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A facile three-component [3+2]-cycloaddition for the regioselective synthesis of highly functionalised dispiropyrrolidines acting as antimycobacterial agents

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ABSTRACT

A series of fourteen dispiropyrrolidines were synthesized using [3+2]-cycloaddition reactions and were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ in HTS (High Throughput Screen). Most of the compounds showed moderate to good activity with MIC of less than 20 μM. Compound 4′-(4-bromophenyl)-1′-methyldispiro[acenaphthylene-1,2′-pyrrolidine-3′,2″-indane]-2,1″(1*H*)-dione (**4c**) was found to be the most active with MIC of 12.50 μM.

Over the last century, tuberculosis (TB) has killed more than 100 million people and this has continued relatively unchanged over the last 50 years, despite the development of effective antitubercular drugs. TB, disproportionally affecting the world's poorest populations, remains one of the biggest public health problems in the 21st century.[1] TB continues to kill young and middle-aged adults faster than any other disease apart from AIDS. In 2010, the number of people who fell ill with TB is 8.8 million cases, including 1.1 million cases among people with HIV. There was an estimated prevalence of 650,000 cases of multidrug-resistant TB (MDR-TB), and in 2008 it was estimated there were 150,000 MDR-TB deaths annually.[2] Today, TB remains one of the leading infectious disease killers around the world. Emerging drug-resistant strains such as multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are presenting a new challenge in the ever changing battle to control and prevent TB.[3] Many published reports[4,5] on the resistance of the current available first line drugs against the virulence strains of MTB incite the researchers to develop new anti TB agents on the priority basis.

Heterocyclic compounds with five and six-membered rings have drawn considerable attention with their highly pronounced biological activities.[6] Spiro compounds especially are known for their antimycobacterial properties and some of them have shown comparable or even better activities than the fist-line TB drugs.[7–9] Hence our group has synthesized and evaluated a series of novel spiro-heterocyles using 1,3-dipolar cycloaddition to obtain novel and more potent antitubercular agents. 1,3-Dipolar cycloaddition is proved to be an efficient method to construct heterocyclic units in a single step without the need to purify the intermediates. Previous studies have shown that chalcone and chalcone-like compounds exert various biological activities including antitubercular,[10] anti-inflammatory,[11] antioxidant,[12] anticancer[13] and antimicrobial.[14] In continuation of our research on the synthesis of biologically active heterocycles and in view of the importance of indanone derivatives in medicinal chemistry, we focused our attention in synthesizing dispiro substituted indanone derivatives. In our previous study[15] we have reported the synthesis of highly functionalised dispiropyrrolidines by [3+2]-cycloaddition reaction of sarcosine and ninhydrin as a source of azomethine ylide with indanone dipolarophiles. As part of the ongoing research program, herein we wish report the synthesis of spiropyrrolidines by the same cycloaddition reaction with different azomethine ylides

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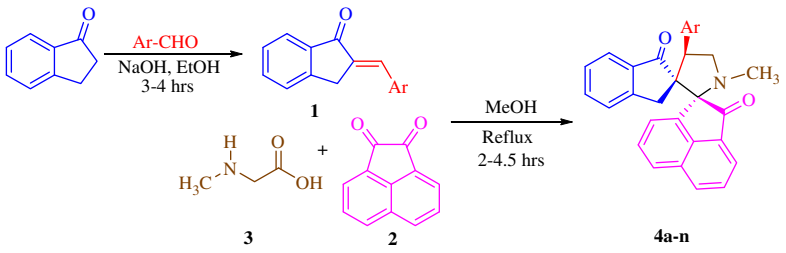


Scheme 1. Protocol for synthesis of titled compound.

generated in situ by decarboxylative condensation of acenaphthoquinone and sarcosine (Scheme 1). The synthesized compounds were screened for antimycobacterial activity and the results are presented in this paper.

