Heterocyclic Letters
Vol. 10| No.4|691-699|Aug-Oct |2020
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

AN ENVIRONMENTAL BENIGN APPROACH TOWARDS SYNTHESIS OF SPIRO[INDOLE-PYRAZOLONES] AND THEIR BIOLOGICAL ACTIVITY

Kanti Sharma*[a],Lokesh K. Sharma[a], Deepak Kumar[b] and RenukaJain[b]

[a]*Department ofChemistry, R.L. Saharia Govt. P.G. College, Kaladera, Jaipur-303801, India*
[b]*Department ofChemistry, University of Rajasthan, Jaipur-302004, India*
E-mail :drkanti@gmail.com

ABSTRACT
The reaction of 3-(2-oxo-2-(2-thienyl)ethylidene)indol-2-oneswith 2-hydrazinobenzothiazole in different media and solvent were investigated. The impact of substitution on indolyl nitrogen was also studied. The chemical structure of the products was proven on the basis of their spectral (IR, ^{1}H-NMR, ^{13}C-NMR, Mass) and analytical studies. All synthesized compounds were screened for antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* bacteria.

KEYWORDS:3-(2-oxo-2-(2-thienyl)ethylidene)indol-2-ones;spiro[indole-pyrazole];2,2,2-trifluoroethanol; antimicrobial activity.

INTRODUCTION :
The development of new approaches for the synthesis of novel heterocycles, substituted with unique functional group, forms the basis of an extensive research activity in synthetic organic chemistry. Isatin is the privileged scaffold in the field of medicinal chemistry which has a wide possibility for chemical modification. [i] Among the diverse heterocyclic systems, nitrogen and sulfur containing heteocycles are of particular attention in the field of biochemistry and medicinal chemistry.Chalcone and their derivatives are attracting increased attention, because of their numerous pharmacological applications and exhibit various biological activities, such as anticancer, anti-inflammatory and antihyperglycemicagents.[ii]Benzothiazole and its derivatives represent an extensive group of heterocyclic compounds, several of which have found in the medical sphere such as antibacterial, antifungal, antiinflammatory, analgesic, anti-HIV, antioxidant, anticonvulsant, antitubercular, antidiabetic, antihistaminic, antimalarialand in agriculturesphere.[iii,iv]Pyrazoles have also gained considerable importance these days in constructing novel bioactive molecules and derivatives are known to exhibit a wide range of biological properties such as analgesic, anti-inflammatory, antipyretic and molecular docking studies,[v] anti-oxidant[vi] and agrochemical herbicides.[vii]Pyrazole analogues have found use as building blocks in organic synthesis for designing pharmaceutical and agrochemicals and as bifunctional ligands for metal catalysis.[viii]Along with indoles a wide spectrum of pharmacological activities are associated with pyrazole derivatives.[ix] These observations

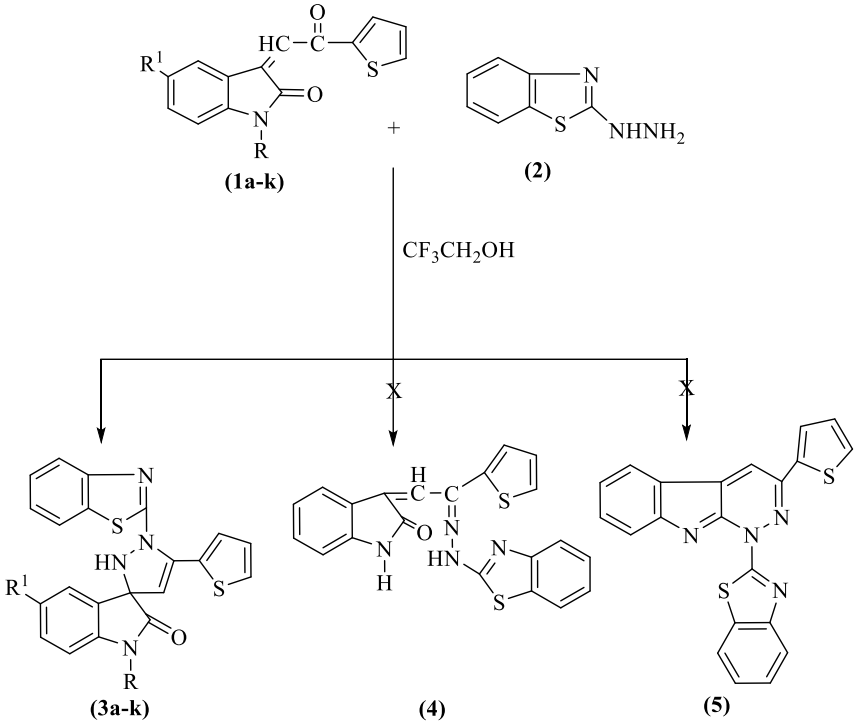
encouraged us to extend our work for the synthesis of pyrazole derivatives with benzothiazole moiety incorporating a spiroindole moiety.

