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Ethyl lactate mediated 1,3-Dipolar Cycloaddition of Azomethine Ylides: A Design, Synthesis and Antibacterial activity of novel Dispiro Heterocycles

Kanti Sharma[a],*, Lokesh K. Sharma[a], Meenakshi Jain[b] and Renuka Jain[b]

[a]*Department of Chemistry, R.L. Saharia Govt. P.G. College, Kaladera, Jaipur-303801, India*
[b]*Department of Chemistry, University of Rajasthan, Jaipur-302004, India*
E-mail : drkanti@gmail.com
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Abstract: A facile, greener and efficient one-pot, three component procedure for the synthesis of novel dispirooxindolopyrrolothiazole derivatives carried out by cycloaddition reaction of 3-methyl-1-phenyl-4-thiophenylidene-5-pyrazolone dipolarophile, azomethine ylides generated in situ, via decarboxylative condensation of isatin with thiazolidine-4-carboxylic acid, has been reported in ethyl lactate, an ethyl lactate as a recyclable solvent in excellent yield without using any catalyst. This green route provides mild reaction conditions, high yields of products in short reaction time, high regio- and stereoselectivity, operational simplicity and environmentally benign synthesis. The synthesized compounds were characterized by analytical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR and FAB mass) data. All synthesized compounds were screened for antibacterial activity against *B. subtilis, S. aureus, E. coli* and *P. aeruginosa* bacteria.

Keywords: 1,3-Dipolar cycloaddition azomethine ylides, 3-methyl-1-phenyl-4-thiophenylidene-5-pyrazolone, spirooxindole, Ethyl lactate, Environmentally benign, antimicrobial activity.

INTRODUCTION :

The last few decades have seen an attentive lens progressively lengthening over the synthesis of pyrrolidine rings given their fascinating array of biological applications - antidiabetic, glucosidase inhibitory [1], potent antiviral [2], antibacterial [3], anticancer activities [4]. Apropos the same, the 1,3-dipolar cycloaddition reaction of azomethine ylide provides a facile synthesis of pyrrolidine and spiropyrrolidine heterocycles [5]. Spiro-oxindole ring systems are the central skeleton for numerous alkaloids as well as pharmacologically important compounds [6]. This comes owing to their potency and plenitude of roles as an antibacterial, antifungal, antimycobacterial, antitumor and acetylcholinesterase inhibitor [7], anti-oxidant [8], antimicrobial [9], anti-malarial [10]. Additionally, as effective nonpeptide inhibitors of the p53-MDM2 small-molecules these reasonably make promising anticancer

therapeutics [11].

Spiropyrrolidinyl-oxindole systems are also found in a number of alkaloids like spirotryprostanin A and B, pteropodin, elacomine, horsfiline, etc.[12] (Fig.1) acting as GPR40 agonists [13].

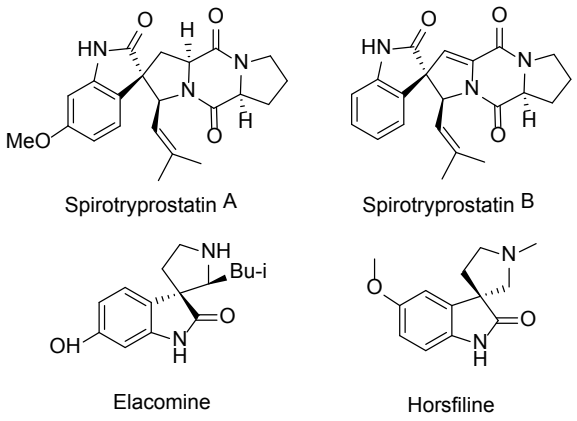

Figure 1. Some compounds containing the spiropyrrolidinyl-oxindole moiety.

Recent challenges to build-up new organic transformations that are not only efficient, selective and high yielding but also environmentally friendly make the choice of a successful synthesis [14]. In this regard, ethyl lactate has emerged among the set of new green solvents to replace the volatile organic solvents. These constitute interesting materials for the earnest chemist due to their physico-chemical properties - negligible vapour pressure, high thermal and chemical stability, easy recyclability, good solvating ability, non-flammability and their potential to enhance reaction rate.

It becomes, therefore, a great countenance to develop an efficient, green and suitable protocol to weigh in the synthesis of compounds containing both spiro-oxindole and pyrrolidine rings as the recent synthesis of ferrocenyl β-C-glycosidic spiro oxindoles via the [3+2] cycloaddition reaction of azomethine ylides by the Raghunathan and co-workers [15] would seek to instantiate.

The synthesis of spiro-oxindole via the 1,3-dipolar cycloaddition reaction of azomethine ylides derived from isatin with various chalcones [16], Baylis–Hillman and Morita–Baylis–Hillman adducts of isatin has been reported [17, 18].

Pyrazolone is a key structure in numerous compounds of therapeutic importance [19]. Pyrazolones are gaining importance especially in drug discovery studies against cerebralischaemia [20] and cardiovascular diseases [21]. Due to its diverse pharmacological properties, the chemistry of pyrazolones is gaining attention, and there have been numerous methodologies reported recently [22]. These reports prompted us to synthesize new compounds containing pyrazolone as one of the constituent units.

