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Syntheses and biological evaluation of new triazole-spirochromone conjugates as inhibitors of *Mycobacterium tuberculosis*

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ABSTRACT

A series of novel 1,2,3-triazole fused spirochromone conjugates have been synthesized bearing both spirochromone moiety as well as a 1,2,3-triazole moiety. Some of the compounds have exhibited potential activity against *Mycobacterium tuberculosis* (virulent strain H37Rv). In particular **5e** proved to be the most potent derivative exhibiting MIC = 0.78 μg/mL.

Tuberculosis (TB) is one of the deadly infectious diseases caused by *Mycobacterium* spp. mainly *Mycobacterium tuberculosis*. This notorious pathogen infects about one-third of the world's population and is responsible for approximately 2 million deaths worldwide per year.[1,2] Furthermore, the emergence of a drug resistant microorganism responsible for TB, especially multidrug-resistant one along with the lethal combination of TB and HIV-1 infection, makes this disease one of the greatest global health challenges facing us today.[3] In the last 50 years, only a few drugs have been approved by the Food and Drug Administration (FDA) to treat TB, reflecting the inherent difficulties in discovery and clinical testing of new agents.[4] Therefore, the discovery and development of new types of anti TB agents acting on novel drug targets are urgently needed.

In recent years, there has been re-stimulated interest in nature's repertoire of structures and many strategies are being adapted to exploit advantageously the features of natural products in the design of novel small-molecules for various biological applications, especially in the area of drug discovery and chemical biology.[5] One such a successful and effective approach to the construction of natural product like small molecules is based upon the concept of 'privileged structures'.[6] Chromone is recognized as a privileged structural motif, observed in a plethora of natural products and in various therapeutic agents.[7] In this context, and in view of our long-standing interest in the chemistry of privileged chromone motif,[8] in particular, the design and synthesis of novel natural products like small molecules based on chromone motif for various biological applications, we describe herein the synthesis of novel triazole fused spirochromone conjugates as potential antimycobacterial agents. The interest in incorporation of 1,2,3-triazole moiety stems from the advent of click chemistry protocol which has been used in various applications such as drug discovery process and chemical biology due to high yield, high selectivity, wide scope, atom-economy and simple purification.[9] Furthermore, these triazole products can readily associate with the biological targets through hydrogen bonding and dipole interactions.[10]

The synthetic strategy followed for the preparation of 1,2,3-triazole fused spirochromone conjugates is given in Scheme 1. Firstly, for the preparation of precursor spirochromanone moiety **2a**,**b** a Kabbe condensation[11] between the cyclohexanone or *N*-Boc piperidone and 2,4-dihydroxy acetophenone was employed. In the Kabbe condensation, the use of acetonitrile as solvent and carrying out the reaction at 50 °C for 24 h in the presence of pyrrolidine as a base is optimum in order to obtain spirochromanone **2a**,**b** in 72% and 75% yields.[12] On the other hand, refluxing with toluene or ethanol as a solvent using Dean–Stark apparatus, the condition generally used in Kabbe condensation did not produce the required products in good yield. Subsequently, the spirochromanone **2a**,**b** were O-alkylated with epichlorohydrin in the presence of K_2CO_3 in refluxing acetone gave epoxides **3a**,**b** in 85% and 82% yields. These epoxides **3a**,**b** that were subjected to ring opening with sodium azide in the presence of ammonium chloride afforded the corresponding azides **4a**,**b** in 88% and 85% yields. Finally, 1,2,3-triazole moiety was incorporated through the 1,3-dipolar cycloaddition of these azides **4a**,**b** with various terminal alkynes to afford

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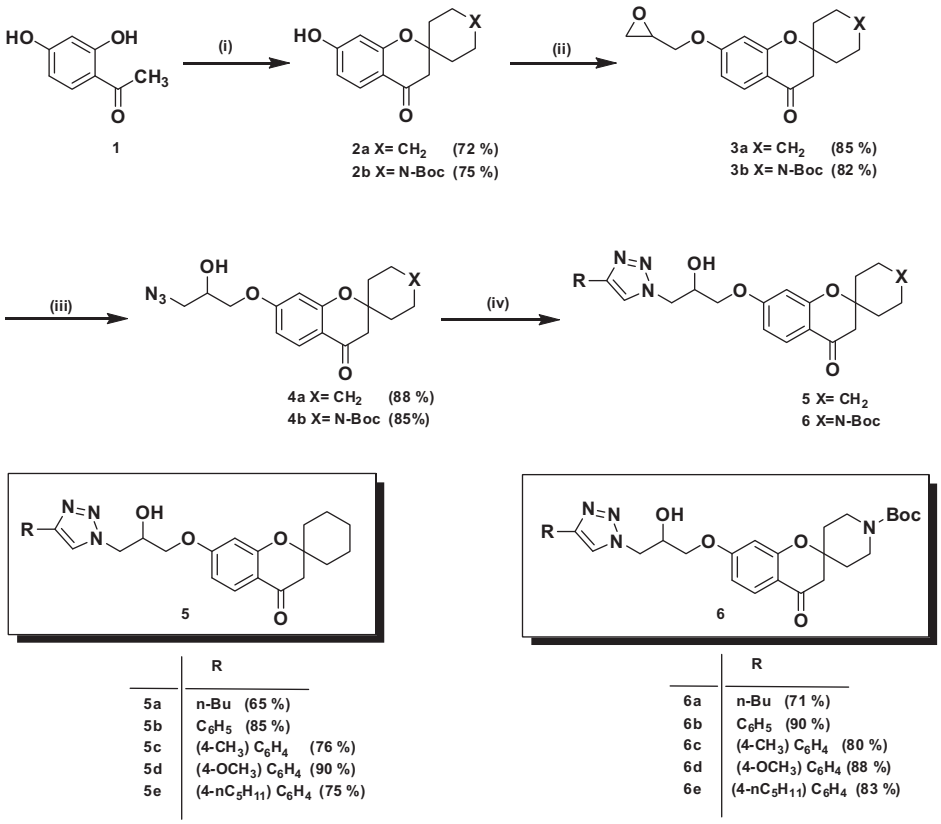