In this regard, development of eco-friendly synthetic protocols for the assembly of new chemical entities is of great importance in recent years. Fluorinated alcohols have frequently been used in the last few years as alternative reaction media for a broad range of chemical transformations. 2,2,2-Trifluoroethanol is emerging as a set of new green solvent to replace the volatile organic solvents contains *viz*. low volatility, high selectivity, high ionizing power, high hydrogen bonding donor ability, negligible vapour pressure, ease of handling, potential for recyclability by simple distillation makes it a well defined polar solvent in green synthesis.[x]

To the best of our knowledge the reaction of 3-aroylmethylene-indol-2-ones with various hydrazine derivatives *viz.*, phenylhydrazines,[xi]thiosemicarbazide[xii]hydrazinobenzimidazole[xiii] and 2-hydrazinobenzothiazole[xiv]have been previously investigated. In continuation of our ongoing program, to develop convenient synthetic protocols for the synthesis of spiroheterocycles by employing green tools[xv-xix]and considering the above urgent need to provide convenient rapid route, we here in, report for the first time,the reaction of 3-(2-oxo-2-(2-thienyl)ethylidene)indol-2-ones[xx]and 2-hydrazinobenzothiazole.There is no report on the synthesis of pyrazolo-benzothiazoles incorporating a spiro-indole moiety with thienyl substituent in 2,2,2-trifluoroethanol medium previously.

Hence, for the aforementioned reasons and in a continuation of our search for better and improved bioactive heterocycles, we carried out the synthesis of novel 1'-benzothiazolo-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol]-2(1*H*)-ones by investing the reaction of 3-(2-oxo-2-(2-thienyl) ethylidene) indol-2-ones[xx] and 2-hydrazinobenzothiazole in different solvent and media (**Scheme-1**) with the assumption that the incorporation of more than one bioactive heterocyclic moiety into a single framework may result in the production of novel heterocycles with enhanced bioactivity.

The effect of substitution on the indolyl nitrogen has also been studied. Furthermore, the newly synthesized compounds were screened for their *in vitro* antimicrobial activities against pathogenic bacteria (Gram-positive and Gram-negative) and fungi.



Compound	R	R^1	Compound	R	R^1
3a	H	H	**3g**	Allyl	H
3b	Me	H	**3h**	H	F
3c	Et	H	**3i**	H	Cl
3d	Benzyl	H	**3j**	H	Br
3e	Vinyl	H	**3k**	H	NO_2
3f	Propargyl	H			

Scheme 1: Synthesis of compounds (3a-k).

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 1'-Benzothiazolo-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3a-k).

Equimolar quantities of 3-(2-oxo-2-(2-thienyl)ethylidene)indol-2(1*H*)-ones **(1a–k,** 0.01mol) and 2-hydrazinobenzothiazole **(2,** 0.01mol) in 2,2,2-trifluoroethanol (10.0 mL) was refluxed for an appropriate time (30-45 minutes).The completion of the reaction was monitored by TLC. The solid precipitate was filtered and washed with trifluoroethanol to furnished pure products **3a-k**. The trifluoroethanol was distilled off to recover for the next reaction.

1'-Benzothiazolo-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3a).Yield : 95 %; M.P. : 265-267 °C; IR (KBr, υ_{max} , cm^{-1}):3400 (indolinone-NH),3290 (pyrazolyl-NH), 1680 (>C=O of indole);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :6.25 (s, 1H, =CH– of pyrazole), 6.70–7.94 (11H, m, Ar-H), 8.99 (1H, s, -NH of pyrazole), 10.51 (1H, s, NH of indole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 76.80 (spiro-C), 101.12, 103.23, 108.85, 109.25(=CH- of pyrazole), 115.33, 118.32, 119.09, 120.67, 121.01, 122.33, 126.08, 128.33, 129.74, 131.69, 131.77, 136.89, 141.85, 159.05 (Ar-C), 166.37 (C=N), 172.79 (CO).MS. Calcd. For $C_{21}H_{14}N_4OS_2$: 402.0609 Found : 402.0612. Anal. Calcd for $C_{21}H_{14}N_4OS_2$: , C, 62.67; H, 3.51; N, 13.92%.Found :C, 62.69; H, 3.54; N, 13.95%.