To the best of our knowledge, 1,3-dipolar cycloaddition reactions of azomethine ylides have been carried out by microwave irradiation [23], ultrasonic irradiation [24], ionic liquid [25] and ethyl lactate [26]. In continuation of our ongoing program, to develop convenient synthetic protocols for the synthesis of spiro heterocycles by employing green tools [27-30] and considering the above urgent need to provide convenient rapid route, we here in, report for the first time, the use of ethyl lactate in 1,3-dipolar cycloaddition reaction involving the olefin segment of Knoevenagel adduct (derived from isatin and unsubstituted 3-methyl-1-phenyl-4-thiophenylidene-5-pyrazolone to synthesize spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole derivatives via the generation of azomethine ylides from isatin and thiazolidine-4-carboxylic acid. This is the first report on Knoevenagel adduct, 3-methyl-1-phenyl-4-thiophenylidene-5-pyrazolone being employed as a dipolarophile in the cycloaddition of azomethine ylides to synthesize dispiro heterocycles. The generated azomethine ylides approached the dipolarophiles regioselectively.

The one-pot regioselective synthesis of spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7-(2-thienyl)tetrahydro-1*H*-pyrrolo[1,2-c][1,3]thiazole **(5a-k)** have been performed by taking indole-2,3-diones (**1a-k**, 1.0 mmol), thiazolidine-4-carboxylic acid (**2**, 1.2 mmol) and 3-methyl-1-phenyl-4-thienylidene-5-pyrazolone (**3**, 1.0 mmol) in ethyl lactate **(Scheme 1)**.

The reaction proceeds through decarboxylative condensation of indole-2,3-diones **(1a-k)** with thiazolidine-4-carboxylic acid **(2)** to generate azomethine ylides **(4a-k)**, which subsequently undergo 1,3-dipolar cycloaddition with the dipolarophiles **(3)** to afford novel cycloadducts **(5a-k)** as a single regioisomer.

Control of the relative stereochemistry at the spiro centre was observed. *Anti*-ylides **(4a)** are involved in the transition state which adds to dipolarophiles to give the observed cycloadducts. Formation of *syn*-ylides is not observed due to the unfavourable steric repulsion between the carbonyl group of oxindole and methyl group of thiazolidine-4-carboxylic acid **(Scheme 2)**.

The regiochemistry in the product formation can be explained by considering secondary orbital interaction **(SOI)** of the carbonyl group of dipolarophile **(3)** with those of the ylide **(4)** [31] as shown in (**Scheme 2)**. Accordingly, the observed regioisomer **(5a)** *via* path A is more favourable due to the presence of **SOI** which is not possible in path B [32, 33] resulting in the formation of only one regioisomer **(5a-k)**.

The choice of an appropriate reaction medium is of crucial importance for successful synthesis. Initially, the three component reaction of isatin 1a, thiazolidine-4-carboxylic acid and 3-methyl-1-phenyl-4-thienylidene-5-pyrazolone, as a simple model substrate, was investigated to establish the feasibility of the strategy and optimize the reaction conditions. Various solvents such as methanol, ethanol, toluene as well as ionic liquid, [bmimPF_6], ethyl lactate were screened and the best results were obtained in ethyl lactate with excellent yield in shorter reaction time (Table 3, entry 7). But the reaction was found to give comparatively lower yield of the product in other petroleum based solvents.

In addition, to establish a high yielding green protocol, the polarity of the solvent was tuned by using water as a co-solvent with ethyl lactate in different percentages. The reaction was carried out in pure ethyl lactate and then the polarity was tuned with water for better results. It is clearly indicated that 80% ethyl lactate in water produced the most favorable polarity of the solvent in which the rapid reaction took place with maximum isolated yield (Table 4, entry 2). This may be attributed to the insolubility of the final product in ethyl lactate–water system.

MATERIALS AND METHODS :

Chemistry

Melting points are uncorrected and were taken in open glass capillaries using a Gallenkamp melting point apparatus. The IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer in KBr pellets and band positions are recorded in wavenumbers (cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ with TMS as an internal reference on a JEOL spectrometer at 300 and 75 MHz, respectively. The mass spectra were recorded on XEVO G2S QTOF-YDA220 mass spectrometer. The elemental analyses were performed at Central Drug Research Institute, Lucknow, India. Chemicals were purchased from *Acros Organics* and used without further purification.

General procedure for synthesis of spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-

pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5a-k)

To a mixture of indole-2,3-dione (**1a-k**, 1.0 mmol), thiazolidine-4-carboxylic acid (**2**, 1.2 mmol) and 3-methyl-1-phenyl-4-thienylidene-5-pyrazolone (**3**, 1.0 mmol) and an ethyl lactate-water system (5.0 mL 80% ethyl lactate in water (v/v)) was stirred magnetically at room temperature for appropriate time (**Table 4**). After completion of the reaction , as indicated by TLC, the reaction mixture was left overnight. The formed precipitate was isolated by filtration and washed with water to furnish pure spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7-(2-thienyl)tetrahydro-1*H*-pyrrolo[1,2-c][1,3]thiazole derivatives in excellent yields, with no need of column chromatographic purification.

Spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl) tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5a).

Yield : 96%; M. P. : 230 ˚C; IR (KBr, υ_{max} , cm^{-1}) : 3385 (NH), 1740 (C=O pyrazolone), 1725 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.63 (s, 3H, CH_3), 3.00 (dd, 1H, *J* = 10.8 Hz, 5.1 Hz, H-1), 3.16 (dd, 1H, *J* =10.8 Hz, 5.1 Hz, H-1) , 3.65 (d, 1H, *J*= 8.4 Hz, H-3), 3.98 (d, 1H, *J*= 8.4 Hz, H-3), 4.01 (d, 1H, *J*= 10.2 Hz, H-7), 4.92-5.35 (m, 1H, H-7a), 7.16-7.97 (m, 12H, Ar-H), 9.93 (s, 1H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 22.75, 39.39, 51.66, 53.83, 69.69, 76.81, 77.12, 110.67, 116.24, 119.06, 128.78, 128.89, 132.05, 138.02, 140.89, 142.05, 145.23, 147.02, 148.06, 150.14, 152.12, 154.21, 162.31, 169.91, 177.97, 183.18. MS. Calcd. For $C_{26}H_{22}N_4O_2S_2$: 486.1184. Found : 486.1186. Anal. Calcd for $C_{26}H_{22}N_4O_2S_2$: C, 64.17; H, 4.56; N, 11.51%. Found : C, 64.30; H, 4.60; N, 11.59%.

