Regio and diastereoselective synthesis of dispiroindole-3,2'-pyrrolidine/pyrrolizines /pyrrolidines-3',3''-indole derivatives through1,3-dipolar addition and their in vitro activity studies against Mycobacterium tuberculosis H37Rv (MTB), multi-drug resistant M. tuberculosis (MDR-TB) and Mycobacterium smegmatis(MC2)

[1]HariKrishnan.P.S.

[1]Associate Professor
[1]Department of Chemistry,
[1]The Madura College, Maduurai, India
[1]harikimku@gmail.com

Abstract— An efficient synthesis of highly medicinally important dispiroindole-3,2'-pyrrolidine/pyrrolizines /pyrrolidines-3',3''-indole derivativeswas achieved by the 1,3-dipolar cycloaddition of azomethineylide generated insitu by the reaction between isatin and proline/L-Phenyl gycine / Sarcosine to (3-Z)-6-chloro-3-(benzylidene-5-(2-chloroethyl-1,3-dihydro-2H-indol-2-one. These dipolaraphiles upon cycloaddition with azomethineylides affords stereoselectively novel dispiroindole-3,2'-pyrrolidine/pyrrolizines /pyrrolidines-3',3''-indole derivatives in high yield, almost quantitatively. These compounds were characterized by IR, NMR spectroscopy. It is planned to screen these compoundsfor their in vitro activity against Mycobacterium tuberculosis H37Rv (MTB), multi-drug resistant M. tuberculosis (MDR-TB) and Mycobacterium smegmatis (MC2) using agar dilution method.

Key words— 1,3-dipolar cycloaddition, azomethine ylide, pyrrolidine.

GENERAL ASPECTS OF DISPIROHETEROCYCLES AND THEIR IMPORTANCE

One of the goals of synthetic organic chemists is the clear stereo defined synthesis of highly complicated structures. Splendidly the very most important great task is the regio- and stereo controlled generation of spiro compounds. Bicyclic organic compounds, comprise rings connected through just one atom are known as Spiro compounds[1], have several unique characteristics, such as 3D structural properties, related to their inherent rigidity, notably, these compounds display a broad range of biological activities.

Nowadays, spiro compounds are attracting increasing attention as scaffolds in modern drug discovery. Spiro compounds are present in numerous number of natural products such as the angiotensin antagonist irbesartan [2] **1**, analgesic morphine [3] **2**, β-vetivone**3**, the antibiotic monensin**4**, (-)-acorenone [4] **5**, opioid receptor agonist oxycodone [5] **6**, Shizuka-acordienol**7**, rosmadial**8**, (-)-cannabispirenone A **9**, trigolute B **10** used in thai medicine, marine alkaloid (+)-dicorhabdin A **11**, and alkaloid citrinalin A **12**.

Taking advantage of their rigidity and extremely precise 3D structure, spiro compounds have emerged as one of the most attractive ligand and catalyst motifs in asymmetric synthesis as well as catalysis. Mainly, these scaffolds are derived from Spinol**13** and show excellenentstereochemical recognition. Some examples such as spiro(oxozoline) **14**, Spinol derived phosphoric acid 15 and the diphosphinespiro P**16** are illustrated in **Fig 1**

