluted. A fixed 0.5 ml volume of overnight culture is added to all the test tubes and then incubated at 37°C for 24 h. After 24 h, these tubes were measured for turbidity. Results are given in Tables 1 and 2.



7–12			
	AR		AR
a	C_6H_5	**d**	4-$CH_3C_6H_4$
b	4-CLC_6H_4	**e**	4-$CH_3OC_6H_4$
c	4-BRC_6H_4		

Scheme 2. Synthesis of spiroisoxazolo[2,3-*b*][1,2,4]thiadiazole-2,2′-thiazolidine-4′-ones and spiroisoxazolo[2,3-*b*][1,2,4]oxadiazole-2,2′-thiazolidine-4′-ones.

Table 1. Antibacterial activity of **9a–9e.**

	MIC[a,b]					
	Gram-positive			Gram-negative		
Compound	*B. subtilis*	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
9a	14	16	17	20	15	18
9b	12	10	8	13	11	14
9c	15	17	18	22	18	17
9d	16	15	19	25	15	13
9e	11	9	13	17	15	13
Ciprofloxacin	20	20	25	30	25	25

Notes: [a]Negative control (acetone) – no activity.
[b]Concentration 100 μg/ml.

Table 2. Antibacterial activity of **12a–12e.**

	MIC[a,b]					
	Gram-positive			Gram-negative		
Compound	*B. subtilis*	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
12a	16	18	20	19	21	23
12b	10	13	16	12	17	14
12c	9	11	15	18	16	12
12d	13	16	19	24	22	19
12e	15	17	11	21	16	12
Ciprofloxacin	20	20	25	30	25	25

Notes: [a]Negative control (acetone) – no activity.
[b]Concentration 100 μg/ml.

The results of antibacterial screening (Tables 1 and 2) reveal that compounds **9a–9e** and **12a–12e** displayed better activity and are more active than the standard drug Ciprofloxacin. In series **9**, compounds **9b** and **9e** possessing chloro and methoxy groups as substituent on the benzene ring showed a better activity, whereas in series **12**, compounds **12b** and **12c** carrying chloro and bromo groups as substituents on the benzene ring imparted remarkable activity. However, the degree of inhibition varied both with the test compound as well as with the bacteria used in the present investigation. In conclusion, almost all the series of compounds, **9a–9e** and **12a–12e** exhibited good activity by inhibiting growth of all the six bacteria to a greater extent in comparison to the standard drug Ciprofloxacin. These remarkable results may be due to the presence of the isoxazole–thiadiazole–thiazolidone and isoxazole–oxadiazole–thiazolidone ring systems. Some of the compounds may be used as bacteriocides after a detailed study.

4. Antifungal activity

Antifungal activities of **9a–9e** and **12a–12e** were determined by using the agar cup bioassay method (*30*) with Clotrimazole as the standard. The compounds were tested for their antifungal activity against five test organisms, *Aspergillus niger*, *Chrysosporium tropicum*, *Rhizopus oryzae*, *Fusarium moniliforma* and *Curvularia lunata* using the agar cup bioassay method at 100 μg/ml concentrations. The ready-made potato dextrose agar medium (HiMedia, 39 g) was suspended in

Table 3. Antifungal activity of **9a–9e.**

Compound	Zone of inhibition[a,b]				
	A. niger	*C. tropicum*	*R. oryzae*	*F. moniliformae*	*C. lunata*
9a	32	39	38	31	29
9b	38	41	37	41	39
9c	41	52	45	51	39
9d	51	48	47	39	41
9e	31	47	35	29	30
Clotrimazole	29	30	28	23	20

Notes: [a]Negative control (acetone) – no activity.
[b]Concentration 100 μg/ml.