1'-Methyl-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5b).

Yield : 95%; M. P.: 248 ˚C; IR (KBr, υ_{max} , cm^{-1}): 1745 (C=O pyrazolone), 1720 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.60 (s, 3H, CH_3), 3.20 (s, 3H, CH_3), 3.25 (dd, 1H, *J*= 10.8, 5.1 Hz, H-1), 3.23 (dd,1H, *J*= 10.8, 5.1 Hz, H-1), 3.64 (d, 1H, *J*= 8.4 Hz, H-3), 3.97 (d, 1H, *J*= 8.4 Hz H-3), 4.01 (d, 1H, *J*= 10.2 Hz, H-7), 4.95-5.38 (m, 1H, H-7a), 7.02-7.86 (m, 12H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 22.71, 39.43, 40.12, 51.62, 53.80, 69.65, 76.84, 77.16, 113.67, 116.60, 122.50, 124.30, 125.30, 127.60, 128.20, 128.50, 128.90, 134.30, 137.20, 143.35, 145.40, 152.80, 154.30, 157.21, 169.87, 177.95, 183.22, MS. Calcd. For $C_{27}H_{24}N_4O_2S_2$: 500.6350. Found : 500.6354. Anal. Calcd for $C_{27}H_{24}N_4O_2S_2$: C, 64.78; H, 4.83; N, 11.19%. Found : C, 64.72; H, 4.78; N, 11.22%.

1'-Ethyl-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5c).

Yield : 94%; M. P.: 194 ˚C; IR(KBr, υ_{max} , cm^{-1}): 1742 (C=O pyrazolone), 1722 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.56 (t, *J*= 7.2, 3H, CH_3), 1.62(s, 3H, CH_3), 3.70 (q, 2H, CH_2), 3.21 (dd, 1H, *J*= 10.8, 5.1 Hz, H-1), 3.25 (dd,1H, *J*= 10.8, 5.1 Hz, H-1), 3.62 (d, 1H, *J*= 8.4 Hz, H-3), 3.95 (d, 1H, *J*= 8.4 Hz H-3), 4.01 (d, 1H, *J*= 10.2 Hz, H-7), 4.95-5.38 (m, 1H, H-7a), 7.01-7.80 (m, 12H, Ar-H).; ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 12.8, 22.67, 39.40, 44.10, 51.60, 53.87, 69.60, 76.80, 77.20, 113.63, 116.65, 122.54, 124.35, 125.34, 127.65, 128.24, 128.57, 128.94, 134.33, 137.24, 143.37, 145.45, 152.84, 154.86, 157.25, 169.83, 177.90, 183.16, MS. Calcd. For $C_{28}H_{26}N_4O_2S_2$: 514.6620. Found : 514.6625. Anal. Calcd for $C_{28}H_{26}N_4O_2S_2$: C, 65.34; H, 5.09; N, 10.89%. Found : C, 65.30; H, 5.12; N, 10.92%.

1'-Benzyl-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5d).

Yield : 93%; M. P.: 237 ˚C; IR(KBr, υ_{max} , cm^{-1}): 1738 (C=O pyrazolone), 1719 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.60 (s, 3H, CH_3), 2.63 (s, 2H, CH_2), 3.02 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.18 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.64 (d, 1H, *J*=8.4 Hz, H-3), 3.96 (d, 1H, *J*=8.4 Hz, H-3), 4.05 (d, 1H, *J*= 10.2 Hz, H-7), 4.99-5.37 (m , 1H, H-7a), 7.19-7.99 (m, 17H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 22.75, 39.39, 48.50, 51.64, 53.87, 69.73, 76.86, 77.17, 110.63, 113.62, 116.60, 122.53, 124.36, 125.30, 126.80, 126.93, 127.62, 128.20, 128.55, 128.98, 134.36, 137.28, 138.86, 143.34, 144.23, 145.43, 152.82, 154.81, 156.28, 157.30, 169.95, 177.92, 183.25, MS. Calcd. For $C_{33}H_{28}N_4O_2S_2$: 576.1654. Found : 576.1657. Anal. Calcd for $C_{33}H_{28}N_4O_2S_2$: C, 68.72; H, 4.89; N, 9.71%. Found : C, 68.70; H, 4.92; N, 9.73%.

1'-Vinyl-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5e).

Yield : 86%; M. P.: 192 ˚C; IR (KBr, υ_{max} , cm^{-1}): 1743 (C=O pyrazolone), 1717 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.62 (s, 3H, CH_3), 3.01 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.19 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.64 (d, 1H, *J*=8.4 Hz, H-3), 3.97 (d, 1H, *J*=8.4 Hz, H-3), 4.01 (d, 1H, *J*= 10.2 Hz, H-7), 4.60 (d, 2H, $=CH_2$), 4.91-5.34 (m , 1H, H-7a), 7.15-7.96 (m, 12H, Ar-H), 8.01 (t, 1H, =CH).; ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 22.74, 39.40, 51.67, 53.82, 69.70, 76.80, 77.11, 90.00, 110.50, 113.63, 116.60, 122.50, 124.34, 125.35, 127.63, 128.24, 128.59, 134.33, 137.30, 138.90, 144.27, 145.40, 152.85, 156.30, 157.30, 169.90, 177.97, 183.18., MS. Calcd. For $C_{28}H_{24}N_4O_2S_2$: 512.1341. Found : 512.1344. Anal. Calcd for $C_{28}H_{24}N_4O_2S_2$: C, 65.60; H, 4.72; N, 10.93%. Found : C, 65.64; H, 4.69; N, 10.89%.

