Bioorganic & Medicinal Chemistry Letters 23 (2013) 1416–1419

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and antitubercular activity of amino alcohol fused spirochromone conjugates

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ARTICLE INFO

Article history:
Received 14 September 2012
Revised 8 December 2012
Accepted 21 December 2012
Available online 5 January 2013

Keywords:
Amino alcohol
Spirochromones
Antitubercular activity
Mycobacterium tuberculosis

ABSTRACT

A series of 21 new amino alcohol fused spirochromone conjugates have been synthesized, characterized with analytical data and evaluated their antimycobacterial activity against *Mycobacterium tuberculosis* (virulent strain H37Rv) in vitro. Some of the compounds exerted significant inhibition, in particular, compound **4f** found to be the most potent derivative exhibiting MIC = 3.13 μg/mL.

Tuberculosis is an infectious pulmonary disease caused by the pathogenic species *Mycobacterium tuberculosis* (Mtb) which is responsible for almost 8.7 million new infections and 1.4 million casualties in 2011 alone.[1] Further, the emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) Mtb strains coupled with HIV co-infection making the disease even more challenging. Although, several compounds are currently in advanced phases of clinical trials, for the last 40 years there is no new compounds have been brought to the market for TB treatment. Considering the global impact of this devastating disease there is an urgent need for the design and development of novel chemical entities endowed with promising antimycobacterial activities.

As a privileged structure in drug discovery, the chromone framework is ubiquitous in a wide variety of naturally occurring and synthetic compounds that exhibit wide range of biological activities.[2] Consequently, interest in the isolation from natural resources, synthesis of chromone derivatives and evaluation of their biological activity with emphasis on their potential medicinal applications has continued. In this context, and in view of our long-standing interest in the chemistry of privileged chromone motif,[3] in particular, the design and synthesis of novel natural products like small molecules based on chromone motif for various biological applications, recently we reported that various spirochromone derivatives possessing 1,2,3-triazole ring system can serve as a lead for developing antitubercular agents.[4] This result has prompted us to take-up this spirochromone motif as an active pharmacophore for further diversification to exploit its anti TB potential. Towards this goal, it was decided to design and synthesise, a series of novel amino alcohol annulated spirochromone conjugates (Fig. 1) by introduction of amino alcohol unit to the phenolic –OH on the C-7 chromone ring and evaluate their anti TB properties. The interest in incorporation of amino alcohol moiety stems from the fact that this motif is an essential component in many antitubercular agents[5] including a well known anti-Tb drug ethambutol.[6] Further, to the best of our knowledge, synthesis and antimycobacterial activities of these amino alcohol annulated spirochromone conjugates is unprecedented.

The synthetic strategy followed for the preparation of amino alcohol fused spirochromone conjugates is given in Scheme 1. Firstly, for the preparation of precursor spirochromanone moiety **2a–c** a Kabbe condensation[7] between various cycloalkanones and 2,4-dihydroxy acetophenone was employed. In the Kabbe condensation, the use of acetonitrile as solvent and carrying out the

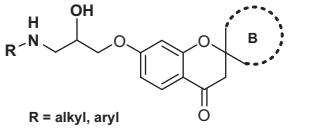

Figure 1. Design of amino alcohol annulated spirochromone conjugates.