Table 4. Antifungal activity of **12a–12e.**

Compound	Zone of inhibition[a,b]				
	A. niger	*C. tropicum*	*R. oryzae*	*F. moniliformae*	*C. lunata*
12a	34	41	51	29	27
12b	33	40	40	29	26
12c	44	48	51	48	50
12d	37	44	38	28	31
12e	49	56	53	44	49
Clotrimazole	29	30	28	23	20

Notes: [a]Negative control (acetone) – no activity.
[b]Concentration 100 μg/ml.

distilled water (1000 ml) and heated until it dissolved completely. The medium and Petri dishes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. The medium was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 ml of (week-old) culture of the test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in acetone (100 μg/ml). Agar inoculation cups were scooped out with a 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, 100 (μg/ml) of the test solution was added. Controls were maintained with acetone and Clotrimazole (100 μg/ml). The treated and the controls were kept at room temperature for 72–96 h. Inhibition zones were determined and their diameter was calculated in millimeter. Three to four replicates were maintained for each treatment. Results are given in Tables 3 and 4.

The antifungal activity results (Tables 3 and 4) indicated that compounds **9a–9e** and **12a–12e** are significantly toxic toward all five fungi and they are lethal even at 100 μg/ml concentration. In series **9**, compounds **9c** and **9d** exhibited high antifungal activity which may be due to the presence of bromo and methyl groups as substituents on the benzene ring. In series **12**, compounds **12c** and **12e** possessing bromo and methoxy groups are highly toxic toward fungi. The antifungal activity of these compounds was compared with the standard drug Clotrimazole, which demonstrated that they have promising activity. In conclusion, almost all the series of compounds, **9a–9e** and **12a–12e** are highly toxic toward the fungi under investigation and they are lethal even at 100 μg/ml concentration in comparison with standard Clotrimazole at the same concentration. This may be due to the presence of isoxazole–thiadiazole–thiazolidone and isoxazole–oxadiazole–thiazolidone ring systems. It is noteworthy that some of the compounds may be exploited after a detailed study for control of wilt diseases of different crops as fungicides.

5. Conclusion

A new synthetic strategy has been utilized for the synthesis of title compounds. The title compounds **9a–9e** and **12a–12e** exhibited promising antimicrobial activity.

6. Experimental

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 and visualization was done by exposing the plates to iodine vapor. 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 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.

6.1. *Synthesis of 1-(5-methyl-3-isoxazolyl)-3-arylthioureas (7a–7e)/1-(5-methyl-3-isoxazolyl)-3-arylureas (10a–10e) – general procedure*

A mixture of 3-amino-5-methylisoxazole (**6**) (0.01 mol) and aryl isothiocyanate (0.01 mol)/aryl isocyanate (0.01 mol) was added to acetonitrile (15 ml) in the presence of K_2CO_3 (0.5 g). The reaction mixture was refluxed while stirring for 4 h. After completion of the reaction (monitored by TLC), the reaction mixture was poured onto crushed ice and the solid filtered off and recrystallized from ethylacetate to produce (**7a–7e**)/(**10a–10e**) in good yields.

6.2. *1-(5-Methyl-3-isoxazolyl)-3-phenylthiourea (7a)*

Pale brown solid; yield 74%, m.p. 134–136°C; IR (KBr) cm^{-1}: 3310 (NH), 1220 (C=S), ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.31 (s, 3H, isoxazole–CH_3), 6.16 (s, 1H, isoxazole–CH), 7.00–8.17 (m, 5H, Ar–H), 8.48 (bs, 1H, NH, D_2O exchangeable), 8.82 (bs, 1H, NH, D_2O exchangeable). EI–MS $[M]^+$ m/z 233. Anal. calcd. for $C_{11}H_{11}N_3OS$: C, 56.63; H, 4.75; N, 18.01%. Found: C, 56.69; H, 4.68; N, 18.04%.

6.3. *1-(5-Methyl-3-isoxazolyl)-3-phenylurea (10a)*

Brown solid; yield 77%, m.p. 138–140°C; IR (KBr) cm^{-1}: 3300 (NH), 1615 (C=O), ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.30 (s, 3H, isoxazole–CH_3), 6.22 (s, 1H, isoxazole–CH), 7.11–8.34 (m, 5H, Ar–H),8.52 (bs, 1H, NH, D_2O exchangeable), 8.78 (bs, 1H, NH, D_2O exchangeable). EI–MS $[M]^+$ m/z 217. Anal. calcd. for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34%. Found: C, 60.78; H, 5.16; N, 19.39%.