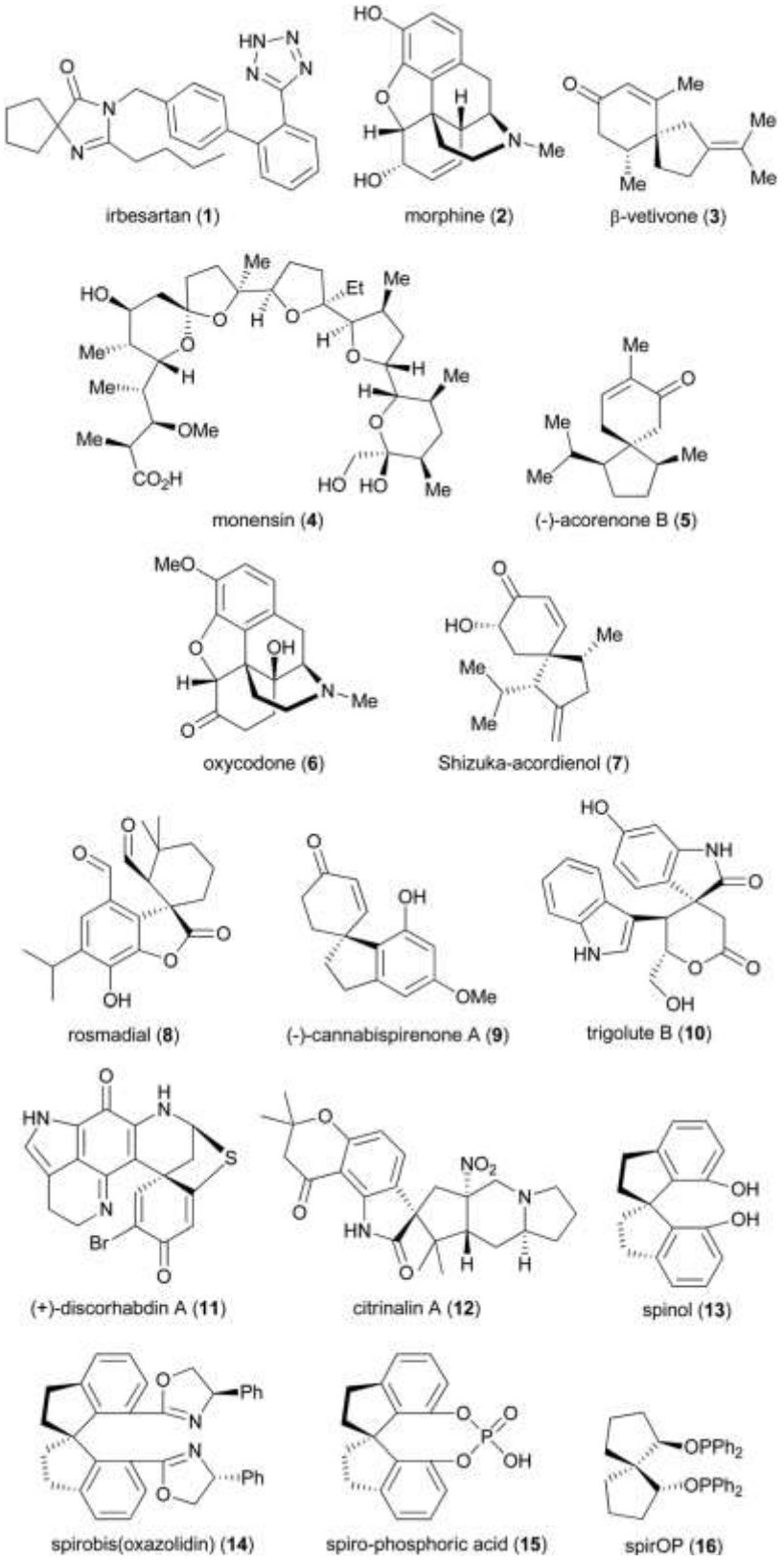
