Med Chem Res (2011) 20:1515–1522
DOI 10.1007/s00044-010-9402-6

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Search of antimycobacterial activities in hybrid molecules with benzopyran skeleton

Rama P. Tripathi · Surendra Singh Bisht · Vivek Parashar Pandey · Sarvesh K. Pandey · Shubhra Singh · Sudhir Kumar Sinha · Vinita Chaturvedi

Received: 9 March 2010 / Accepted: 26 July 2010 / Published online: 6 August 2010

Abstract A series of hybrid molecules (**7–9**, **12**, **13**, and **14–18**) consisting of chromans and pyrolidines or cyclic amines were prepared either by (3 + 2) cycloaddition of nitrostyrenes and azomethine ylide or by three component reactions of chromanyl aldehydes, acetylenes, and cyclic amines. All the synthesized compounds were evaluated against both avirulent (H37Ra) and virulent (H37Rv) strains of *Mycobacterium tuberculosis*. Out of all the compounds screened, compounds **16**, **17**, and **18** were found to be active against the virulent strain *M. tuberculosis* H37Rv displaying MIC in the range of 6.25–1.56 μg/ml against.

Keywords *Mycobacterium tuberculosis* · Tuberculosis · Benzopyran · (3 + 2) Cycloaddition · Multi-component reaction

Introduction

Mycobacterium tuberculosis, responsible for tuberculosis (TB) in human, causes the death of almost 3 million people annually, and it is positioned as the leading bacterial infectious agent (Tripathi *et al*., 2005; Dwivedi *et al*., 2008; Cegielski *et al*., 2002; Spigelman, 2007). Despite the availability of four drug regimens to treat TB, loss of human lives is essentially unabated as a result of poverty, synergy with the HIV/AIDS pandemic, and the emergence of multidrug resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis*. Moreover, nowadays more people die from TB (Spigelman, 2007); therefore, the present scenario involves the development of new drugs regimens which are effective against MDR and XDR TB and latent TB. New chemotherapeutic agents that will shorten the duration of current chemotherapy are also needed as the treatment schedule of the disease is 6–12 months. Although some recent progresses have been made due to 10 potent leads in antiTB drug pipeline, yet none of them are expected to hit the market before 2012, the stipulated time. Therefore, extensive efforts are being made globally to develop new chemical entities against this devastating pathogen taking into account both the serendipitous and rational drug design approach. Very recently compounds with chromeno and chromano dibenzofurans (**A** and **B**) skeletons were shown to posses' potent antitubercular activity in vitro (Prado *et al.,* 2006; Prado *et al.,* 2007). To establish structure activity relationship, a number of other derivatives of benzofuran having fused benzopyran were prepared, but the compounds had lower antitubercular activity and they were also cytotoxic. Apart from this, certain chromene-based compounds have also shown antibacterial activities (Fukushima *et al*., 2000; Nicolaou *et al*., 2001; Babu *et al*., 2003; Prado *et al*., 2006; Prado *et al*., 2007; Brown *et al*., 2004) (Fig. 1).

Pyrrolidine derivatives on the other hand showed a wide range of biological activities (Pandey *et al*., 2007; Tsou, *et al*., 2008) including the noteworthy antitubercular activities (He Xin *et al*., 2006). Receiving impetus from above reports and guided by the principle that the combination of two or more biologically versatile moieties in a single molecular frame often enhances the biological profile of molecule many folds (Morphy *et al*., 2004),

R. P. Tripathi (✉) · S. S. Bisht · V. P. Pandey · S. K. Pandey
Medicinal and Process Chemistry Division, Central Drug Research Institute, CSIR, Lucknow 226001, India
e-mail: rpt.cdri@gmail.com

S. Singh · S. K. Sinha · V. Chaturvedi
Drug Target Discovery and Development Division, Central Drug Research Institute, CSIR, Lucknow 226001, India