1'-Propargyl-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5f).

Yield : 85%; M. P.: 254 ˚C; IR(KBr, υ_{max} , cm^{-1}) (KBr) υ_{max} : 1742 (C=O pyrazolone), 1716 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.61 (s, 3H, CH_3), 2.30 (t, 1H, ≡CH), 3.02 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.18 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.63 (d, 1H, *J*=8.4 Hz, H-3), 3.96 (d, 1H, *J*=8.4 Hz, H-3), 4.02 (d, 1H, *J*= 10.2 Hz, H-7), 4.50 (d, 2H, NCH_2), 4.90-5.33 (m , 1H, H-7a), 7.14-7.95 (m, 12H, Ar-H).; ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 22.73, 36.79, 39.55, 51.66, 53.81, 69.71, 73.87, 76.81, 77.12, 90.12, 110.64, 113.66, 116.62, 122.53, 124.38, 125.37, 127.65, 128.28, 128.60, 134.36, 137.35, 138.93, 144.30, 145.42, 152.87, 156.32, 169.91, 177.96, 183.17, MS. Calcd. For $C_{29}H_{24}N_4O_2S_2$: 524.1341. Found : 524.1344. Anal. Calcd for $C_{29}H_{24}N_4O_2S_2$: C, 66.39; H, 4.61; N, 10.68%. Found : C, 66.43; H, 4.64; N, 10.68%.

1'-Allyl-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5g).

Yield : 88%; M. P.: 255 ˚C; IR (KBr, υ_{max} , cm^{-1}): 1740 (C=O pyrazolone), 1716 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.62 (s, 3H, CH_3), 3.01 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.15 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.64 (d, 1H, *J*=8.4 Hz, H-3), 3.97 (d, 1H, *J*=8.4 Hz, H-3), 4.02 (d, 1H, *J*= 10.2 Hz, H-7), 4.23 (dd, 2H, NCH_2), 4.91-5.34 (m , 1H, H-7a), 5.18 (m, 2H, $=CH_2$), 5.68 (m, 1H, =CH), 7.15-7.96 (m, 12H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 22.73, 39.40, 42.02, 51.65, 53.80, 69.64, 76.83, 77.15, 110.65, 113.68, 116.65,118.48,

122.55, 124.40, 125.39, 127.66, 128.30, 128.62, 130.17, 134.38, 137.36, 138.95, 144.32, 145.44, 152.89, 156.34, 169.95, 177.94, 183.14., MS. Calcd. For $C_{29}H_{26}N_4O_2S_2$: 526.1497. Found : 526.1494. Anal. Calcd for $C_{29}H_{26}N_4O_2S_2$: C, 66.13; H, 4.98; N, 10.64%. Found : C, 66.16; H, 4.94; N, 10.68%.

5'-Fluoro-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5h).

Yield : 91%; M. P.: 242 ˚C; IR (KBr, υ_{max} , cm^{-1}): 3385 (NH), 1742 (C=O pyrazolone), 1725 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.65 (s, 3H, CH_3), 3.03 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.19 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.66 (d, 1H, *J*=8.4 Hz, H-3), 3.97 (d, 1H, *J*=8.4 Hz, H-3), 4.03 (d, 1H, *J*= 10.2 Hz, H-7), 4.91-5.38 (m , 1H, H-7a), 7.14-7.95 (m, 11H, Ar-H), 10.05 (s, 1H, NH).; ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 23.75, 40.39, 50.66, 54.83, 70.69, 75.81, 77.42, 110.58, 116.26, 119.09, 128.80, 128.91, 132.09, 138.06, 140.91, 142.07, 145.25, 147.04, 148.10, 150.16, 152.15, 154.23, 162.34, 169.97, 177.87, 183.50. MS. Calcd. For $C_{26}H_{21}FN_4O_2S_2$: 504.1090. Found : 504.1094. Anal. Calcd for $C_{26}H_{21}FN_4O_2S_2$: C, 61.89; H, 4.19; N, 11.10%. Found : C, 61.88; H, 4.22; N, 11.06%.

5'-Chloro-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5i).

Yield : 88%; M. P.: 240 ˚C; IR(KBr, υ_{max} , cm^{-1}): 3387 (NH), 1741 (C=O pyrazolone), 1723 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.62 (s, 3H, CH_3), 3.05 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.17 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.65 (d, 1H, *J*=8.4 Hz, H-3), 3.96 (d, 1H, *J*=8.4 Hz, H-3), 4.05 (d, 1H, *J*= 10.2 Hz, H-7), 4.93-5.37 (m , 1H, H-7a), 7.12-7.90 (m, 11H, Ar-H), 10.06 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 23.72, 40.38, 50.67, 54.80, 70.71, 75.82, 77.40, 110.55, 116.28, 119.12, 128.83, 128.94, 132.12, 138.10, 140.09, 142.10, 145.26, 147.07, 148.13, 150.18, 152.18, 154.25, 162.36, 169.96, 177.88, 183.52. MS. Calcd. For $C_{26}H_{21}ClN_4O_2S_2$: 520.0794.Found : 520.0796. Anal. Calcd for $C_{26}H_{21}ClN_4O_2S_2$: C, 59.93; H, 4.06; N, 10.75%. Found : C, 59.90; H, 4.10; N, 10.70%.

5'-Bromo-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5j).