1'-Benzothiazolo-1-methyl-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3b).

Yield : 94 %; M.P. : 255-257 °C; IR (KBr, υ_{max} , cm^{-1}):3300 (NH of pyrazole), 1690 (>C=O of indole);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :3.46 (s, 3H, -NCH_3), 6.22 (s, 1H, =CH- of pyrazole), 6.72–7.96 (m, 11H, Ar-H), 9.00 (s, 1H, NH of pyrazole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 32.3(-NCH_3), 76.77 (spiro-C), 101.15, 103.25, 108.88, 109.28(=CH- of pyrazole), 109.30, 115.35, 118.40, 119.25, 120.70, 121.04, 122.36, 126.10, 128.36, 129.78, 131.70, 136.90, 141.88, 159.09 (Ar-C), 166.41(C=N), 172.82(CO).MS. Calcd. For $C_{22}H_{16}N_4OS_2$: 416.5170 Found : 416.5173. Anal. Calcd for $C_{22}H_{16}N_4OS_2$: , C, 63.44; H, 3.87; N, 13.45 %. Found : C, 63.46; H, 3.89; N, 13.47 %.

1'-Benzothiazolo-1-ethyl-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3c).

Yield : 93 %; M.P. : 260-262˚C; IR (KBr, υ_{max} , cm^{-1}):3310 (NH of pyrazole), 1685(>C=O of indole);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :1.40 (t, 3H, -NCH_2**CH_3**), 3.50 (q, 2H, -N**CH_2**CH_3), 6.23 (s, 1H, =CH of pyrazole), 6.70–7.95 (m, 11H, Ar-H), 9.01 (s, 1H, NH of pyrazole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 12.50(N-CH_2-**CH_3**), 44.20(N-**CH_2**-CH_3),75.80(spiro-C), 101.15, 103.28, 108.25, 108.90,109.33, 115.38, 118.44, 119.28, 120.73, 121.08, 122.38, 126.13, 128.38, 129.80,131.74, 136.93, 141.90, 160.05 (Ar-C), 165.37(C=N), 173.79(CO).MS. Calcd. For $C_{23}H_{18}N_4OS_2$: 430.5440 Found : 430.5442. Anal. Calcd for $C_{23}H_{18}N_4OS_2$: C, 64.16; H, 4.21; N, 13.01 % Found : C, 64.18; H, 4.23; N, 13.03 %.

1'-Benzothiazolo-1-benzyl-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3d).

Yield : 90 %; M.P. : 270-272 ˚C; IR (KBr, υ_{max} , cm^{-1}):3315 (NH of pyrazole), 1690(>C=O of indole);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :4.00 (s, 2H, N-**CH_2**-), 6.22 (s, 1H, =CH- of pyrazole), 6.72–7.96 (m, 16H, Ar-H), 9.00 (s, 1H, NH of pyrazole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 49.00(N-CH_2-), 76.80(spiro-C), 101.16, 103.30, 108.20, 108.89, 109.25(=CH- of pyrazole), 109.35, 115.40, 118.43, 119.26, 120.72, 121.10, 122.36, 126.12, 128.35, 129.82, 131.72, 136.92, 138.20, 141.92, 142.31, 148.44, 160.07,162.33, 165.35 (Ar-C), 166.37 (C=N), 172.79 (CO).MS. Calcd. For $C_{28}H_{20}N_4OS_2$: 492.6150 Found : 492.6153. Anal. Calcd for $C_{28}H_{20}N_4OS_2$: C, 68.27; H, 4.09; N, 11.37 %. Found : C, 68.25; H, 4.11; N, 11.39 %.

1'-Benzothiazolo-1-vinyl-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3e).