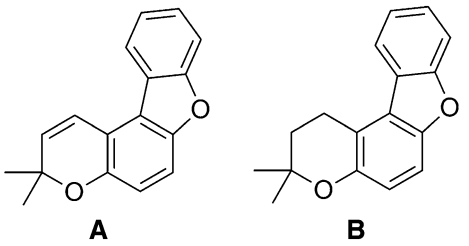


Fig. 1 Antitubercular chromeno and chromano benzofurans **A** and **B**

hybridization of two or more than two pharmacophores was envisaged. Herein, we have synthesized certain hybrid molecules consisting of chromans, chromenes, cyclic amines, and pyrrolidine and evaluated them for their antitubercular profiles against *M. tuberculosis*.

Results and discussion

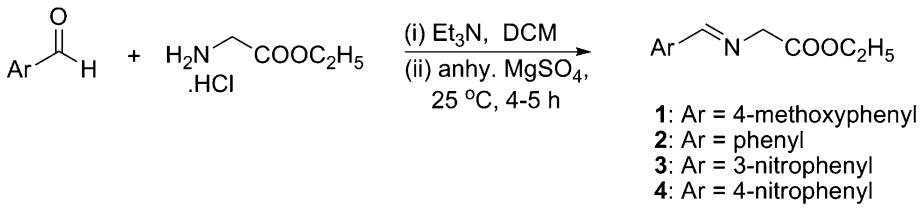
In the first set of 3 + 2 cycloaddition reactions to access chromanyl and chromenyl pyrrolidines, we have carried out reactions of (benzylidene) amino acetic acid ethyl esters with different nitrostyrenes. The (benzylidene) amino acetic acid ethyl esters **1–4** were prepared (Scheme 1) by reaction of different aromatic aldehydes and ethylglycinate hydrochlorides by our earlier reported method (Pandey *et al*., 2007) and used as such for further reaction. The starting nitrostyrene **(6)** was prepared (Scheme 2) by reaction of preformed 2,2-dimethyl-chroman-6-carbaldehyde **(5)** (obtained by the reaction of 4-hydroxy benzaldehyde with isoprene in the presence of *ortho*-phosphoric acid, Ahluwalia and Arora 1981) with nitromethane in the presence of ammonium acetate in acetic acid (Cheng *et al.*, 2009) (Scheme 2).

Thus, cycloaddition of the above nitrostyrene **6** with [(4-methoxybenzylidene)-amino]-acetic acid ethyl ester (**1**) in acetonitrile in the presence of DBU–LiBr as a catalyst led to the formation of the expected ethyl-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-(4-methoxyphenyl)pyrrolidine-2-carboxylate **(7)** as a major compound along with small amount of other products as observed on TLC (Scheme 3) (Annunziata *et al*., 1991; Galley *et al*., 1995; Pätzel *et al*., 1993). The compound **7** was isolated by column chromatography and characterized on the basis of its spectroscopic data. The relative stereochemistry in compound **7** was speculated on the basis of literature precedents and the mechanism (Annunziata *et al*., 1991; Galley *et al*., 1995). It has earlier been reported that in such cycloaddition reactions involving a chiral dienophile and azomethine ylide, the relative orientation of substituents in major isomer at C2/C3 and C3/C4 is *anti* and at C4/C5 is *syn*. Furthermore, the relative stereochemistry is attained via regiospecific endo cycloaddition of the W-shaped dipole to the *E* configured dipolarophiles in the ester series.