Yield : 87%; M. P.: 208 ˚C; IR (KBr, υ_{max} , cm^{-1}): 3385 (NH), 1742 (C=O pyrazolone), 1722 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.63 (s, 3H, CH_3), 3.01 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.14 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.64 (d, 1H, *J*=8.4 Hz, H-3), 3.97 (d, 1H, *J*=8.4 Hz, H-3), 4.02 (d, 1H, *J*= 10.2 Hz, H-7), 4.91-5.36 (m , 1H, H-7a), 7.15-7.96 (m, 11H, Ar-H), 9.97 (s, 1H, NH).^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 22.76, 39.40, 51.65, 53.82, 69.68, 76.80, 77.13, 110.66, 117.02, 120.02, 128.85, 128.96, 132.15, 138.13, 140.12, 142.14, 145.28, 147.10, 148.16, 150.20, 152.22, 154.27, 162.38, 169.90, 177.96, 183.17. MS. Calcd. For $C_{26}H_{21}BrN_4O_2S_2$: 564.0289. Found : 564.0291. Anal. Calcd for $C_{26}H_{21}BrN_4O_2S_2$: C, 55.22; H, 3.74; N, 9.91%. Found : C, 55.26; H, 3.70; N, 9.88%.

5'-Nitro-spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7(2-thienyl)tetrahydro-1*H*-pyrolo[1,2-c] [1,3] thiazole (5k).

Yield : 86%; M. P.: 210 ˚C; IR(KBr, υ_{max} , cm^{-1}): 3385 (NH), 1742 (C=O pyrazolone), 1726 (C=O oxindole); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.64 (s, 3H, CH_3), 3.07 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1), 3.19 (dd, 1H, *J*= 10.8 Hz, 5.1 Hz, H-1),

3.66 (d, 1H, *J*=8.4 Hz, H-3), 3.97 (d, 1H, *J*=8.4 Hz, H-3), 4.07 (d, 1H, *J*= 10.2 Hz, H-7), 4.94-5.39 (m , 1H, H-7a), 7.13-7.92 (m, 11H, Ar-H), 10.07 (s, 1H, NH), ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm) : 23.71, 40.37, 50.65, 54.81, 70.72, 75.81, 77.41, 110.54, 117.06, 120.05, 128.86, 128.98, 132.18, 138.16, 140.16, 142.13, 145.30, 147.13, 148.18, 150.24, 152.25, 154.29, 162.40, 169.95, 177.87, 183.53. MS. Calcd. For $C_{26}H_{21}N_5O_4S_2$: 531.1035. Found : 531.1038. Anal. Calcd for $C_{26}H_{21}N_5O_4S_2$: C, 58.74; H, 3.98; N, 13.17%. Found : C, 58.70; H, 3.94; N, 13.20%.

ANTIBACTERIAL ACTIVITY

Dispirooxindolopyrrolothiazole derivatives (**5a-k**) were evaluated for antibacterial activity. The discs diffusion method was used to screen for the antibacterial activity.

Microorganism:

The microorganisms selected, namely Gram +ive : *Bacilllus subtilis* (ATCC 6633), *Staphylococcus aureus* (MTCC 740) and Gram –ve : *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25668) were obtained from IMTECH Chandigarh, India. These cultures were grown and maintained on Nutrient Broth medium (NBM) at 27 °C for 48 h.

Agar disc diffusion assay

For this purpose DMSO was used as diluents, Mueller-Hilton (Himedia, India) agar medium was prepared and sterilized by autoclaving at 121˚C at 15 psi for 15 min. The medium was poured into sterile petri dishes under aseptic condition using laminar airflow chamber. After the solidification of medium the suspension of the test organism (10^6 cfu ml^{-1}) was swabbed onto the individual media plates using a sterile glass spreader. A sterile discs (9.0 mm diameter) impregnated with compounds was placed over media surface and the plates were incubated at 37˚C for 18-24 h under dark condition. The determination as to whether the organism is susceptible, intermediate, or resistant was made by measuring the zone of inhibition in comparison with standard antibiotic.

Minimum Inhibitory Concentration

For antibacterial assay the zone of inhibition was performed at 128.0 μg ml^{-1} concentration for all the compounds (**5a-k**) using discs diffusion method, MIC assay was performed to determine the lowest concentration of compound necessary to inhibit a test organism. MIC values were evaluated for all the compounds **(5a-k)** using broth micro-dilution method as per the standard guideline. Assay was carried out for the compounds at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 and 128.0 μg ml^{-1} concentrations. A set of tubes containing Muller Hilton broth medium with different concentrations of compounds were prepared. The tubes were inoculated with bacterial culture (10^6 cfu ml^{-1}) and incubated on a rotator shaker (180 rpm) at 37 °C for 18-24 h under dark condition. MIC values were defined as lowest concentration of compound that prevented the visible growth of bacteria after the incubation period. All the experiments were performed in three replicates.

Results and discussion

The compounds **5a-k** were screened for antibacterial activities against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* (**Table 1** and **2**). It was observed that compounds **5h** and **5i** shows better zone of inhibition and lower MIC μg ml^{-1} values than standard. Compound **5h** shows equal MIC value than standard . Other compounds shows good to moderate zone of inhibition and MIC values. Among all the compounds **5h** show good results due to fluoro substituent and can be used as a potential broad

spectrum antibacterial agent.