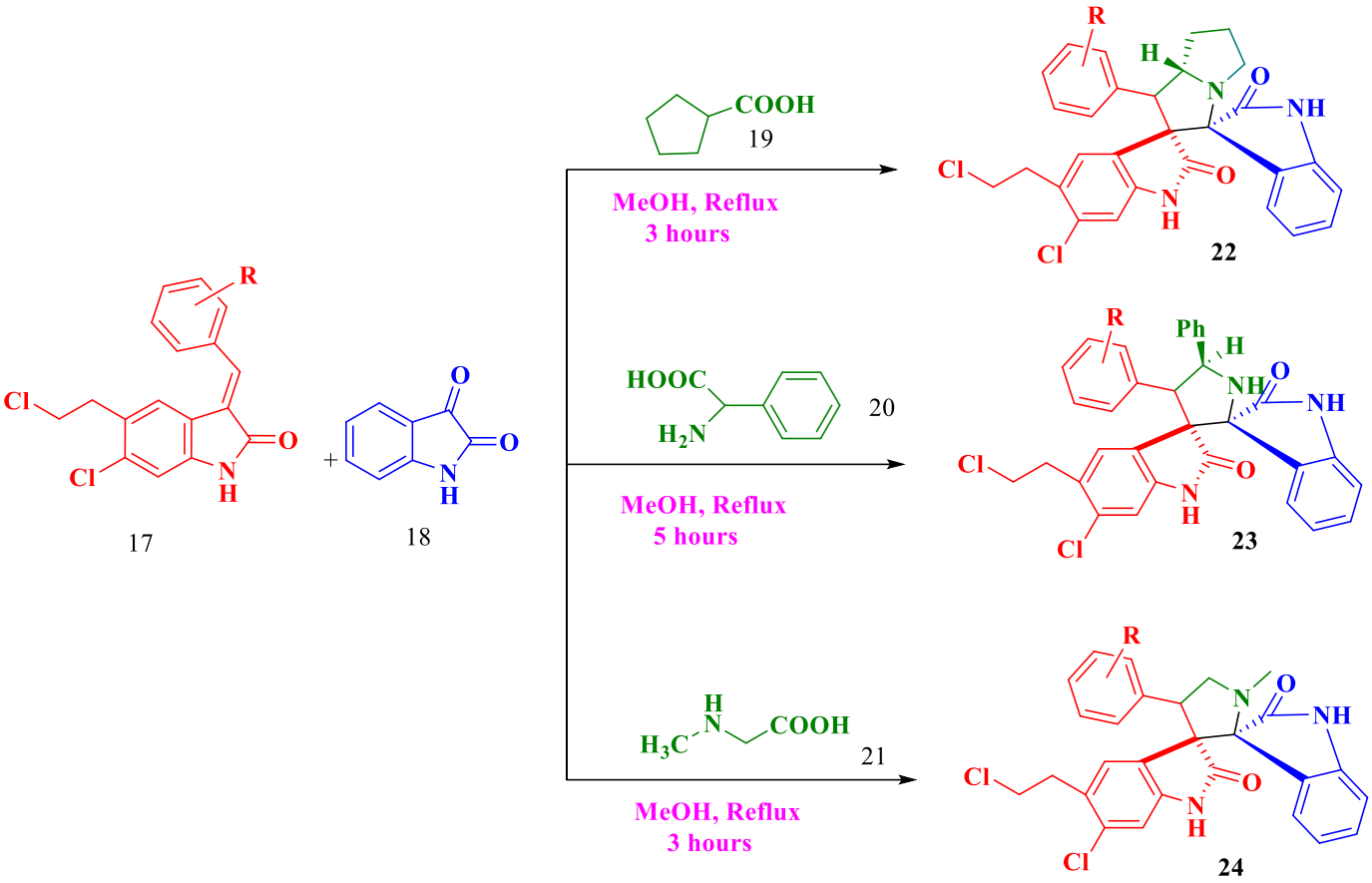