2-[(*E*)-1-Arylmethylidene]-1-indanones (**1**) were synthesized according to the literature method.[16] An equimolar mixture of the starting materials were refluxed in methanol until completion of the reaction (as evident from TLC) and the pyrrolidine derivatives were purified by crystallization.

The purity of the newly synthesized dispiropyrrolidines was checked using NMR, CHN, mass spectrometry and X-ray analysis. All the analytical and spectral data showed that the synthesized compounds were in full agreement with the proposed structures. In the ^{1}H NMR spectrum, the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. ^{1}H NMR spectrum of **4c** showed a singlet at δ 2.22 ppm corresponding to N–CH_3 group; a triplet at 4.43 ppm with J = 9.0 Hz is due to H-4′. The triplets at 3.74 ppm (J = 9.0 Hz) and 4.16 ppm (J = 9.3 Hz) are due to protons of 5′-CH_2 while the doublets at 2.63 ppm (J = 17.7 Hz) and 2.91 ppm (J = 17.7 Hz) were due to protons of 3″-CH_2. The aromatic protons appear around 6.80–8.01 ppm. In ^{13}C NMR spectrum, the N–CH_3 signal appears at 35.6 ppm, aliphatic ring carbons appear at 35.0, 50.5 and 59.9 ppm while the peaks at 67.6 and 82.0 ppm are due to the spiro carbons. The C=O of acenaphthenequinone ring appears at 207.1 ppm while the C=O of indanone appears at 208.4 ppm. A selected ^{1}H and ^{13}C chemical shift of **4c** is shown in Figure 1. The product **4c** was further confirmed by mass spectrometry with the molecular ion peak at m/z 509 [M^+]. The regio- and stereochemical outcome of the cycloaddition reaction was ascertained by single crystal X-ray analysis[18] of **4c** (Fig. 2). This molecular structure is stabilized by intermolecular and intramolecular C–H···O hydrogen bonds.

The mechanism for the formation of the novel pyrrolidine derivative is proposed and summarized in Scheme 2. The reaction of acenaphthenequinone and sarcosine affords the azomethine ylide (**5**), which adds to C=C bond of the dipolarophile from the bottom to form the corresponding cycloadduct. The reaction proceeds with complete regioselectivity affording regioisomer (**4**) and also with

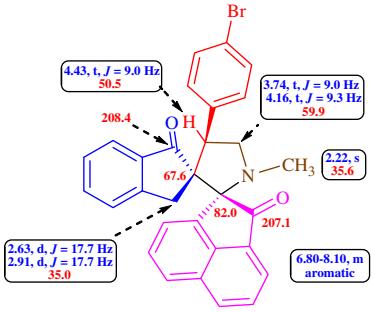


Figure 1. Selected ^{1}H and ^{13}C NMR chemical shifts of **4c**.