RESULTS AND DISCUSSION :

Chemistry

The IR spectrum of **5a** exhibits absorptions at 1725, 1740 and 3385 cm^{-1} due to C=O oxindole, C=O pyrazolone and N–H stretching vibrations, respectively. The ^1H NMR spectrum of the compound **5a** exhibits a doublet of doublets at δ 3.00 and δ 3.16 ppm (J = 10.8 and 5.1 Hz) corresponding to the 1-CH_2 group, as well as two mutually coupled doublets at δ 3.65 and δ 3.98 ppm (J = 8.4 Hz) assigned to the 3-CH_2 group. Furthermore, H-7 and H-7a give rise to a doublet at δ 4.01 ppm (J = 10.2 Hz) and a multiplet at δ 4.92-5.35 ppm, respectively. This excludes the presence of the hypothetical inverse regioisomer **6a** (Scheme 2), for which the ^1H NMR spectrum should exhibit a singlet and doublet of doublet pattern for the pyrrolizidinyl protons H-7 and H-7a, respectively. The >NH proton of the oxindole ring appears as a broad singlet at δ 9.93 ppm. The ^{13}C NMR spectrum of **5a** displays two peaks at δ 34.7 and 50.5 ppm indicating the presence of two methylene groups. The peaks at δ 53.83 and 76.81 ppm are assigned to the pyrrolizidinyl carbons C-7 and C-7a, respectively. The two spirocarbons C-6 and C-5 resonate at δ 69.69 and 77.12 ppm, respectively. In addition, two carbonyl carbons are recognized at δ 177.97 and 183.18 ppm and are assigned to the pyrazolone and oxindole carbonyl groups, respectively. Further mass spectrum shows M^+ at M/Z 486.1184 which further supported the formation of cycloadduct **5a**.

CONCLUSION :

In what seeks to conclude the compendium of the efforts substantiated unto the research, a green synthesis of a novel spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7-(2-thienyl)tetrahydro-1*H*-pyrrolo[1,2-c][1,3]thiazole derivatives **5a-k** by the reaction of indole-2,3-dione (**1a-k**, 1.0 mmol), thiazolidine-4-carboxylic acid (**2**, 1.2 mmol) and 3-methyl-1-phenyl-4-thienylidene-5-pyrazolone (**3**, 1.0 mmol) with ethyl lactate-water system was carried out in excellent yield. Synthesized compounds can be used as a potential broad spectrum antibacterial agent.

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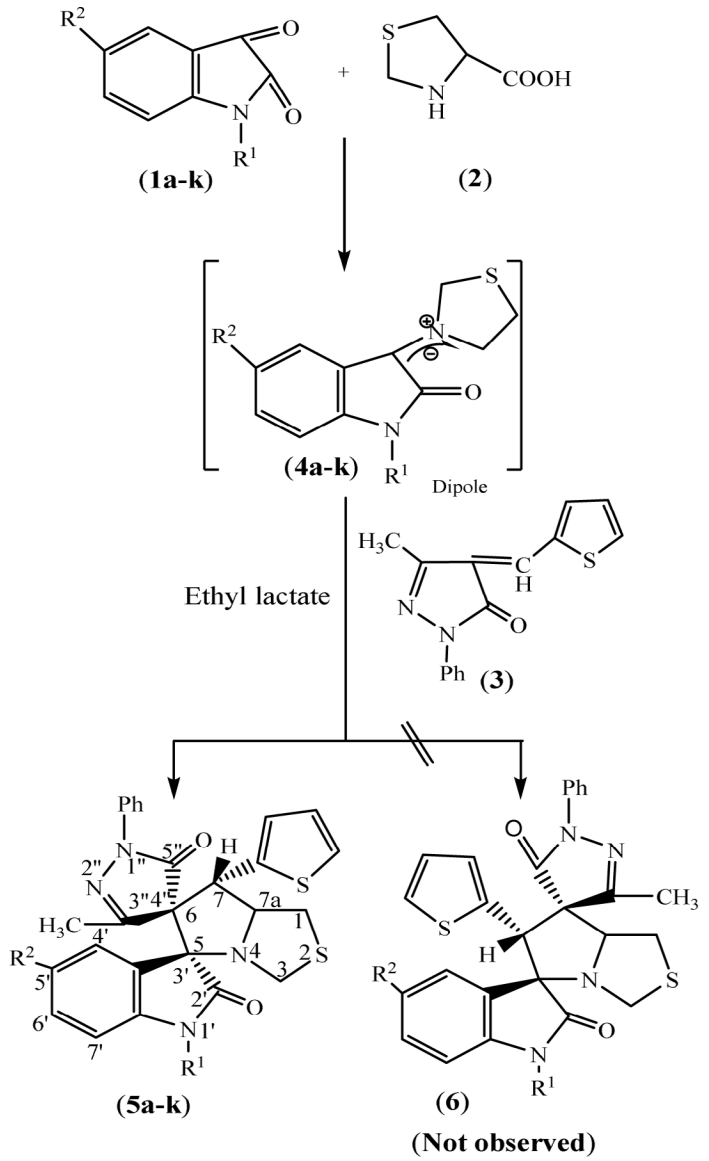
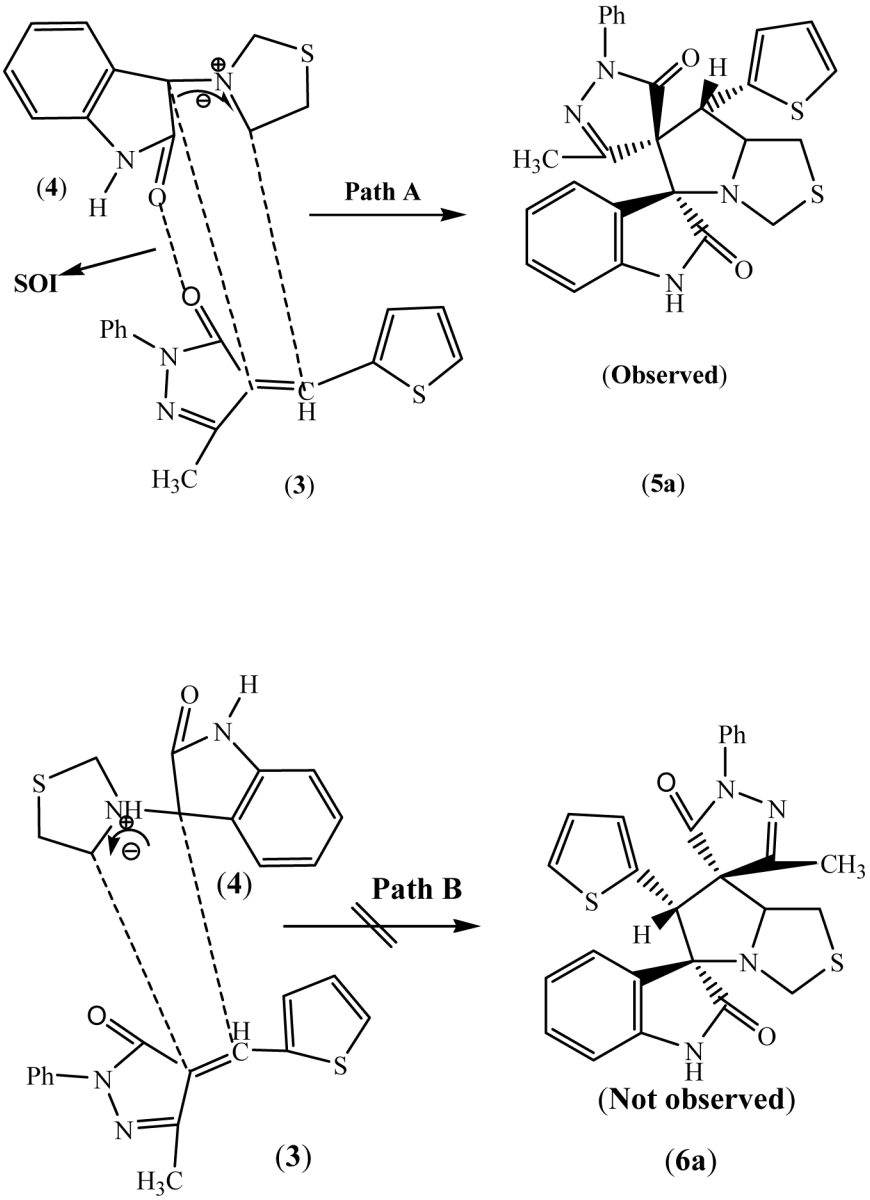
REFERENCES :