Fig.1 Examples of Compounds containing Spiro cyclic ring.

RESULTS & DISCUSSION

In the present investigation, 1,3 d dipolar cycloaddition of azomethineylide generated insitu by the reaction between isatin**18** and proline**19/** L-Phenyl gycine**20**/ Sarcosine**21** to (3-Z)-6-chloro-3-(benzylidene-5-(2-chloroethyl-1,3-dihydro-2H-indol-2-one **17** results in highly regio and stereo selective formation of highly medicinally important dispirocompound **22, 23 & 24** in good yield



Scheme-1

Initially, the reaction of 6-chloro-5-(2-cholroethyl)-1,3-dihydro-2H-indol-2-onewith various aldehydes in the presence of Potassium carbonate using methanol as the solvent results in the monobenzylidine compound **17** in good yield. This undergoes 1,3dipolar cycloaddition to give the diastereoisomer**22, 23 & 24** in good yield (**Table 1**).

Table 1.Synthesis of dispirocmopund 22, 23& 24

Entry	Comp.	Ar	Yield (%)		
			22	**23**	**24**
1	**A**	*p*-$OCH_3C_6H_4$	92	88	85
2	**B**	C_6H_5	82	85	86
3	**C**	*p*-$CH_3C_6H_4$	95	93	92
4	**D**	*m*-BrC_6H_4	78	80	85
5	**E**	*o*-ClC_6H_4	82	75	98
6	**F**	*p*-BrC_6H_4	84	85	94
7	**G**	*p*-*N*,*N*-DimethylC_6H_4	86	88	96
8	**H**	*m*-$NO_2C_6H_4$	82	95	95
9	**I**	*p*-$NO_2C_6H_4$	88	94	86
10	**J**	*p*-ClC_6H_4	92	98	88

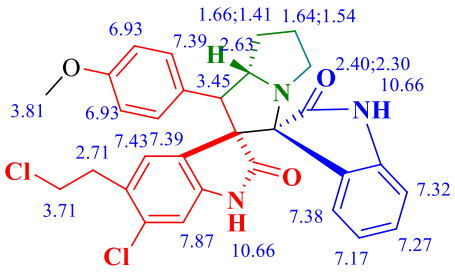


Figure 2. Selected proton chemical shifts of 22a

The structure of dispiro compound **22** was elucidated using ^{1}H, and ^{13}C NMR spectroscopic data as described for a representative example, **22a** (**Figure 2**). The ^{1}H NMR spectrum of **22a** has three diastereostopic protons present in the pyrrolidine ring (C-20, 21 &22) appear as a doublet of doublet at 2.40;2.30 ppm, 1.64;1.54ppm and 1.66;1.41ppm respectively, with coupling constant J = 7.1 Hz and 7.0 Hz. The H-37 appear as a multiplet at 2.63 ppm. The C-13 proton appears at 3.45 ppm as a doublet with J = 7. Hz. A triplet at 2.71ppm is due to to the $–CH_2$ protons and a triplet at 3.71 ppm is because of $–CH_2Cl$ protons. The –NH protons at indoline and isatin ring present at 10.66 and 10.68 ppm respectively. The methoxy protons appears as a singlet at 3.81 ppm.

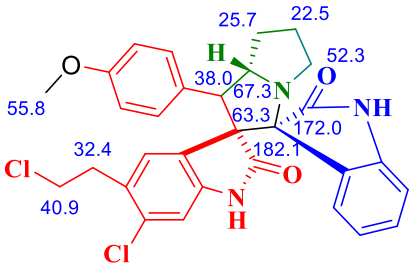


Figure 3. Selected ^{13}C chemical shifts of 22a

^{13}C NMR spectroscopic data as described for a representative example, **22a** (**Figure 3**). The carbonyl carbons of indoline and isatin appears at 182.1 and 172.ppm. The chemical shift of spiro carbons C-13, C-8 and C-12 are 38.0, 63.3 and 67.3 ppm respectively. A peak at 55.5 ppm is due to methoxy carbon (C-39). The C-33 carbon ($-CH_2$) and C-34 carbon ($-CH_2Cl$) appears at 32.4 and 40.9 ppm.

ANTIMYCROBACTERIAL ACTIVITY OF DISPIROINDOLE-3,2'-PYRROLIDINE/PYRROLIZINES /PYRROLIDINES-3',3''-INDOLE DERIVATIVES AGAINST M. TUBERCULOSIS H37RV