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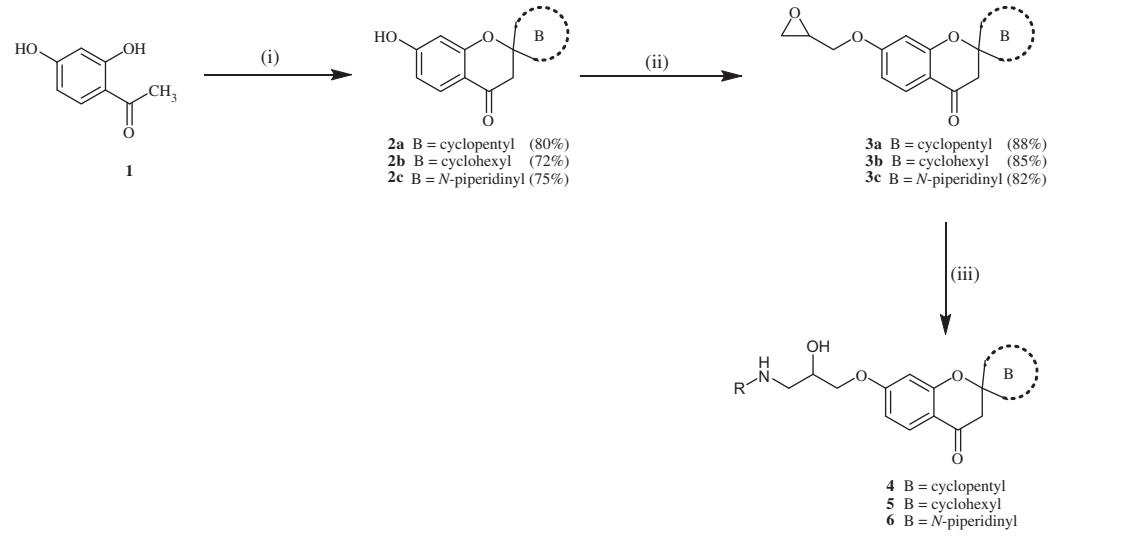
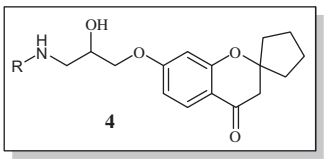
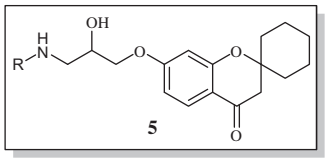
http://dx.doi.org/10.1016/j.bmcl.2012.12.073

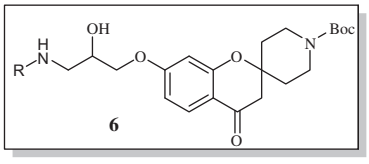




	R	
4a	C_6H_5	(85%)
4b	(2-CH_3) C_6H_4	(72%)
4c	(2-Cl) C_6H_4	(35%)
4d	(2-iPr) C_6H_4	(67%)
4e	(2-OMe) C_6H_4	(79%)
4f	(3-F) C_6H_4	(56%)
4g	(4-OMe) C_6H_4	(76%)
4h	(4-^{i}Pr) C_6H_4	(90%)
4i	(2-OH, 4-NO_2) C_6H_3	(55%)
4j	-CH_2(4-OMe) C_6H_4	(38%)
4k	morpholinyl	(81%)



	R	
5a	C_6H_5	(88%)
5b	(2-CH_3) C_6H_4	(80%)
5c	(2-OMe) C_6H_4	(82%)
5d	(4-OMe) C_6H_4	(84%)
5e	(4-^{i}Pr) C_6H_4	(85%)



	R	
6a	C_6H_5	(75%)
6b	(2-CH_3) C_6H_4	(73%)
6c	(2-OMe) C_6H_4	(80%)
6d	(4-OMe) C_6H_4	(84%)
6e	(4-^{i}Pr) C_6H_4	(80%)

Scheme 1. Reagents and conditions: (i) cyclopentanone/cyclohexanone/*N*-Boc-piperidone, pyrrolidine, acetonitrile, 50 °C, 24 h; (ii) epichlorohydrin, K_2CO_3, acetone, reflux, 12 h; (iii) alkyl/aryl amine, LiBr, MeOH, rt, 12 h.

reaction at 50 °C for 24 h in the presence of pyrrolidine as a base is optimum in order to obtain spirochromanone **2a–c** in 72–80% yields.[8] On the other hand, refluxing with toluene or ethanol as a solvent using DS apparatus, the condition generally used in Kabbe condensation did not produce the required products in good yield. Subsequently, the spirochromanone **2a–c** were O-alkylated with epichlorohydrin in the presence of K_2CO_3 in refluxing acetone gave epoxides **3a–c** in 82–88% yields. Finally, the amino alcohol moiety was incorporated through nucleophilic ring opening of this spirochromone epoxides **3a–c** with various aromatic/aliphatic amines to afford amino alcohol fused spirochromone conjugates **4–6**, in moderate to good yields. The structure of all the new products **4–6** (21 compounds) were confirmed by the IR, ^{1}H NMR, ^{13}C NMR and mass spectral data (Supplementary data).[9] In the IR spectrum (compound **4h** as a representative example), a signal corresponding to the chromanone carbonyl was observed at 1675 cm^{-1}. The signal corresponding to the C-3 protons of chromanone skeleton was observed as a singlet at δ 2.78 ppm in the ^{1}H NMR spectrum and the corresponding ^{13}C resonance signal was observed at δ 47 ppm. In the ^{13}C NMR spectrum, the spirocarbon was discernible at δ 90.4 ppm. Similarly, the characteristic signal appeared as a multiplet at δ 4.25–4.31 ppm was ascribable to the methine proton attached to secondary –OH. Conclusive evidence for its structure was obtained from single-crystal X-ray analysis (Fig. 2; Supplementary data).[10]