1. Y. Natori, T. Imahor, Y. Yoshimura, *Yuki Gosei Kagaku Kyokaishi*, **2016**, 74, 335-349.
2. T. Tsubogo, S. Saito, K. Seki, Y. Yamashita, S. Kobayashi, *J. Am. Chem. Soc.* **2008**, 130, 13321-13332.
3. A. Barakat, S. M. Soliman, A. M. Al-Majid, M. Ali, M. S. Islam, Y. A. M. M. Elshaier, H. A. Ghabbour, *J. Mol. Str.* **2018**, 1152, 101-114.
4. D. Konyar, C. A. Andac, E. Buyukbingol, *Lett. Drug Des. Dis.* **2018**, 15, 37-45.
5. A. Dandia, S. Khan, P. Soni, A. Indora, D. K. Mahawar, P. Pandya, C. S. Chauhan, *Bioorg. Med. Chem. Lett.* **2017**, 27, 2873-2880.
6. J. Yu, F. Shi, L.-Z. Gong, *Acc. Chem. Res.* **2011**, 44, 1156-1171.
7. P. Saraswat, G. Jeyabalan, M. Z. Hassan, M. U. Rahman, N. K. Nyola, *Synth. Commun.* **2016**, 46, 1643-1664.
8. N. Karal, O. Guzel, N. Ozsoy, S. Ozbey, A. Salman, *Eur. J. Med. Chem*. **2010**, 45, 1068-1077.
9. A. Nandakumar, P. Thirumurugan, P. T. Perumal, P. Vembu, M. N. Ponnuswamy, P. Ramesh, *Bioorg. Med. Chem. Lett.* **2010**, 20, 4252-4259.
10. B. K. S. Yeung, B. Zou, M. Rottmann, S. B. Lakshminarayana, S. -H. Ang, S. Y. Leong, J. Tan, J. Wong, S. Keller-Maerki, C. Fischli, A. Goh, E. K. Schmitt, P. Krastel, E. Francotte, K. Kuhen, D. Plouffe, K. Henson, T. Wagner, E. A. Winzeler, F. Petersen, B. Reto, V. Dartois, T. T. Diagana, T. H. J. Keller, *Med. Chem.* **2010**, 53, 5155-5164.
11. A. K. Gupta, M. Bharadwaj, A. Kumar, R. Mehrotra, *Top. Curr. Chem*. **2017**, 375, 1-25.
12. S. T. Hilton, T. C. T. Ho, G. Pljevaljcic, K. Jones, *Org. Lett*. **2000**, 2, 2639-2641.
13. E. A. Jurica, X. Wu, K. N. Williams, A. S. Hernandez, D. S. Nirschl, R. A. Rampulla, A. Mathur, M. Zhou, G. Cao, C. Xie, *J. Med. Chem.* **2017**, 60, 1417-1431.



Compound	R^1	R^2	Compound	R^1	R^2
5a	H	H	**5g**	Allyl	H
5b	Me	H	**5h**	H	F
5c	Et	H	**5i**	H	Cl
5d	Benzyl	H	**5j**	H	Br
5e	Vinyl	H	**5k**	H	NO_2
5f	Propargyl	H			

Scheme 1. Synthesis of dispiropyrrolidine derivatives **5a-k**.



SOI - Secondary orbital interaction

Scheme 2.

Table 1. Antibacterial activities of Spiro[5,3']-oxindole-spiro[6,4"]-3"-methyl-1"-phenyl-pyrazol-5"-one-7-(2-thienyl)tetrahydro-1*H*-pyrrolo[1,2-c][1,3]thiazole (5a-k).