Scheme 1. Reagents and conditions: (i) cyclohexanone/*N*-Boc piperidone, pyrrolidine, acetonitrile, 50 °C, 24 h; (ii) epichlorohydrin, potassium carbonate, acetone, reflux, 12 h; (iii) sodium azide, ammonium chloride, methanol, reflux, 5 h; (iv) alkyne, $CuSO_4$, sodium ascorbate, ^{t}butanol/water (1:1), 60 °C, 12 h.

Table 1
In vitro antimycobacterial activity of the 1,2,3-triazole fused spirochromone conjugates

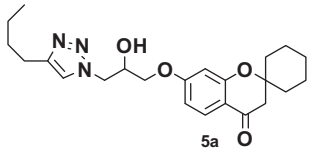
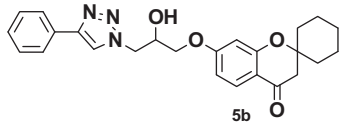
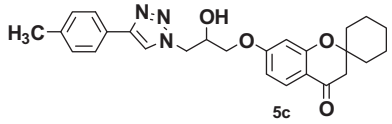
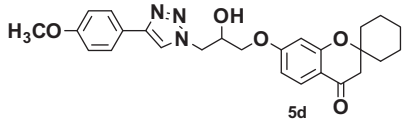
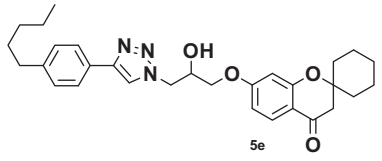
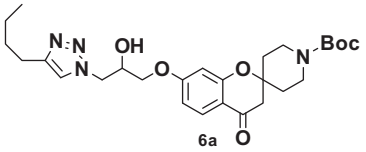
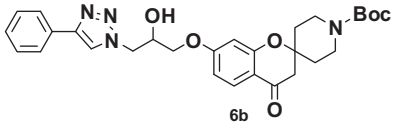
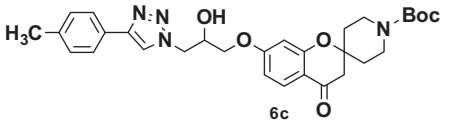
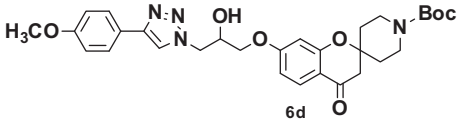
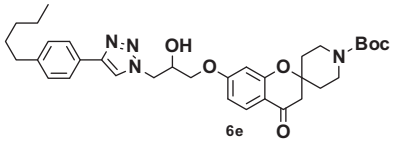
Entry	Compound	MIC (μg/mL)
1		12.5
2		6.25
3		3.13
4		12.5
5		**0.78**

Table 1 (*continued*)

Entry	Compound	MIC (μg/mL)
6		25.0
7		12.5
8		6.25
9		25.0
10		3.13
11	Rifampicin	0.2
12	Ethambutol	1.56

1,2,3-triazole fused spirochromones conjugates **5a–e**, **6a–e** in 65–90% yields with high purity.[13] In this reaction, the copper(I) catalyst was generated in situ by the reduction of copper(II)sulfate with sodium ascorbate, as described by Sharpless.[14] The structure of all the new compounds **5a–e**, **6a–e** were confirmed by the ^{1}H NMR, ^{13}C NMR and mass spectral data (Supplementary data)

All the new triazole fused spirochromone conjugates were screened for their in vitro antimycobacterial activity against *M. tuberculosis* H37Rv (ATCC27294) using an agar dilution method.[15] The minimum inhibitory concentration (MIC; μg/mL) was determined for each compound. The MIC is defined as the minimum concentration of compound required to completely inhibit the bacterial growth. Rifampicin and ethambutol were used as reference compounds. The MIC values of the synthesized compounds along with the standard drugs for comparison were reported in Table 1. Most of the compounds (**5b–c**, **5e**, **6c**, **6e**) showed a significant in vitro activity against *M. tuberculosis*, MIC in the range of 0.78–6.25 μg/mL. Among them, compound **5e** is found to be more active having MIC 0.78 μg/mL among all the compounds screened and the potency is better than first line antibacterial drug ethambutol. Importantly, compound **5e** represents a novel structural chemotype for which antitubercular properties have not been previously noted. Preliminary structure–activity relationship of the triazole fused spirochromone conjugates reveals that compounds possessing cyclohexyl group at 2nd position of the chromone ring favor better activity than piperidinyl moiety. Furthermore, aromatic substitution at 4th position of the triazole is favorable than alkyl substitution.