6.4. *Synthesis of (Z)-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]thiadiazol-2-ylidene)-anilines (8a–8e) and (Z)-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]oxadiazol-2-ylidene)-anilines (11a–11e) – general procedure*

To a solution of 1-(5-methyl-3-isoxazolyl)-3-arylthioureas (**7a**) (0.01 mol)/1-(5-methyl-3-isoxazolyl)-3-arylureas (**10**) (0.01 mol) in ethanol (10 ml), a solution of I_2 (0.01 mol) in 10 ml

of ethanol was added and the contents are stirred. After completion of the reaction (monitored by TLC), the solvent was removed under pressure and 30 ml of water was added to the residue. The resulting solution was then extracted with ethyl acetate to give a residue after evaporation that was purified by recrystallization from methanol to produce **8a–8e**/**11a–11e** in high yields.

6.5. *(Z)-N-(6-Methyl-2H-isoxazolo[2,3-b][1,2,4]thiadiazol-2-ylidene)-aniline (8a)*

Brown solid; yield 75%, m.p. 182–184°C; IR (KBr) cm^{-1}: 1615 (C=N), ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.32 (s, 3H, isoxazole–CH_3), 6.21 (s, 1H, isoxazole–CH), 7.20–8.00 (m, 5H, Ar–H). EI–MS $[M]^+$ m/z 231.^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 16.42, 90.61, 124.31, 125.13, 126.42, 127.31, 127.86, 150.13, 163.42, 165.36, 186.41. Anal. calcd. for $C_{11}H_9N_3OS$: C, 57.13; H, 3.92; N, 18.17%. Found: C, 57.10; H, 3.97; N, 18.20%.

6.6. *(Z)-4-Chloro-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]thiadiazol-2-ylidene)-aniline (8b)*

Brown solid; yield 78%, m.p. 180–182°C; IR (KBr) cm^{-1}: 1620 (C=N), ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.28 (s, 3H, isoxazole–CH_3), 6.14 (s, 1H, isoxazole–CH), 7.00–8.04 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 265.^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 16.11, 90.81, 124.01, 125.34, 126.65, 127.52, 132.58, 150.01, 162.98, 165.54, 186.32. Anal. calcd. for $C_{11}H_8N_3OSCl$: C, 49.72; H, 3.03; N, 15.81%. Found: C, 49.77; H, 3.00; N, 15.88%.

6.7. *(Z)-4-Bromo-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]thiadiazol-2-ylidene)-aniline (8c)*

Brown solid; yield 75%, m.p. 185–187°C; IR (KBr) cm^{-1}: 1627(C=N), ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.30 (s, 3H, isoxazole–CH_3), 6.20 (s, 1H, isoxazole–CH), 7.01–7.83 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 309. Anal. calcd. for $C_{11}H_8N_3OSBr$: C, 42.60; H, 2.60; N, 13.55%. Found: C, 42.58; H, 2.65; N, 13.51%.

6.8. *(Z)-4-Methyl-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]thiadiazol-2-ylidene)-aniline (8d)*

Brown solid; yield 69%, m.p. 183–185°C; IR (KBr) cm^{-1}: 1618 (C=N), ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.26 (s, 3H, isoxazole–CH_3), 2.51 (s, 3H, Ar–CH_3), 6.18 (s, 1H, isoxazole–CH), 7.03–8.11 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 245. Anal. calcd. for $C_{12}H_{11}N_3OS$: C, 58.76; H, 4.52; N, 17.13%. Found: C, 58.80; H, 4.50; N, 17.18%.

6.9. *(Z)-4-Methoxy-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]thiadiazol-2-ylidene)-aniline (8e)*

Brown solid; yield 72%, m.p. 179–181°C; IR (KBr) cm^{-1}: 1622 (C=N), ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.31 (s, 3H, isoxazole–CH_3), 3.64 (s, 3H, OCH_3), 6.22 (s, 1H, isoxazole–CH), 6.98–8.01 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 261. Anal. calcd. for $C_{12}H_{11}N_3O_2S$: C, 55.16; H, 4.24; N, 16.08%. Found: C, 55.20; H, 4.20; N, 16.12%.