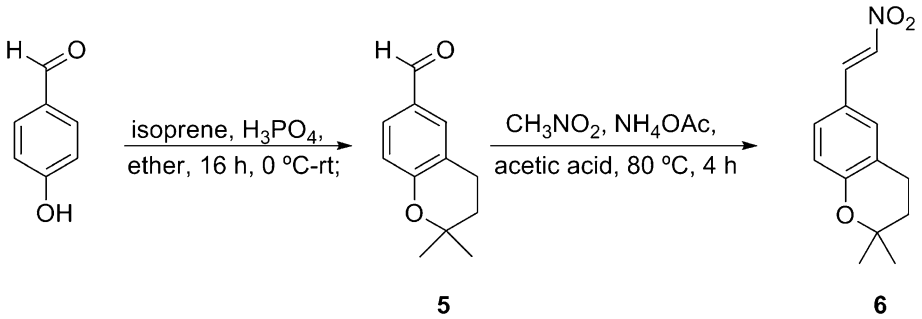
Similarly, 3 + 2 cycloaddition of reaction of the in situ generated azomethine ylides from the [(benzylidene)-amino]-acetic acid ethyl ester **(2)** and [(3-nitrobenzylidene)-amino]-acetic acid ethyl ester **(3)** with the above nitrostyrene derivative **6** led to the formation of respective chromanyl pryrrolidine derivatives **8** and **9** in good yields (Scheme 3).

In yet another attempt to prepare fused chromanyl pyrrolidines, the 3 + 2 cycloaddition of azomethine ylide of [(4-methoxybenzylidene)-amino]-acetic acid ethyl ester **1** and [(4-nitrobenzylidene)-amino]-acetic acid ethyl ester **4** with 3-nitrochromene derivative **11** was carried out. Compound **11**, in turn, was prepared by recently reported reaction of 4-methoxy nitrostyrene **10** and salicylaldehyde

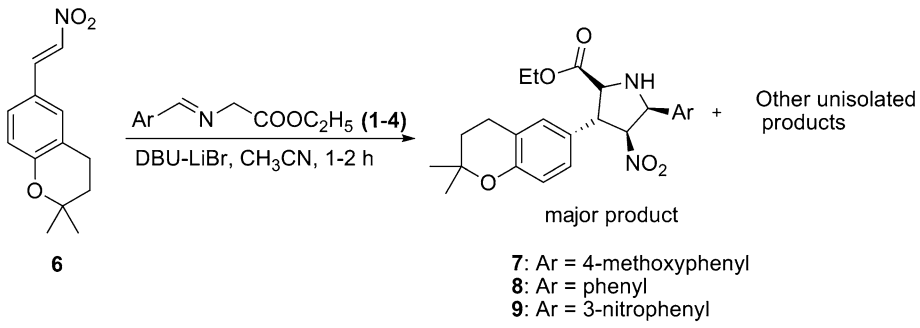
Scheme 1 Synthesis of (benzylidene) amino acetic acid ethyl esters (**1–4**)



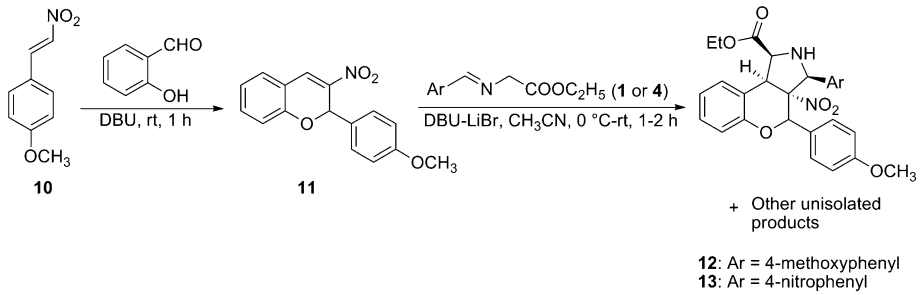
Scheme 2 Synthesis of (E)-2, 2-dimethyl-6-(2-nitrovinyl)chroman (**6**)



Scheme 3 Synthesis of chromanyl pyrrolidine derivatives (**7**, **8**, and **9**)



Scheme 4 Synthesis of fused chromanyl pyrrolidines (**12** and **13**)



(Viranyi *et al*., 2006). Thus, 3 + 2 cycloaddition of **1** and **4** separately with **11** in acetonitrile in the presence of DBU–LiBr led to the formation of respective ethyl-4-(4-methoxyphenyl)-3a-nitro-3-(4-methoxyphenyl)-1,2,3,3a,4,9b-hexahydrochromeno[3,4-c]pyrrole-1-carboxylates **(12)** and ethyl-4-(4-nitrophenyl)-3a-nitro-3-(4-methoxyphenyl)-1,2,3,3a,4,9b-hexahydrochromeno[3,4-c]pyrrole-1-carboxylates **(13)**, respectively in good yields (Scheme 4). The structures of the compounds **12** and **13** were in full agreement with the spectroscopic data.