Yield : 89 %; M.P. : 269-271˚C; IR (KBr, υ_{max} , cm^{-1}):3295 (NH of pyrazole), 1680 (>C=O of indole);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :4.70 (d, 2H, N-CH=**CH_2**), 6.23 (s, 1H, =CH- of pyrazole), 6.70–7.94 (m, 11H, Ar-H), 7.80 (t, 1H, N-**CH**=CH_2), 9.01 (s, 1H, NH pyrazole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 76.80(spiro-C), 100.50(N-CH=**CH_2**), 101.14, 103.32, 108.24, 109.23 (=CH- of pyrazole), 115.42, 118.40, 119.23, 120.70, 121.07, 122.37, 125.50(N-**CH**=CH_2), 126.14, 128.34, 131.70, 136.90, 138.23, 141.93, 142.33, 159.10 (Ar-C), 166.37 (C=N), 172.79 (CO), MS. Calcd. For $C_{23}H_{16}N_4OS_2$: 428.0766 Found : 428.0768. Anal. Calcd for $C_{23}H_{16}N_4OS_2$: C, 64.46; H, 3.76; N, 13.07 %. Found : C, 64.48; H, 3.74; N, 13.05 %.

1'-Benzothiazolo-1-propargyl-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3f).

Yield : 89 %; M.P. : 285-287 ˚C; IR (KBr, υ_{max} , cm^{-1}):3290 (NH of pyrazole), 1685(>C=O of indole);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :2.35 (s, 1H, N-CH_2-C≡**CH**), 4.30 (s, 2H, -N-**CH_2**-C≡CH), 6.25 (s, 1H, =CH- of pyrazole), 6.72–7.95 (m, 11H, Ar-H), 9.02 (s, 1H, NH pyrazole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 35.50 (N-**CH_2**-), 73.00 (N-CH_2-C≡**CH**), 75.10 (N-CH_2-**C**≡CH), 76.82 (spiro-C), 101.13, 103.31, 108.25, 109.24 (=CH- of pyrazole), 115.40, 118.42, 119.25, 120.72, 121.10, 122.39, 125.51, 126.16, 128.39, 131.72, 136.93, 138.25,141.95, 142.35, 159.15 (Ar-C), 166.37 (C=N), 172.79 (CO);MS. Calcd. For $C_{24}H_{16}N_4OS_2$: 440.5390 Found : 440.5393. Anal. Calcd for $C_{24}H_{16}N_4OS_2$: C, 65.43; H, 3.66; N, 12.72 %. Found : C, 65.47; H, 3.69; N, 12.75 %.

1-Allyl-1'-benzothiazolo-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3g).

Yield : 88 %; M.P. : 290-292˚C; IR (KBr, υ_{max} , cm^{-1}):3300 (NH of pyrazole), 1690 (>C=O of indole);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :4.20 (d, 2H, N-**CH_2**), 5.20 (d, 2H, -N-CH_2-CH=**CH_2**), 5.65 (m, 1H, -N-CH_2-**CH**=CH_2), 6.23 (s, 1H, =CH- of pyrazole), 6.73–7.96 (m, 11H, Ar-H), 9.03 (s, 1H, NH pyrazole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) :

43.50 (N-**C**$\mathbf{H_2}$-), 76.85 (spiro-C*)*, 101.15, 103.33, 108.27, 109.27 (=CH- of pyrazole), 115.43, 117.50 (-N-CH_2-CH=**C**$\mathbf{H_2}$), 118.40, 119.23, 120.70, 121.08, 122.41, 125.53, 126.18, 128.40, 131.30 (-N-CH_2-**CH**=CH_2), 133.73, 136.95, 138.23, 141.92, 159.15 (Ar-C), 166.37 (C=N), 172.79 (CO), MS. Calcd. For $C_{24}H_{18}N_4OS_2$: 442.5550 Found : 442.5553. Anal. Calcd for $C_{24}H_{18}N_4OS_2$: C, 65.14; H, 4.10; N, 12.66 %. Found : C, 65.17; H, 4.13; N, 12.64 %.

1'-Benzothiazolo-5-fluoro-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3h).

Yield : 90 %; M.P. : 190-192°C; IR (KBr, υ_{max} , cm^{-1}):3410 (NH of indole), 3295 (NH of pyrazole), 1685 (CO);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :6.23 (s, 1H, =CH- of pyrazole), 6.72–7.95 (m, 10H, Ar-H), 9.00 (s, 1H, NH pyrazole), 10.53 (s, 1H, NH indole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 76.82 (spiro-C), 101.12, 103.30, 108.22, 109.24 (=CH- of pyrazole) , 115.40, 118.42, 119.20, 120.72, 122.39, 125.52, 126.16, 128.36, 131.73, 136.92, 138.20, 141.90, 142.90, 159.08 (Ar-C), 166.35 (C=N), 172.80 (CO), MS. Calcd. For $C_{21}H_{13}FN_4OS_2$: 420.4804 Found : 420.4800. Anal. Calcd for $C_{21}H_{13}FN_4OS_2$: C, 59.99; H, 3.12; N, 13.32 %. Found : C, 59.96; H, 3.10; N, 13.30 %.