6.10. *(Z)-N-(6-Methyl-2H-isoxazolo[2,3-b][1,2,4]oxadiazol-2-ylidene)-aniline (11a)*

Brown solid; yield 70%, m.p. 165–167°C; IR (KBr) cm^{-1}: 1617 (C=N), ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.30 (s, 3H, isoxazole–CH_3), 6.19 (s, 1H, isoxazole–CH), 6.82–8.00 (m, 5H, Ar–H). EI–MS $[M]^+$ m/z 215.^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 17.01, 89.98, 124.15, 125.55,

126.47, 127.14, 132.42, 150.53, 163.11, 165.65, 186.12. Anal. calcd. for $C_{11}H_9N_3O_2$: C, 61.39; H, 4.22; N, 19.53%. Found: C, 61.43; H, 4.26; N, 19.49%.

6.11. *(Z)-4-Chloro-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]oxadiazol-2-ylidene)-aniline (11b)*

Brown solid; yield 74%, m.p. 171–173°C; IR (KBr) cm^{-1}: 1613 (C=N), ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.27 (s, 3H, isoxazole–CH_3), 6.21 (s, 1H, isoxazole–CH), 6.93–7.65 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 249.^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 16.67, 90.14, 124.01, 125.42, 126.11, 127.51, 132.37, 150.27, 162.65, 165.77, 186.57. Anal. calcd. for $C_{11}H_8N_3O_2Cl$: C, 52.92; H, 3.23; N, 14.20%. Found: C, 52.87; H, 3.19; N, 14.26%.

6.12. *(Z)-4-Bromo-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]oxadiazol-2-ylidene)-aniline (11c)*

Brown solid; yield 71%, m.p. 190–192°C; IR (KBr) cm^{-1}: 1621(C=N), ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.31 (s, 3H, isoxazole–CH_3), 6.18 (s, 1H, isoxazole–CH), 7.00–7.87 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 293. Anal. calcd. for $C_{11}H_8N_3O_2Br$: C, 44.92; H, 2.74; N, 14.29%. Found: C, 44.89; H, 2.68; N, 14.35%.

6.13. *(Z)-4-Methyl-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]oxadiazol-2-ylidene)-aniline (11d)*

Brown solid; yield 76%, m.p. 173–175°C; IR (KBr) cm^{-1}: 1621 (C=N), ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.23 (s, 3H, isoxazole–CH_3), 2.58 (s, 3H, Ar–CH_3), 6.19 (s, 1H, isoxazole–CH), 7.13–8.16 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 229. Anal. calcd. for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; N, 18.33%. Found: C, 62.81; H, 4.90; N, 18.38%.

6.14. *(Z)-4-Methoxy-N-(6-methyl-2H-isoxazolo[2,3-b][1,2,4]oxadiazol-2-ylidene)-aniline (11e)*

Brown solid; yield 75%, m.p. 177–179°C; IR (KBr) cm^{-1}: 1612 (C=N), ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.29 (s, 3H, isoxazole–CH_3), 3.62 (s, 3H, OCH_3), 6.21 (s, 1H, isoxazole–CH), 6.88–7.86 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 261. Anal. calcd. for $C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13%. Found: C, 58.72; H, 4.58; N, 17.18%.

6.15. *Synthesis of 6-methyl-3′-aryl[spiro[2,3-b][1,2,4] thiadiazole-2,2′-thiazolidin]-4′-ones (9a–9e) and 6-methyl-3′-aryl[spiro[2,3-b][1,2,4]oxadiazole-2, 2′-thiazolidin]-4′-ones (12a–12e) – general procedure*

To solutions of (*Z*)-*N*-(6-methyl-2*H*-isoxazolo[2,3-*b*][1,2,4]thiadiazol-2-ylidene)-anilines (**8**) (0.01 mol)/(*Z*)-*N*-(6-methyl-2*H*-isoxazolo[2,3-*b*][1,2,4]oxadiazol-2-ylidene)-anilines (**11**) (0.01 mol) in ethanol (15 ml), mercaptoacetic acid (0.01 mol) was added and the contents were refluxed for 6 h. After completion of the reaction (monitored by TLC), the solvent was removed under pressure and 30 ml of water was added to the residue. The resulting solution was then extracted with ethyl acetate which when evaporated gave a residue that was purified by recrystallization from ethyl acetate to produce **9a–9e**/**12a–12e** in high yields.