Further, in order to see the antitubercular activity profiles in the hybrid compounds of three moieties the acetylenes, chroman, and cyclic amines, a three component reactions of the above chromanyl carbaldehyde **5** with different acetylenes and cyclic amines in the presence of zinc(II) acetate (Ramu *et al*., 2007) were carried out separately to get the desired 4-(1-(2,2-dimethylchroman-6-yl)-3-phenylprop-2-ynyl) cyclic amines **(14–18)** in good yields (Scheme 5).

Biological activity

The above synthesized compounds **7–9**, **12**, **13**, and **14–18** were evaluated against *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv strains following earlier protocols (Collins and Franzblan, 1997; Saito *et al.,* 1991). The results are depicted in Table 1. As evident from Table 1 among all the compounds screened, except compounds **7** and **12**, all were active against the virulent strain, *M. tuberculosis* H37Rv with MIC values ranging from 12.5–1.56 μg/ml, while these compounds were inactive against the avirulent strain, indicating the selectivity of these compounds toward virulent strain only. Among the active compounds, only three compounds **15**, **16**, and **17** from 4-(1-(2,2-dimethylchroman-6-yl)-3-phenylprop-2-ynyl) cyclic amines series showed potent antitubercular activity with MIC of 6.12, 1.56, and 3.12, respectively. Thus, the most potent compound of the series, compound **16** (MIC 1.56 μg/ml) opens up new door to optimize this

Scheme 5 Synthesis of 4-(1-(2,2-dimethylchroman-6-yl)-3-phenylprop-2-ynyl) cyclic amines (**14**–**18**)

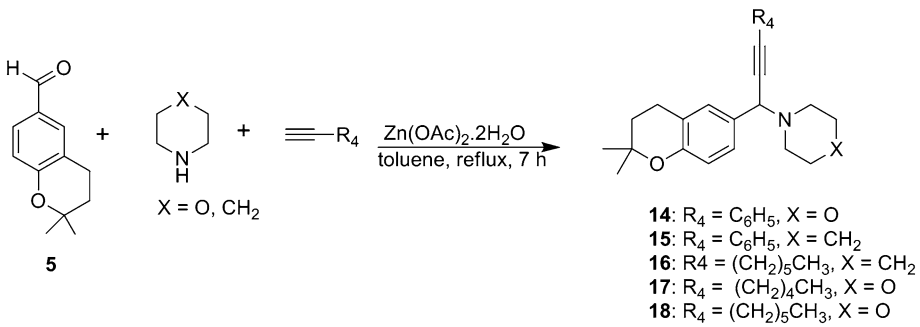


Table 1 Antitubercular activity of synthesized compounds **7–9**, **12**, **13**, and **14–18** against *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv

S. No.	Compound	MIC (μg/ml) against *M. tuberculosis* H37Rv	MIC (μg/ml) against *M. tuberculosis* H37Ra
1	**7**	>12.5	>12.5
2	**8**	12.5	>12.5
3	**9**	12.5	>12.5
4	**12**	>12.5	>12.5
5	**13**	12.5	>12.5
6	**14**	12.5	>12.5
7	**15**	6.25	>12.5
8	**16**	1.56	>12.5
9	**17**	3.12	>12.5
10	**18**	ND[a]	>12.5
11	**Isoniazid**	0.75	–
12	**Ethambutol**	3.25	–

[a] *ND* not done, *MIC* minimum inhibitory concentration

series for new class of antituberculars. Further, the compounds being specific to the virulent strains only, also point out the target important for infective mycobacteria, which is yet to be determined.