All the compounds were subjected to High Throughput Screening (HTS) for in vitro antimycobacterial activity against Mycobacterium tuberculosis H37Rv using microdilution Alamar Blue (AB) broth assay reported by Collins and Franzblau. Five standard drugs were used as references along with the synthesized compounds for the assay. All the dispiro hybrid heterocycles, except 22c, 22e, 22j and 22f, showed IC50 >29.9 lM (Table 2) viz. higher potency than the standard pyrimethamine (IC50 = 37.35 lM) and all the compounds, except 6f, were more potent than pyrimethamine (IC90 = 74.96 - lM). The IC50 values of nine compounds (22a, 22b, 22h, 22i, 22b and 22l) are in the range of 1.07–11.07 lM indicating that they are more active than cycloserine (IC50 = 12.47 lM). Compound 23g, having an IC50 value of 12.87 lM, are as potent as cycloserine. The IC90 values of four compounds (22b and 22h) reveal that their activities are close to that of cycloserine. Compound 22h with an IC50 value of 1.07 is more active than the standard ethambutol (IC50 <1.56 lM). The IC90 values of fifteen compounds (22a, 22b, 22d, 22h, 22i, 23a, 23b, 23d, 23g, 23h, 23j), ranging from 1.98 lM to 27.86 lM, have higher activity than ethambutol (IC90 = 32.79 lM). The MIC values (Table 3. 2) of compounds 22h and 22j show better activity than the standard drugs like cycloserine, ethambutol and pyrimethamine. Compounds 46h and 22i possess potency equal to that of cycloserine and more potent than pyrimethamine. Compounds 22a–c, 23g, 23a–c, 23e, 23g–j, 22l, 22m and 22n show higher potency than pyrimethamine (MIC = 100.00 lM) and compounds 23e, 23f, 23j, 23n, 22d and 22f are found to be as potent as pyrimethamine. 22h has emerged as the most active derivative with an IC50 value of 1.07 lM, being more potent than cycloserine about 12 times, pyrimethamine about 37 times and ethambutol. Similarly 23j with IC50 = 2.87 lM is more active than both cycloserine about 4 times and pyrimethamine about 12 times.

Table 2. Antimycrobacterial activity of dispiroindole-3,2'-pyrrolidine/pyrrolizines /pyrrolidines-3',3''-indole derivatives against M. tuberculosis H37Rv

Entry	Compound	IC50 (lM)	IC90 (lM)	MIC (lM)	CY (lg/ml)	Compound	IC50 (lM)	IC90 (lM)	MIC (lM)	CYT (lg/ml)
1	22a	11.07	21.98	50.00	>6.25	23a	13.19	25.90	50.00	>6.25
2	22b	08.46	12.42	50.00	>6.25	23b	07.20	15.50	50.00	>6.25
3	22c	29.95	41.87	50.00	>6.25	23c	21.91	47.93	50.00	>6.25
4	22d	15.17	27.86	100	>6.25	23d	16.30	25.50	100.0	>6.25
5	22e	47.20	66.50	100.0	>6.25	23e	28.57	47.72	50.00	>6.25
6	22f	25.87	44.60	100.0	>6.25	23f	44.95	74.96	100.0	>6.25
7	22g	18.28	36.50	50.00	>6.25	23g	12.87	24.60	50.00	>6.25
8	22h	04.28	09.86	25.00	>6.25	23h	16.38	21.97	50.00	>6.25
9	22i	5.81	16.13	25.00	>6.25	23i	17.20	35.50	50.00	>6.25
10	22j	47.20	66.50	100.0	>6.25	23j	08.57	14.72	50.00	>6.25
Standard										
Amikacin							0.07	0.08	0.16	>6.25

Cycloserine							12.47	13.49	25.00	>6.25
Ethambutol							<1.56	32.79	12.50	>6.25
Isoniazid							0.18	0.29	0.31	>6.25

CONCLUSION

In the present investigation, 1,3 d dipolar cycloaddition of azomethineylide generated insitu by the reaction between isatin and proline to (3-Z)-6-chloro-3-(benzylidene-5-(2-chloroethyl-1,3-dihydro-2H-indol-2-one results in highly regio and stereo selective formation of highly medicinally important dispiro compound in good yield. The synthesized compounds were subjected to in vitro activity studies against Mycobacterium tuberculosis H37Rv (MTB), multi-drug resistant M. tuberculosis (MDR-TB) and Mycobacterium smegmatis(MC2). Many compounds shows considerable activity.

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