In conclusion, a new class of 1,2,3-triazole fused spirochromone conjugates have been synthesized. All the compounds were obtained in good yield and were tested as new potential antitubercular agents. One of those compounds, **5e** showed good activity against MTB. Further optimization of this series is ongoing and will be reported shortly.

Acknowledgment

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Supplementary data

Supplementary data (experimental procedures, compound characterization data and copies of ^{1}H NMR, ^{13}C NMR and HPLC chromatograms of compounds **5b**, **5e**, **6a**, **6d**) associated with this Letter can be found, in the online version, at doi:10.1016/j.tetlet.2011.02.099.

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13. *Typical procedure for the synthesis of compound* **5e**: To a stirred solution of azido spirochromone **4a** (0.23 g; 0.7 mmol) and 1-ethynyl-4-pentylbenzene (0.13 g; 0.77 mmol) in ^{t}butanol (3 mL) was added sequentially copper sulphate pentahydrate (0.035 g; 0.14 mmol), sodium ascorbate (0.028 g; 0.14 mmol) and distilled water (3 mL). The resulting reaction mixture was stirred for 12 h at 60 °C. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with CH_2Cl_2 (10 mL) and then washed with water (2 × 5 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography over silica gel with ethylacetate/hexanes (1:1) as eluent to furnish compound **5e** as a colorless solid, 0.264 g (75%). Other compounds were synthesized similarly, and the spectroscopic data of selected compounds are as follows:
Analytical data for compound **5e**: colorless solid; mp 156–57 °C; ; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, J = 6.4 Hz, 3H),1.22–1.72 (m, 14H), 1.93–2.0 (m, 2H), 2.56–2.60 (m, 2H), 2.63 (s, 2H), 4.08–4.11 (br d, J = 4.6 Hz, 2H), 4.43–4.74 (m, 3H), 4.96 (d, J = 4.2 Hz, 1H), 6.43 (d, J = 2.3 Hz, 1H), 6.52–6.57 (dd, J = 8.6, 2.4 Hz, 1H), 7.14 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 9.0 Hz, 1H), 7.79 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 191.3, 164.7, 161.5, 147.4, 143.1, 128.8, 128.3, 127.3, 125.4, 121.1, 115.1, 109.2, 102.0, 80.5, 69.3, 68.4, 53.2, 47.8, 35.6, 34.7, 31.4, 31.0, 25.1, 22.5, 21.4, 14.0; ESI-MS: m/z 526 $[M+Na]^+$.
Analytical data for compound **6c**: colorless solid; mp 128–29 °C ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.50–1.66 (m, 2H), 1.90–1.96 (m, 2H), 2.36 (s, 3H), 2.65 (s, 2H), 3.13–3.25 (m, 2H), 3.82–3.88 (m, 2H), 4.07–4.10 (br d, J = 4.9 Hz, 2H), 4.45–4.73 (m, 4H), 6.44 (d, J = 2.3 Hz, 1H), 6.55–6.61 (dd, J = 8.9, 2.4 Hz, 1H), 7.16 (d, J = 8.2 Hz, 2H), 7.55 (d, J = 7.6 Hz, 2H), 7.79(d, J = 7.6 Hz, 1H), 7.81 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 190.2, 164.8, 160.9, 154.7, 147.5, 138.1, 129.5, 128.5, 127.2, 125.4, 121.1, 115.0, 109.7, 102.1, 79.8, 78.3, 69.4, 68.4, 60.4, 53.2, 47.6, 39.1, 34.0, 28.4, 21.3; ESI-MS: m/z 571 $[M+Na]^+$.
Analytical data for compound **6d**: colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.51–1.66 (m, 2H), 1.85–2.04 (m, 2H), 2.65 (s, 2H), 3.13–3.25 (m, 2H), 3.83 (s, 3H), 3.84–3.89 (m, 2H), 4.08–4.10 (br d, J = 4.2 Hz, 2H), 4.45–4.73 (m, 4H), 6.43 (d, J = 2.3 Hz, 1H), 6.56–6.61 (dd, J = 8.7, 2.2 Hz, 1H), 6.89 (d, J = 9.3 Hz, 2H), 7.59 (d, J = 9.3 Hz, 2H), 7.76 (s, 1H), 7.80 (d, J = 8.7 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 190.1, 164.8, 160.9, 159.6, 154.7, 147.3, 128.5, 126.8, 122.7, 120.6, 115.1, 114.2, 109.7, 102.1, 79.8, 78.3, 69.3, 68.5, 55.3, 53.1, 47.6, 39.0, 34.0, 28.4; ESI-MS: m/z 587 $[M+Na]^+$.
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