6.16. *6-Methyl-3 '-phenyl spiro[isoxazolo[2,3-b][1,2,4]thiadiazole-2,2′-thiazolidin]-4′-one (9a)*

Brown solid; yield 70%, m.p. 194–196°C; IR (KBr) cm^{-1}: 1675 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.30 (s, 3H, isoxazole–CH_3), 4.11 (s, 2H, thiazole–CH_2), 6.11 (s, 1H, isoxazole–CH), 7.11–8.20 (m, 5H, Ar–H). EI–MS $[M]^+$ m/z 305. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 16.12, 30.45, 86.13, 90.53, 124.35, 125.33, 126.02, 127.52, 127.67, 150.21, 165.48, 172.43, 186.30. Anal. calcd. for $C_{13}H_{11}N_3O_2S_2$: C, 51.13; H, 3.63; N, 13.76%. Found: C, 51.18; H, 3.68; N, 13.81%.

6.17. *3′-(4-Chlorophenyl)-6-methyl-spiro[isoxazolo[2,3-b][1,2,4]thiadiazole-2, 2′-thiazolidin]-4′-one (9b)*

Brown solid; yield 76%, m.p. 201–203°C; IR (KBr) cm^{-1}: 1655 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.28 (s, 3H, isoxazole–CH_3), 4.18 (s, 2H, thiazole–CH_2), 6.21 (s, 1H, isoxazole–CH), 6.98–8.00 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 339. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 17.02, 30.15, 84.13, 90.31, 124.65, 125.46, 126.08, 127.12, 127.72, 150.38, 165.33, 172.00, 186.45. Anal. calcd. for $C_{13}H_{10}N_3O_2S_2Cl$: C, 45.95; H, 2.97; N, 12.37%. Found: C, 45.89; H, 2.93; N, 12.45%.

6.18. *3′-(4-Bromophenyl)-6-methyl-spiro[isoxazolo[2,3-b][1,2,4]thiadiazole-2,2′-thiazolidin]-4′-one (9c)*

Brown solid; yield 76%, m.p. 198–200°C; IR (KBr) cm^{-1}: 1671 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.30 (s, 3H, isoxazole–CH_3), 4.21 (s, 2H, thiazole–CH_2), 6.11 (s, 1H, isoxazole–CH), 6.88–7.75(m, 4H, Ar–H). EI–MS $[M]^+$ m/z 383. Anal. calcd. for $C_{13}H_{10}N_3O_2S_2Br$: C, 40.63; H, 2.62; N, 10.94%. Found: C, 40.58; H, 2.68; N, 10.87%.

6.19. *6-Methyl-3′-p-tolyl-spiro[isoxazolo[2,3-b][1,2,4]thiadiazole-2, 2′-thiazolidin]-4′-one (9d)*

Brown solid; yield 71%, m.p. 207–209°C; IR (KBr) cm^{-1}: 1666 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.27 (s, 3H, isoxazole–CH_3), 2.57 (s, 3H, Ar–CH_3), 4.25 (s, 2H, thiazole–CH_2), 6.18 (s, 1H, isoxazole–CH), 6.93–7.67 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 319. Anal. calcd. for $C_{14}H_{13}N_3O_2S_2$: C, 52.65; H, 4.10; N, 13.06%. Found: C, 52.59; H, 4.16; N, 13.11%.

6.20. *3′-(4-Methoxyphenyl)-6-methyl-spiro[isoxazolo[2,3-b][1,2,4]thiadiazole-2,2′-thiazolidin]-4′-one (9e)*

Brown solid; yield 76%, m.p. 203–205°C; IR (KBr) cm^{-1}: 1646 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.21 (s, 3H, isoxazole–CH_3), 3.60 (s, 3H, OCH_3), 4.18 (s, 2H, thiazole–CH_2), 6.10 (s, 1H, isoxazole–CH), 6.88–7.97 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 335. Anal. calcd. for $C_{14}H_{13}N_3O_3S_2$: C, 50.13; H, 3.91; N, 12.53%. Found: C, 50.09; H, 9.86; N, 12.58%.