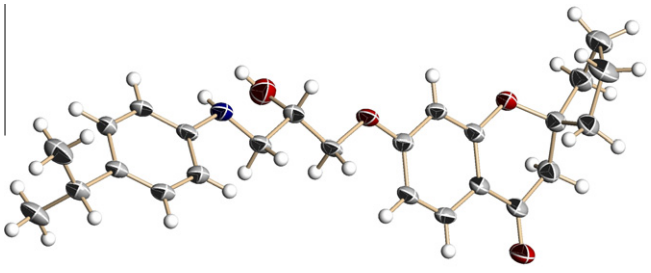
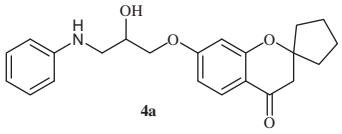
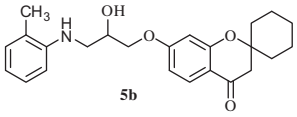
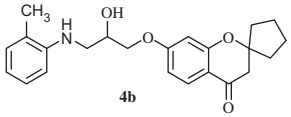
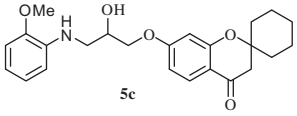
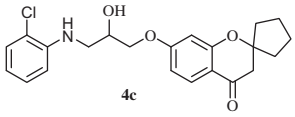
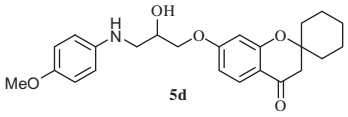
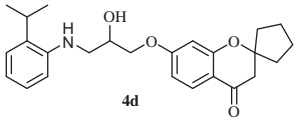
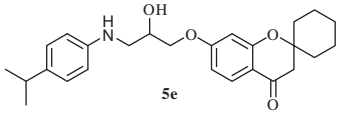
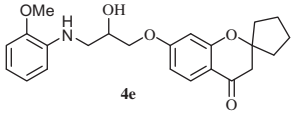
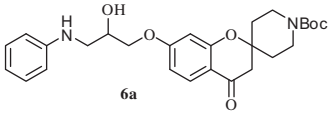
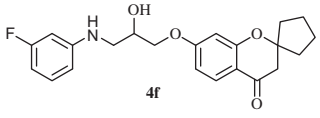
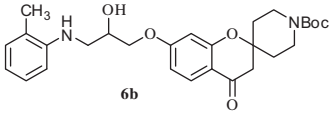
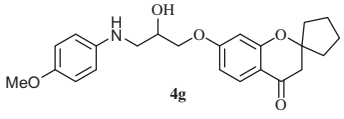
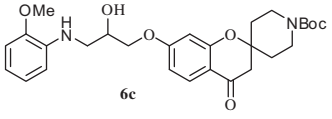
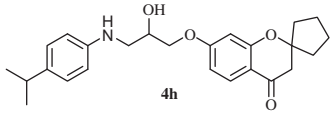
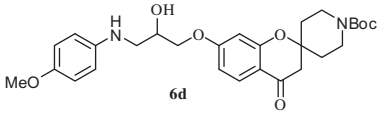
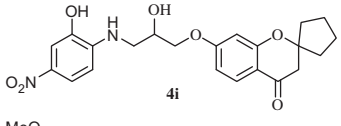
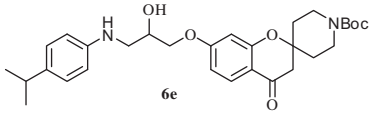
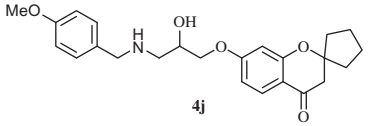
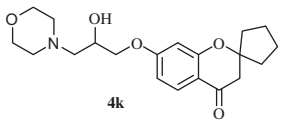
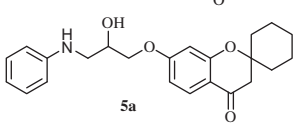


Figure 2. ORTEP diagram of the compound **4h** (thermal ellipsoids are drawn at 50% probability level).