1'-Benzothiazolo-5-chloro-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3i).

Yield : 89 %; M.P. : 196-198°C; IR (KBr, υ_{max} , cm^{-1}) : 3415 (NH of indole), 3292 (NH of pyrazole), 1686 (CO);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) : 6.22 (s, 1H, =CH- of pyrazole), 6.71–7.96 (m, 10H, Ar-H), 9.03 (s, 1H, NH pyrazole), 10.55 (s, 1H, NH indole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 76.80 (spiro-C), 101.10, 103.33, 108.24, 109.22, 115.43, 118.45, 119.23 (=CH- of pyrazole), 120.70, 122.36, 125.50, 126.15, 128.33, 131.71, 136.94, 138.23, 141.93, 142.88, 159.10 (Ar-C), 166.38 (C=N), 172.83 (CO), MS. Calcd. For $C_{21}H_{13}ClN_4OS_2$: 436.9320 Found : 436.9323. Anal. Calcd for $C_{21}H_{13}ClN_4OS_2$: C, 57.73; H, 3.00; N, 12.82 %. Found : C, 57.75; H, 3.02; N, 12.85 %.

1'-Benzothiazolo-5-bromo-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3j).

Yield : 88 %; M.P. : 201-203°C; IR (KBr, υ_{max} , cm^{-1}):3413 (NH of indole), 3295 (NH of pyrazole), 1680 (CO); ^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) :6.24 (s, 1H, =CH- of pyrazole), 6.73–7.97 (m, 10H, Ar-H), 9.03 (s, 1H, NH pyrazole), 10.54 (s, 1H, NH indole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 76.82 (spiro-C*)*, 101.13, 103.36, 108.25, 109.23 (=CH- of pyrazole), 120.72, 122.35, 125.52, 126.16, 128.34, 131.72, 136.95, 138.25, 141.95, 142.89, 159.13 (Ar-C), 166.34 (C=N), 172.84 (CO), MS. Calcd. For $C_{21}H_{13}BrN_4OS_2$: 481.3860 Found : 481.3862. Anal. Calcd for $C_{21}H_{13}BrN_4OS_2$: C, 52.40; H, 2.72; N, 11.64 %. Found : C, 52.42; H, 2.74; N, 11.66 %.

1'-Benzothiazolo-5-nitro-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol] 2(1*H*)-ones (3k).

Yield : 87 %; M.P. : 210-212°C; IR (KBr, υ_{max} , cm^{-1}):3415 (NH of indole), 3290 (NH of pyrazole), 1685 (CO);^{1}H-NMR (δ, $CDCl_3$+DMSO-d_6, 300.15 MHz) : 6.25 (s, 1H, =CH- of pyrazole), 6.76–7.98 (m, 10H, Ar-H), 9.02 (s, 1H, NH pyrazole), 10.53 (s, 1H, NH indole); ^{13}C-NMR (δ, $CDCl_3$+DMSO-d_6, 75.47 MHz) : 76.85 (spiro-C*)*, 101.15, 103.35, 108.24, 109.24 (=CH- of pyrazole), 115.40, 118.42, 119.25, 120.70, 122.32, 125.50, 126.15, 128.32, 131.70, 136.92, 138.24, 141.93, 142.90, 159.15 (Ar-C), 166.35 (C=N), 172.85 (CO), MS. Calcd. For $C_{21}H_{13}N_5O_3S_2$: 447.4870 Found : 447.4873. Anal. Calcd for $C_{21}H_{13}N_5O_3S_2$: C, 56.37; H, 2.93; N, 15.65 %. Found : C, 56.35; H, 2.95; N, 15.66 %.

ANTIMICROBIALACTIVITY :

Materials and method

Source of test organisms

Bacteria : Pure cultures of test bacteria, namelyGram +ve : *Bacilllussubtilis*(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 and Mueller-Hinton agar culture plates were prepared for the test [Hi Media Laboratories Pvt. Ltd; Mumbai, India].

Fungi : Pure cultures of test fungi namely *Aspergillusflavus* (ATCC 16870), *A. niger* (ATCC 322), *Candida albicans* (ATCC 4718) and *Penicilliumchrysogenum* (ATCC 5476) were obtained from IARI, New Delhi, India and cultured on Sabouraud Dextrose Broth (SDB) at 37 °C for 48 h and Sabouraud dextrose agar [SDA] culture plates was prepared for the test [Hi Media Laboratories Pvt. Ltd; Mumbai, India].