In conclusion, we have synthesized hybrid molecules with chroman, pyrrolidine, and cyclic amines as combination of pharmacophores using either (3 + 2) cycloaddition or three component reaction. The compounds were screened against avirulent and virulent strains of *M. tuberculosis*. Three of the compounds **15**, **16**, and **17** displayed MIC in the range of 6.25–1.56 μg/ml, a criterion for further optimization of the series for new antitubercular agents.

Experimental section

Chemistry

Commercially available reagent grade chemicals were used as received. All the reaction were followed by TLC on E. Merck Kieselgel 60 F_{254}, with detection by UV light, spraying a 20% $KMnO_4$ aq solution. Column chromatography was performed on silica gel (60–120 mesh and 100–200 mesh, E. Merck). IR spectra were recorded as thin films or in KBr soln with a Perkin Elmer Spectrum RX—1 (4000–450 cm^{-1}) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-300 in $CDCl_3$. Chemical shift values are reported in ppm relative to TMS (tetramethylsilane) as internal reference, unless otherwise stated, s (singlet), d (doublet), t (triplet), m (multiplet); *J* in hertz. ESI mass spectra were performed using Quattro II (Micromass).

General procedure for preparation of benzylidene ethyl glycinates (**1–4**)

To a solution of ethyl glycinate hydrochloride salt (2 eq.) and anhydrous $MgSO_4$ in dry CH_2Cl_2 was added aromatic aldehydes (1 eq.), and the reaction was stirred for 3–4 h at ambient temperature. After the completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to get the desired benzylidene ethyl glycinates (**1–4**). The freshly prepared benzylidine ethyl glycinates (**1–4**) were used as such.

Preparation of 2,2-di-methyl chroman-6-carbaldehyde (**5**)

To a stirring solution of 4-hydroxybenzaldehyde (1 g, 8.19 mmol), orthophosphoric acid (0.94 ml, 16.39 mmol) in petroleum ether (10 ml) was added isoprene (1.64 ml, 16.39 mmol) at 0°C. The reaction mixture was stirred at room temperature for 16 h. After completion of reaction (TLC), the reaction mixture was poured into beaker containing crushed ice and sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over sodium sulfate then concentrate under reduced pressure. Purification of the residue by silica gel chromatography (EtOAc/n-hexane, 1:3) provided 2,2-di-methyl chroman-6-carbaldehyde **5** as liquid, 0.8 g, 51.3%. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.35 (s, 6H, $CH_3\times2$), 1.81–1.85 (m, 2H, CH_2), 2.82 (t, 2H, $J = 6.7$ Hz, OCH_2), 6.85 (d, 1H, $J = 9.0$ Hz, ArH), 7.58–7.61(m, 2H, ArH), 9.80 (s, 1H, CHO).

Preparation of (E)-2,2-dimethyl-6-(2-nitrovinyl) chroman (**6**)

To a stirred solution of 2,2-dimethylchroman-6-carbaldehyde **5** (1 g, 5.26 mmol), nitromethane (5 ml, as reagent and as solvent), ammonium acetate (0.41 g, 5.26 mmol), and acetic acid (1 ml) were heated at 80°C for 4 h. After completion of reaction (TLC), the reaction mixture was poured onto ice; excess of acetic acid was neutralized by sodium bicarbonate extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to get compound **6** as syrup. Yield 0.8 g (65.23%). FT-IR (KBr, cm^{-1}) 1657, 1256; ^1H NMR (200 MHz, $CDCl_3$) δ 1.35 (s, 6H, $C(CH_3)_2$), 1.86 (t, 2H, $J = 6.4$ Hz, CH_2), 2.82 (t, 2H, $J = 6.4$ Hz, CH_2), 6.80 (d, 1H, $J = 8.3$ Hz, ArH), 7.25–7.29 (m, 2H, ArH), 7.42 (d, 1H, $J = 13.4$ Hz, =CH), 7.85 (d, 1H, $J = 13.4$ Hz, =CH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.6 (CH_2), 27.3 (CH_3), 32.8 (CH_2), 118.9 (ArCH), 122.1 (ArC), 129.0 (ArCH), 131.7, 135.0, 139.4 (ArCH), 158.4 (ArC); ESMS (m/z): 234 $[M + H]^+$.