Compounds	*B. subtilis*	*S. aureus*	*E.coli*	*P. aeruginosa*
	Zone of inhibition (mm)			
5a	28	21	23	25
5b	23	20	20	21
5c	22	20	19	20
5d	21	18	18	19
5e	24	24	21	21
5f	24	24	20	18
5g	24	24	20	19
5h	32	29	26	30
5i	30	28	25	28
5j	26	23	23	25
5k	21	19	17	18
Chloromphenicol	26	24	23	25

Table 2. MIC (µg ml^{-1}) values (5a-k).

Compound	*B. subtilis*	*S. aureus*	*E.coli*	*P. aeruginosa*
	MIC (µgml^{-1})			
5a	64	64	64	64
5b	128	128	128	128
5c	128	128	128	128
5d	128	128	128	128
5e	64	64	64	64
5f	64	64	64	64
5g	64	64	64	64
5h	16	16	16	16
5i	32	32	32	32
5j	64	64	32	32
5k	128	128	128	128
Chloromphenicol	16	16	16	16

Table 3. Optimization of reaction conditions for the synthesis of 5a[b]

Entry	Solvent	Time (h)	Yield[a] (%)
1.	Toluene	24	23
2.	Acetonitrile	20	18
3.	THF	14	27
4.	DMF	18	32
5.	Methanol	24	[c]
6.	Ethanol	24	[c]
7.	Ethyl lactate	1	96

[a]Isolated yield.

[b]All the reaction were carried out at room temperature.

[c]No reaction.

Table 4. Effect of solvent polarity on the yield for the synthesis of 5a[a].

S.No.	Solvent ratio (Ethyl lactate : Water)	Yield[b] (%)
1.	100 : 00	83
2.	80 : 20	96
3.	60 : 40	89
4.	40 : 60	82
5.	20 : 80	47

[a]All the reaction were carried out at room temperature.

[b] Isolated yield.

14. C. S. M. Pereira, V. M. T. M. Silva, A. E. Rodrigues, *Green Chem.* **2011**, 13, 2658-2671.
15. R. Prasanna, S. Purushothaman, M. Suresh, R. Raghunathan, *Tetrahedron Lett.* **2011,** 52, 792-797.
16. (a) R. Murugan, S. Anbazhagan, S. S. Narayanan, *Eur. J. Med. Chem.* **2009**, 44, 3272-3279; (b) H. Liu, G. Dou, D. J. Shi, *Comb. Chem*. **2010**, 12, 292-294; (c) R. Durga, R. S. Manian, J. Jayashankaran, S. S. Kumar, R. Raghunathan, *Tetrahedron Lett.* **2006**, 47, 829-832.
17. P. Shanmugam, B. Viswambharan, S. Madhavan, *Org. Lett*. **2007**, 9, 4095-4098.
18. P. Shanmugam, B. Viswambharan, K. K. Selva, S. Madhavan, *Tetrahedron*

Lett. **2008**, 49, 2611-2614.

19. G. Mariappan, B. P. Saha, I. Sutharson, A. Haldar, *Indian. J. Chem*. **2010**, 49B, 1671-1674.
20. J. R. Walker, K. E. Fairfull-Smith, K. Anzai, *Med. Chem. Comm*. **2011**, 2**,** 436-441.
21. G. Kuçukguze, S. Rollas, H. Erdeniz, M. Kiraz, A. Ekinci, V. A. Cevdet, *Eur. J. Med. Chem.* 2000, 35**,** 761-771.
22. (a) L. Baciu-Atudosie, A. Ghinet, D. Belei, *Tetrahedron Lett.* **2013,** 45, 6127-6130.;(b) X. Tu, H. Feng, M. Tu, *Tetrahedron Lett*. 2012, 53**,** 3169-3172.; (c) P. P. Ghosh, G. Pal, S. Paul, *Green Chem*. **2012,** 14, 2691-2698.
23. (a) S. Cuddick, *Tetrahedron,* **1995**, 51**,** 10403-10432; (b) A. Loupy, A. Petit, J. Hamelin, F. Texier-Boullet, P. Jacquault, D. Mathe, *Synthesis*, **1998**, 1213-1234.
24. A. R. Suresh Babu, R. Raghunathan, *Tetrahedron Lett*. **2007**, 48**,** 6809-6813.
25. J. F. Dubreuil, J. P. Bazureau, *Tetrahedron Lett*. **2000**, 41, 7351-7355.
26. A. Dandia, A. K. Jain, A. K. Laxkar, *Tetrahedron Lett*. **2013,** 54, 3929-3932.
27. K. Sharma, R. Jain, *J. Serb. Chem. Soc*. **2013**, 78, 165-172.
28. D. Kumar, L. K. Sharma, K. Sharma, R. Jain, J. Heterocycl. Chem. **2017**, 54, 570-574.
29. K. Sharma, L. K. Sharma, R. Jain, *J. Heterocyclic. Lett.* **2015**, 5, 383-390.
30. K. Sharma, L. K. Sharma, D. Kumar, R. Jain, *Chemistry & Biology Interface*. **2017**, 7, 124-133.
31. S. S. Bhella, A. P. S. Pannu, M. Elango, A. Kapoor, M. S. Hundal, M. P. S. Ishar, *Tetrahedron.* **2009**, 65, 5928-5935.
32. R. Murugan, S. Anbazhagan, S. S. Narayanan, *Eur. J. Med. Chem.* **2010**, 45, 3518.
33. R. T. Pardasani, P. Pardasani, V. Chaturvedi, S. K. Yadav, A. Saxena, I. Sharma, *Heteroat. Chem*. **2003**, 14, 36-41.