Cultures of test microbes

Stock cultures were maintained at 4 °C on slops of nutrient agar. Active cultures for experiments were prepared by transferring an inoculating loop of cultures from the stock cultures to test tubes of NB medium for bacteria and SD Broth for fungi which were incubated without agitation of 24 h at 37 °C and 25 °C, respectively.

Antibacterial and Antifungal Assay

For both antibacterial and antifungal assay, agar well diffusion method was adopted, because of its re-productivity and precision. The plates were prepared by pouring 15.0 mL of molten media into sterile petri plates. The plates were allowed to solidify for 5 min. Thereafter, 40 µl (in case of bacteria) and 80 µl (in case of fungi) suspension was spread uniformly with the help of a sterile glass spreader and dried for 5 min. The zone of inhibition were measured around sterilized dried well, which were containing 4 mg/well of the test compounds and control (streptomycin for bacteria and Itraconazole for fungi) as reference drugs separately. Such treated well were air-dried at room temperature, to remove any residual solvent which might interfere with the determination, sterilized and inoculated. Before incubation, these plates were placed at low temperature for 1 h so as to allow maximum diffusion of the compound from the test discs into the agar plate and later, these were kept for incubation at 37 °C for 24 h in case of bacteria and 36 h for fungi. At the end of incubation, inhibition zones formed around the well were measured with a transparent scale in millimeters. The experiments were performed in triplicate and the mean values of the diameter of inhibition were taken.

Table 1. Antibacterial activity of 1'-benzothiazolo-5'-thienyl-2',4'-dihydrospiro [indole-3,3'-pyrazol]-2(1*H*)-ones (3a-k).

Compounds	***B. subtilis***		***S. aureus***		***E. coli***		***P.aeruginosa***	
	IZ	**AI**	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**
3a	23.33	1.02	23.66	1.09	22.66	1.03	20.66	0.95
3b	20.33	0.89	19.00	0.87	20.00	0.90	19.66	0.90
3c	19.66	0.86	20.33	0.93	22.00	1.00	18.33	0.84
3d	17.00	0.75	16.66	0.76	18.66	0.84	17.00	0.78
3e	21.33	0.94	20.00	0.92	19.66	0.89	18.00	0.83
3f	20.33	0.89	21.33	0.98	19.00	0.86	17.33	0.80
3g	22.66	1.00	21.66	1.00	21.33	0.96	20.66	0.95
3h	28.66	1.26	29.33	1.35	30.00	1.36	22.66	1.04
3i	25.00	1.10	26.33	1.21	24.66	1.12	21.33	0.98
3j	23.33	1.02	24.00	1.10	23.66	1.07	20.00	0.92

3k	18.00	0.79	19.00	0.87	17.00	0.77	16.66	0.77

IZ = Inhibition zone (in mm) including the diameter of well (6 mm, mean);
AI = Activity index = Inhibition zone of sample/Inhibition zone of standard;
Standard : streptomycin.

Table 2. Antifungal activity of 1'-benzothiazolo-5'-thienyl-2',4'-dihydrospiro [indole-3 ,3'-pyrazol]-2(1*H*)-ones (3a-k).

Compd.	***A. flavus***		***A. niger***		***C. albicans***		***P. chrysogenum***	
	IZ	**AI**	**IZ**	**AI**	**IZ**	**AI**	**IZ**	**AI**
3a	25.66	1.04	25.33	1.04	25.66	1.08	24.00	1.02
3b	21.66	0.87	20.66	0.87	19.66	0.83	19.00	0.81
3c	20.33	0.82	20.00	0.82	18.33	0.77	19.33	0.83
3d	21.66	0.87	20.33	0.87	19.33	0.81	18.66	0.79
3e	23.66	0.96	24.33	0.96	22.33	0.94	22.66	0.97
3f	21.66	0.88	20.33	0.88	18.00	0.76	19.66	0.84
3g	23.00	0.87	20.33	0.93	17.66	0.75	20.00	0.85
3h	29.33	0.93	28.66	1.18	26.66	1.12	27.00	1.15
3i	26.66	1.18	26.66	1.08	25.33	1.07	25.66	1.09
3j	25.00	1.01	24.66	1.01	24.33	1.02	24.00	1.02
3k	20.66	0.83	20.33	0.83	19.33	0.81	17.66	0.75

IZ = Inhibition zone (in mm) including the diameter of well (6 mm);
AI = Activity index = Inhibition zone of sample/Inhibition zone of standard;
Standard :Itraconazole.