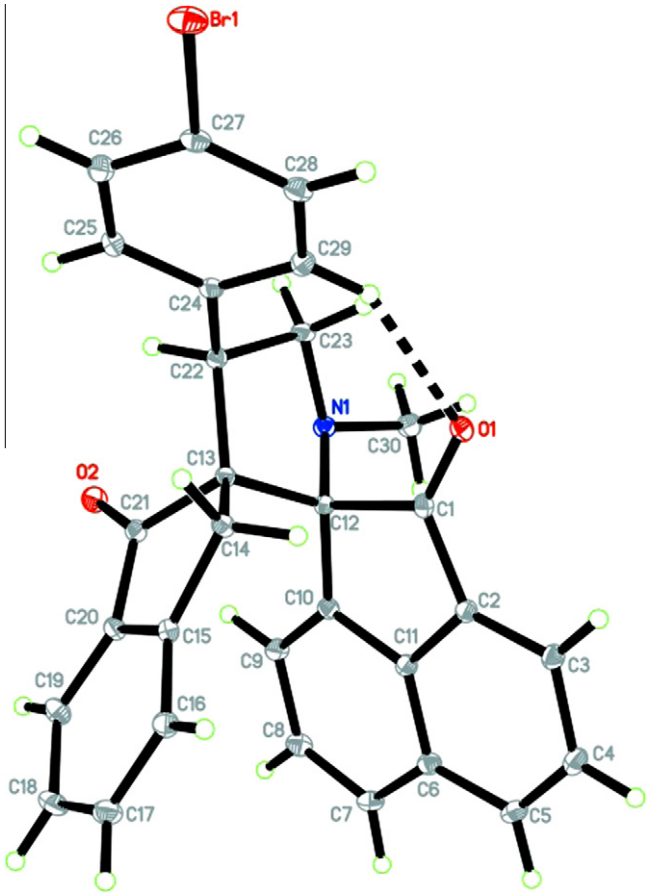
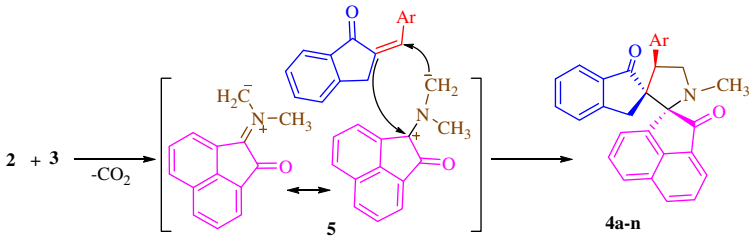


Figure 2. ORTEP diagram of **4c**.

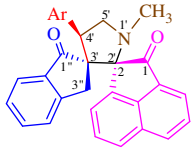
complete stereoselectvity furnishing only one stereoisomer of the cycloadduct despite of the presence of three stereocentres.

All the newly synthesized compounds **4a–n** were screened for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}R_V$ (MTB-H_{37}Rv) in a HTS (High Throughput Screen) using an assay adapted from the microdilution alamar Blue (AB) broth assay reported by Collins and Franzblau[17] and additionally used an alternative method for end-point detection was assessed using the Promega reagent BacTiter-Glo™ Microbial Cell Viability (BTG). The BTG assay is a quantitative ATP assay for bacteria using luciferase production as an end-point detection point. Six standard drugs were used to test together with the synthesized compound as references for the assay. Data was analyzed using the IDBS Activity Base software and the dose response result was analyzed using a four parameter logistic fit to the data (Excel Fit equation 205) with the maximum and minimum locked at 100 and 0. From these curves, EC_{90} and EC_{50} values were calculated. The MIC was defined as the minimum concentration of compound required to



Scheme 2. Mechanism for the regioselective formation of dispiropyrrolidine.