6.21. *6-Methyl-3′-phenyl spiro[isoxazolo[2,3-b][1,2,4]oxadiazole-2,2′-thiazolidin]-4′-one (12a)*

Brown solid; yield 68%, m.p. 204–206°C; IR (KBr) cm^{-1}: 1663 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.27 (s, 3H, isoxazole–CH_3), 4.23 (s, 2H, thiazole–CH_2), 6.27 (s, 1H, isoxazole–CH), 6.94–8.11 (m, 5H, Ar–H). EI–MS $[M]^+$ m/z 289. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 16.83, 31.01, 90.25, 110.21, 124.23, 125.54, 126.21, 127.62, 127.81, 150.11, 165.27, 172.59, 186.45.

Anal. calcd. for $C_{13}H_{11}N_3O_3S$: C, 53.97; H, 3.83; N, 14.52%. Found: C, 53.91; H, 3.87; N, 14.48%.

6.22. *3′-(4-Chlorophenyl)-6-methyl-spiro[isoxazolo[2,3-b][1,2,4]oxadiazole-2,2′-thiazolidin]-4′-one (12b)*

Brown solid; yield 75%, m.p. 211–213°C; IR (KBr) cm^{-1}: 1675 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.30 (s, 3H, isoxazole–CH_3), 4.18 (s, 2H, thiazole–CH_2), 6.11 (s, 1H, isoxazole–CH), 7.12–8.34 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 339. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 17.01, 32.06, 91.00, 110.45, 124.11, 125.36, 126.45, 127.17, 127.92, 150.32, 165.28, 172.23, 186.67. Anal. calcd. for $C_{13}H_{10}N_3O_3SCl$: C, 48.23; H, 3.11; N, 12.98%. Found: C, 48.18; H, 3.18; N, 12.91%.

6.23. *3′-(4-Bromophenyl)-6-methyl-spiro[isoxazolo[2,3-b][1,2,4]oxadiazole-2,2′-thiazolidin]-4′-one (12c)*

Brown solid; yield 79%, m.p. 208–210°C; IR (KBr) cm^{-1}: 1663 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.31 (s, 3H, isoxazole–CH_3), 4.27 (s, 2H, thiazole–CH_2), 6.18 (s, 1H, isoxazole–CH), 7.11–8.34 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 367. Anal. calcd. for $C_{13}H_{10}N_3O_3SBr$: C, 42.41; H, 2.74; N, 11.41%. Found: C, 42.48; H, 2.80; N, 11.37%.

6.24. *6-Methyl-3′-p-tolyl-spiro[isoxazolo[2,3-b][1,2,4]oxadiazole-2,2′-thiazolidin]-4′-one (12d)*

Brown solid; yield 73%, m.p. 217–219°C; IR (KBr) cm^{-1}: 1660 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.20 (s, 3H, isoxazole–CH_3), 2.61 (s, 3H, Ar–CH_3), 4.11 (s, 2H, thiazole–CH_2), 6.10 (s, 1H, isoxazole–CH), 7.10–7.97 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 303. Anal. calcd. for $C_{14}H_{13}N_3O_3S$: C, 55.43; H, 4.32; N, 13.85%. Found: C, 55.38; H, 4.38; N, 13.91%.

6.25. *3′-(4-Methoxyphenyl)-6-methyl-spiro[isoxazolo[2,3-b][1,2,4]oxadiazole-2, 2′-thiazolidin]- 4′-one (12e)*

Brown solid; yield 78%, m.p. 215–217°C; IR (KBr) cm^{-1}: 1677 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.23 (s, 3H, isoxazole–CH_3), 3.58 (s, 3H, OCH_3), 4.24 (s, 2H, thiazole–CH_2), 6.16 (s, 1H, isoxazole–CH), 7.03–8.13 (m, 4H, Ar–H). EI–MS $[M]^+$ m/z 319. Anal. calcd. for $C_{14}H_{13}N_3O_4S$: C, 52.66; H, 4.10; N, 13.16%. Found: C, 52.71; H, 4.17; N, 13.11%.

Acknowledgements

The authors are thankful to the Head, Department of Chemistry, Kakatiya University, Warangal for facilities and to the Director, Indian Institute of Chemical Technology, Hyderabad for recording ^1H NMR, ^{13}C NMR and Mass Spectra.

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