Results and discussion

The synthesized 1'-benzothiazolo-5'-thienyl-2',4'-dihydrospiro [indole-3,3'-pyrazol]-2(1*H*)-one derivatives (**3a-k**) have been subjected to in vitro antimicrobial activity against various plant and human pathogenic bacteria and fungi and results are summarized in the **Tables 1** and **2** for antibacterial and antifungal, respectively.

The antibacterial screening data revealed that all the tested compounds (**3a-k**) showed moderate to significant bacterial inhibition (**Table 1**). Compounds 3a, 3h, 3i and 3j were more potent even than the standard, (streptomycin) against *B. subtilis*, *S. aureus*and*E. coli* bacteria. compounds 3a, 3h, 3i and 3j showed better results. Compound 3h showed significant bacterial inhibition with *P.aeruginosa* even then standard. Compound 3c is equally potent with *E. coli* and compound 3g is equally potent with standard with *B. subtilis* and *S. aureus*.

The antifungal screening data of the compounds (**3a-k**) also revealed promising to moderate activity against Aspergillus species (**Table 2**). Compounds 3a, 3h, 3i and 3j exhibited significant inhibitory activity against all fungi even better comparison to Itraconazole (standard). Compound 3e is equally potent with *A. niger*.

Among all compounds 3h showed the good zone of inhibition in comparison with standard bacteria as well as fungi. This is attributed due to fluoro substituent in benzene ring of indole moiety.

RESULTS AND DISCUSSION :

Chemistry

The present investigation describes the synthesis of some new functionalized spiro[indole-pyrazoles], 1'-benzothiazolo-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol]-2(1*H*)-ones**(3a-k)**by the reaction of 3-(2-oxo-2-(2-thienyl)ethylidene)indol-2-ones (**1a-k**) and 2-hydrazino-benzothiazole **(2)** in 2,2,2-trifluoroethanol as a recyclable as well as green solvent in excellent yields **(Scheme 1)**. All the synthesized compounds have been fully characterized by their analytical as well as spectral studies.

To optimize the reaction conditions for the synthesis of1'-benzothiazolo-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol]-2(1*H*)-ones**(3)**, we first investigated the reaction of 3-(2-oxo-2-(2-thienyl)ethylidene)indol-2-ones (**1a**)and 2-hydrazinobenzothiazole **(2)** as a model reaction in various solvents (**Scheme 1** and **Table 3**).

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

S.No.	Solvent	Temperature(˚C)	Time (h)	Yield[b](%)
1.	Methanol	Reflux	7	70
2.	Ethanol	Reflux	8	75
3.	Acetonitrile	Reflux	6	65
4.	THF	Reflux	7	66
5.	1,4-Dioxane	Reflux	6	69
6.	2,2,2-Trifluoroethanol	Reflux	45 min	95

[a] Reaction conditions: 1 mmol of **1a** and **2**.

[b] Isolated yields.

Theoretically this reaction offers three different possibilities (**Scheme 1**), *viz.*, formation of 1'-benzothiazole-5'-thienyl-2',4'- dihydrospiro[indole-3,3'-pyrazole]-2(1*H*)-ones (**3a-k**), 3-thienyl-(2-benzothiazolyl-hydrazone) methylene-indol-2(1*H*)-ones (**4**) and 3-benzothiazolyl-1-thienyl-9*H*-pyridazino[3,4-b]indole (**5**). Since we are using CF_3CH_2OH as a self catalytic polar green solvent therefore we got only 1'-benzothiazole-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazole] 2(1*H*) ones (**3a-k**) as a product neither **4** nor **5**.

Exploration for the formation of **3a-k** is based on spectral and analytical analysis. Compound **3a** in the IR spectrum shows peaks at 3400 cm^{-1} (indolinone-NH), 3290 cm^{-1} (pyrazolyl-NH) and 1680 due to >C=O of indole are observed. In the ^{1}H NMR spectrum peaks appeared at δ 6.25 ppm for =CH of pyrazolyl, 8.99 ppm for NH of pyrazolyl and 10.51 ppm for indolyl NH. ^{13}C NMR of **3a** exhibited peaks at δ 76.80 (spiro-C), 109.25 (=CH), 101.12-159.05 (18 Ar-C), 166.37 (C=N ofthiazolyl) and 172.79 (CO of indolinone). Further, mass spectrum also corresponded to the molecular mass M^+ appears at m/z 402.06.