Table 1
Antimycobacterial activity of pyrrolidine derivatives **4a**–**n**



Compd	Ar	EC_{50} (μM) BTG	EC_{90} (μM) BTG	MIC (μM) BTG	Std Dev EC_{50} BTG	EC_{50} (μM) AB	EC_{90} (μM) AB	MIC (μM) AB	Std Dev EC_{50} AB	Cytotoxicity (μg/ml)
4a	Phenyl	>100.00	>100.00	NA	0.000	>100.00	>100.00	NA	>9999	>62.5
4b	4-Chlorophenyl	6.27	29.62	50.00	0.377	68.46	>100.00	NA	31.96	>62.5
4c	4-Bromophenyl	2.20	10.76	12.50	0.163	15.07	>100.00	NA	4.54	>62.5
4d	4-Trifluoromethylphenyl-	3.21	12.29	50.00	0.258	23.43	>100.00	NA	6.41	>62.5
4e	4-Trifluoromethoxyphenyl-	8.51	26.08	50.00	0.463	36.60	>100.00	NA	5.24	>62.5
4f	5-[(4-Fluorophenyl)-pyridine	64.46	>100.00	NA	27.106	>100.00	>100.00	NA	4304.53	>62.5
4g	4-Carboxy phenyl	20.74	>100.00	100.00	4.062	69.21	99.70	100.00	5.09	>62.5
4h	3-Nitrophenyl	10.06	>100.00	NA	1.514	77.50	>100.00	NA	11.80	>62.5
4i	4-Methoxyphenyl	13.43	62.37	100.00	1.121	40.90	>100.00	NA	3.64	>62.5
4j	Benzo[*d*][1,3]dioxole	11.51	>100.00	100.00	1.478	63.84	>100.00	NA	5.79	>62.5
4k	4-Dimethylaminophenyl	9.87	55.34	100.00	1.104	55.99	>100.00	NA	10.18	>62.5
4l	1-(4-(Morpholine-1-yl)phenyl)	18.85	>100.00	NA	1.867	>100.00	>100.00	NA	>9999	>62.5
4m	2,5-Dimethoxyphenyl	9.56	>100.00	NA	1.065	67.96	>100.00	NA	14.20	>62.5
4n	1-(4-(Piperidine-1-yl)phenyl)-	17.75	>100.00	NA	1.760	>100.00	>100.00	NA	23.72	>62.5
Cycloserine	—	23.55	26.38	100	1441.00	24.76	28.01	100.00	358.28	>62.5
Isoniazid	—	0.13	0.2	0.31	0.04	0.19	>5.00	NA	0.04	>62.5
Rifampin	—	0.02	0.03	0.04	0.00	0.02	0.02	0.16	0.40	>62.5
Amikacin	—	0.07	0.12	0.16	0.00	0.12	0.14	0.16	1458.49	>62.5
Pyrimethamine	—	24.27	46.37	100.00	1.18	25.09	28.00	100.00	275	>62.5
Ethambutol	—	1.50	1.64	6.25	3542.14	3.45	>200.00	NA	1.60	>62.5

inhibit 90% of bacterial growth and all the biological results were reported in Table 1.

Among the fourteen compounds that have been newly synthesized, compound **4c** (4′-(4-bromophenyl)-1′-methyldispiro[acenaphthylene-1,2′-pyrrolidine-3′,2″-indane]-2,1″(1*H*)-dione) was found to be the most active with EC_{50} of 2.20 μM and MIC at 12.50 μM using BTG. The compound with electron withdrawing group sustituents, **4d**, **4b** and **4e** showing 50% inhibition at concentration of 3.21, 6.27 and 8.51 μM. However substituents with 2,5-dimethoxy (**4m**), 4-dimethylamino (**4k**), 3-nitro (**4h**), methylene dioxy (**4j**), 4-methoxy (**4i**), piperidine (**4n**) and morpholino (**4l**) groups showed moderate to low inhibitory with EC_{50} less than 20 μM. It is to be noted that the halogen substituent in the phenyl ring causes remarkable improvement in antimycobacterial activity, especially bromo group. In particular, the compound 4′-(4-bromophenyl)-1′-methyldispiro[acenaphthylene-1,2′-pyrrolidine-3′,2″-indane]-2,1″(1*H*)-dione **(4c)** is endowed with maximum potency in the titled compounds, being, respectively, 10.70-, 11.0-fold at EC_{50}, 2.45-, 4.30-fold at EC_{90} and more than 8-fold of MIC than the standard drugs cycloserine and pyrimethamine. However none of the compounds screened in the present derivatives are found to be more potent than other used standard drugs.

All the compounds were also tested for cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μg/mL or 10 times. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 nonradioactive cell proliferation assay. All of the active compounds were found to be non-toxic up to 62.5 μg/mL. The compounds were bactericidal and the cytotoxic profile was within an acceptable range. Therefore, a lead optimization programme was initiated with the goal of achieving potent antitubercular activity.

These reports clearly showed that the presence of halogen substitution cause remarkable improvement in antimycobacterial activity. It is conceivable that derivatives showing antimycobacterial activity can be further modified to exhibit better potency than standard drugs. Further studies to acquire more information about quantitative structure–activity relationships (QSAR) are in progress in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.069.

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