All the new aminoalcohol fused spirochromone conjugates were screened for their in vitro antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (ATCC27294) using an agar dilution method.[11] 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. Among the 21 synthesized compounds, seven compounds (**4c**, **4d**, **4f**, **4h**, **4i**, **5b**, **5e)** were found to be active with MIC in the range of 3.13–6.25 μg/mL. The compound **4f**, is found to be more active having MIC 3.13 μg/mL among all the compounds screened. Importantly, compound **4f** represents a novel structural chemotype for which antitubercular properties have not been previously noted. Preliminary structure activity relationship of the aminoalcohol fused spirochromone conjugates reveals that compounds possessing cycloalkyl group at 2nd position of the chromone ring favors better activity than piperidinyl moiety. Among cycloalkyl groups, not much difference in activity has been observed between cyclopentyl and cyclohexyl groups. Only in few

Table 1
In vitro antimycobacterial activity of the amino alcohol fused spirochromone conjugates

Entry	Compound	MIC (μg/mL)	Sr.No	Compound	MIC (μg/mL)
1	 **4a**	25	13	 **5b**	6.25
2	 **4b**	12.5	14	 **5c**	12.5
3	 **4c**	6.25	15	 **5d**	12.5
4	 **4d**	6.25	16	 **5e**	6.25
5	 **4e**	12.5	17	 **6a**	>25
6	 **4f**	**3.13**	18	 **6b**	25
7	 **4g**	25	19	 **6c**	25
8	 **4h**	6.25	20	 **6d**	>25
9	 **4i**	6.25	21	 **6e**	12.5
10	 **4j**	12.5	22	Rifampicin	0.2
11	 **4k**	>25	23	Ethambutol	1.56
12	 **5a**	12.5			

cases (**5a**, **5b**, **5d**) cyclohexyl group favors better activity. Furthermore, the halide substitution at aromatic ring of amino alcohol favors better activity (**4c**, **4f**). In addition, it is also observed that, isopropyl group in the aromatic ring is very favorable in enhancing the activity (**4d**, **4h**, **5e**).

In conclusion, a series of amino alcohol fused spirochromone conjugates were synthesized for the first time via an easy and convenient synthetic procedure starting from 2,4-dihydroxy acetophenone and all these new compounds were confirmed by ^{1}H NMR, ^{13}C NMR and MS spectra. The single X-ray diffraction study was used to confirm the molecular structure of a representative compound **4f** unambiguously. The in vitro antimycobacterial evaluation showed that most of the synthesized amino alcohol fused spirochromone conjugates exhibited moderate to good antimycobacterial activity. Noticeably, compound **4f** is most potent compound in vitro with MIC of 3.13 μg/mL, against MTB. These findings demonstrated that amino alcohol fused spirochromone conjugates have biological significance; further optimization of this series as well as preparation of chiral isomers is ongoing in our laboratory.

Acknowledgments

M.Mujahid thanks CSIR, New Delhi for a research fellowship. Financial support from the CSIR network projects (OSDD, NAPAHA & ORIGIN) are gratefully acknowledged.

Supplementary data

Supplementary data (experimental procedures, compound characterization data and copies of ^{1}H NMR, ^{13}C NMR and mass spectra of selected compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.073.