The formation of hydrazone derivatives (**4**) is ruled out due to absence of hydrazono>NH peak at downfield 10-11 ppm. Also the formation of pyradizino derivatives (**5**) is ruled out as there is a >NH peak in all the compounds. If it is formed then there should not be a peak for >NH and =CH should be merged with aromatic proton. But there is a clear peak for pyrazolyl =CH around δ 6.25 ppm. All these observation indicated that reaction of 3-(2-oxo-2-(2-thienyl)ethylidene)indol-2-ones with 2-hydrazinobenzothiazole gives exclusively the compounds **3a-k**.

CONCLUSION :

The present investigation describes the synthesis of some new functionalized spiro[indole-pyrazoles],1'-benzothiazolo-5'-thienyl-2',4'-dihydrospiro[indole-3,3'-pyrazol]-2(1*H*)-ones**(3a-k)**by the reaction of 3-(2-oxo-2-(2-thienyl)ethylidene)indol-2-ones (**1a-k**) and 2-hydrazino-benzothiazole **(2)** in 2,2,2-trifluoroethanol as a recyclable as well as green solvent

in excellent yields for the first time. Further, the synthesized compounds (**3a-k**) were screened for their *in vitro* antimicrobial activity and the data revealed that some compounds were even more active than the standard and could act as potential antimicrobial agents.

ACKNOWLEDGEMENTS:
We are thankful to CDRI, Lucknow, India for elemental and spectral analysis.

REFERENCES :

i. N. V. Huan, L. V. Cuong, J. Kim, Y. G. Kim, S. B. Han, N. H. Nam,Bioorg. Chem.71, 160 (2017).
ii. M. V. P. de Mello, B. A. Abrahim-Vieira , T. F. S. Domingos, J. B. de Jesus, A. C. C. de Sousa, C. R. Rodrigues, A. M. Teles de Souza, Eur. J. Med. Chem. 150, 920 (2018),
iii. A. Srivastava, A. P. Mishra, S. Chandra, A. Bajpai, Int.J.Pharm.Sci.Res. 10, 1553 (2019).
iv. A. Shaista, A. Parle, Int. J. Pharm. Sci. Res.8, 4909**(**2017).
v. M. F. El Shehrya,, E. F. Ewiesb, E. M. Zayed, Russ. J. Gen. Chem.89, 492 (2019).
vi. A. Ansari, A. Ali, M. Asif, New J. Chem. 41, 16(2017).
vii. K. Karrouchi, S. Radi, Y. Ramli, J. Taoufik,Y. N. Mabkhot, F. A. Al-aizari, M. Ansar, Molecules.23, 134 (2018).
viii. A. K. Kumar, P. Jayaroopa, Int. J. Pharm Tech. Res., 5,1473 (2013).
ix. R. Jain, K. Sharma, D. Kumar, J. Indian Chem. Soc.91, 1517 (2014).
x. A. Dandia, R. Singh, J. Joshi, S. Kumari, J. Fluorine chem. 156, 283 (2013).
xi. K.C. Joshi, R. Jain, S. Garg, Pharmazie, 40, 21 (1984).
xii. K. C. Joshi, R. Jain, K. Sharma, Heterocycles,31, 473 (1990).
xiii. K. Sharma, R. Jain, K. C.Joshi, Indian J. Heterocycl. Chem.1, 189 (1992).
xiv. R. Jain, K. Sharma, D. Kumar, J. Chem. Sci., 125, 299 (2013).
xv. K. Sharma, R. Jain, J. Serb. Chem. Soc. 78, 165 (2013).
xvi. D. Kumar, L. K. Sharma, K. Sharma, R. Jain, J. Heterocycl. Chem. 54, 570 (2017).
xvii. K. Sharma, L. K. Sharma, R. Jain, J. Heterocyclic. Lett. 5, 383 (2015).
xviii. K. Sharma, L. K. Sharma, D. Kumar, R. Jain, Chemistry & Biology Interface. 7, 124 (2017).
xix. K. Sharma, L. K. Sharma, M. Jain, R. Jain, Chemistry & Biology Interface. 8, 138 (2018).
xx. K. Sharma, L. K. Sharma, R. Jain, Heterocyclic Lett. 5, 383 (2015).

Received on September 3, 2020.