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9. *General procedure for the synthesis of compound **4**–**6**:* To a stirred solution of epoxide **3a/3b/3c** (0.5 mmol) and LiBr (0.1 mmol) in methanol (3 mL) was added an appropriate amine (0.55 mmol) and the resulting reaction mixture was stirred at rt for 12 h. After completion of the reaction (monitored by TLC), solvent was removed in vacuo and the reaction mixture was diluted with ethyl acetate (20 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 (ethyl acetate/petroleum ether 3:7 (v/v)) afforded pure product **4**–**6**. Spectroscopic data of selected compounds are as follows:
*Analytical data for compound **4h**:* Colorless solid; mp 117–18 °C; IR ($CHCl_3$, cm^{-1}): ν_{max} 3414, 2935, 1608, 1441; ^{1}H NMR (200 MHz, $CDCl_3$): δ_H = 1.22 (d, *J* = 6.7 Hz, 6H), 1.60–1.83 (m, 6H), 2.02–2.10 (m, 2H), 2.77 (s, 2H), 2.81–2.88 (m, 1H), 3.32 (dd, *J* = 12.8, 6.9 Hz, 1H), 3.39 (dd, *J* = 12.8, 4.0 Hz, 1H), 4.06–4.14 (m, 2H), 4.25–4.30 (m, 1H), 6.30 (d, *J* = 1.3 Hz, 1H), 6.52–6.65 (m, 3H), 7.08 (d, *J* = 8.0, 2H), 7.82 (d, *J* = 8.9 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ_C 191.4 (CO), 164.9 (C), 162.3 (C), 145.9 (C), 138.8 (C), 128.5 (CH), 127.2 (CH, 2 carbons), 115.2 (C), 113.4 (CH, 2 carbons), 109.4 (CH), 102.2 (CH), 90.5 (C), 70.4 (CH_2), 68.5 (CH), 46.9 (CH_2), 46.7 (CH_2), 37.5 (CH_2, 2 carbons), 33.1 (CH), 24.2 (CH_3, 2 carbons), 23.8 (CH_2, 2 carbons); MS: *m*/*z* 410 $[M+H]^+$, 432 $[M+Na]^+$.
*Analytical data for compound **5a**:* Yellow oil; IR ($CHCl_3$, cm^{-1}): ν_{max} 3434, 2935, 1605, 1505, 1443, 1271; ^{1}H NMR (200 MHz, $CDCl_3$): δ_H = 1.33–1.72 (m, 8H), 1.94–2.00 (m, 2H), 2.64 (s, 2H), 3.30 (dd, *J* = 13.1, 7.0 Hz, 1H), 3.42 (dd, *J* = 13.1, 4.2 Hz, 1H), 4.07–4.13 (m, 2H), 4.22–4.33 (m, 1H), 6.44 (d, *J* = 2.2 Hz, 1H), 6.56 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.65–6.78 (m, 3H), 7.15–7.26 (m, 2H), 7.80 (d, *J* = 8.6 Hz, 1H)); ^{13}C NMR (50 MHz, $CDCl_3$): δ_C = 191.4 (CO), 165.0 (C), 161.6 (C), 147.9 (C), 129.3 (CH, 2 carbons), 128.3 (CH), 118.1 (CH), 115.0 (C), 113.3 (CH, 2 carbons), 109.3 (CH), 102.0 (CH), 80.5 (C), 70.3 (CH_2), 68.5 (CH), 47.8 (CH_2), 46.5 (CH_2), 34.8 (CH_2, 2 carbons), 25.1 (CH_2), 21.5 (CH_2, 2 carbons); MS: *m*/*z* 382 $[M+H]^+$, 404 $[M+Na]^+$.
*Analytical data for compound **6b**:* Colorless oil; IR ($CHCl_3$, cm^{-1}): ν_{max} 3447, 1674, 1607, 1541 1522, 1426; ^{1}H NMR (200 MHz, $CDCl_3$): δ_H = 1.46 (s, 9H), 1.59–1.67 (m, 2H), 1.98–2.05 (m, 2H), 2.18 (s, 3H), 2.66 (s, 2H), 3.14–3.26 (m, 2H), 3.35 (dd, *J* = 13.0, 6.9 Hz, 1H), 3.47 (dd, *J* = 13.0, 4.2 Hz, 1H), 3.83–3.90 (m, 2H), 4.11–4.14 (m, 2H), 4.28–4.38 (m, 1H), 6.47 (d, *J* = 2.1 Hz, 1H), 6.58 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.65–6.74 (m, 2H), 7.06–7.17 (m, 2H), 7.84 (d, *J* = 8.9 Hz, 1H)); ^{13}C NMR (50 MHz, $CDCl_3$): δ_C = 190.1 (CO), 165.1 (C), 160.9 (C), 154.7 (C), 145.8 (C), 130.3 (CH), 128.5 (CH), 127.2 (CH), 122.7 (C), 117.8 (CH), 115.0 (C), 110.1 (CH), 109.8 (CH), 102.1 (CH), 79.9 (C), 78.3 (C), 70.6 (CH_2), 68.4 (CH), 47.7 (CH_2, 2 carbons), 46.4 (CH_2, 2 carbons), 34.0 (CH_2, 2 carbons), 28.4 (CH_3, 3 carbons), 17.5 (CH_3); MS: *m*/*z* 497 $[M+H]^+$, 519 $[M+Na]^+$.
10. The crystallographic data of compound **4h** has been deposited with the Cambridge Crystallographic Data Center as deposition No. CCDC 895296. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (1223) 336033; e-mail: deposit@ccdc.cam.ac.uk].
11. NCCLS-National Committee for Clinical Laboratory Standards, Antimycobacterial susceptibility testing for *Mycobacterium tuberculosis*. Proposed standard M24-T; Villanova, PA, 1995.