Preparation of 2-(4-methoxyphenyl)-3-nitro-2H-chromene **(11)**

To a stirred solution of (*E*)-1-methoxy-4-(2-nitrovinyl) benzene **10** (1 g, 5.58 mmol), salicylaldehyde (0.88 g, 6.13 mmol), 0.242 ml of DBU (30 mol %) was added at room temperature. After completion of reaction (TLC), the reaction mixture was extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to get compound **11** as yellow solid. Yield 1.3 g (82.2%). FT-IR (KBr, cm^{-1}) 1657, 1256; ^1H NMR (200 MHz, $CDCl_3$) δ 3.75 (s, 3H, OCH_3), 6.51 (s, 1H, H-2), 6.79–6.85 (m, 3H, ArH), 6.98–7.02 (m, 1H, ArH), 7.25–7.34 (m, 4H, ArH), 8.03 (s, 1H, H-4); ESMS (m/z): 234 $[M + H]^+$.

General procedure for preparation of compounds (**7**, **8**, **9**, **12**, and **13**)

To a stirring solution of DBU (10 mol%) and LiBr (10 mol%) in acetonitrile was added [(benzylidene)amino]-acetic acid ethyl ester (**1–4**, 1 eq.) at 0°C and stirring continued for further 30 min and followed by addition of compound **3** or compound **8** (1 eq.). After complete addition, the reaction mixture was stirred at room temperature for further 1 h. After completion of the reaction (TLC), the reaction mixture was extracted with ethyl acetate (2× 70 ml); the organic layer was dried (anh. Na_2SO_4) and evaporated under reduced pressure to get a crude product. The latter was chromatographed (SiO_2 column, 60–120 mesh) using a gradient of hexane: ethyl acetate (6:4) to give the desired compounds (**7**, **8**, **9**, **12**, and **13**).

Ethyl-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-(4-methoxyphenyl)pyrrolidine-2-carboxylate **(7)**

It was obtained by the reaction of compound **6** (0.5 g, 2.14 mmol), [(4-methoxybenzylidene) amino]-acetic acid ethyl ester **1** (0.47 g, 2.14 mmol), and DBU–LiBr (1:1, 10 mol%) as liquid, 0.65 g, in 66.6% yield. FT-IR (KBr, cm^{-1}) 1657, 1730, 3402; ^1H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3), 1.22–1.33 (m, 6H, $C(CH_3)_2$), 1.79 (m, 2H, CH_2), 2.16 (bs, 1H, NH), 2.75 (t, 2H, $J = 6.7$ Hz, CH_2), 3.75–3.82 (m, 5H, OCH_3 and CH×2), 4.27–4.32 (m, 2H, OCH_2), 4.65 (d, 1H, $J = 8.5$ Hz, CH), 5.11 (m, 1H, CH), 6.70 (d, 1H, $J = 8.2$ Hz, ArH), 6.89–6.96 (m, 4H, ArH), 7.48 (d, 2H, $J = 8.7$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.68 (CH_3), 22.8 (CH_2), 27.3, 27.2 (CH_3), 33.14 (CH_2), 55.4 (OCH_3), 61.28 (OCH_2), 64.36, 67.53 (CH), 95.30 (C), 114.50, 114.80 (ArC), 117.98 (ArCH), 121.13 (ArC), 127.20 (ArCH), 128.31 (ArC), 128.53, 129.30 (ArCH), 129.96 (ArC), 154.31, 160.42 (ArC), 172.11 (*C*OOEt); ESMS (m/z): 455 $[M + H]^+$.

Ethyl-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-phenylpyrrolidine-2-carboxylate **(8)**

It was obtained by the reaction of compound 6 (0.5 g, 2.14 mmol), [(benzylidene) amino]-acetic acid ethyl ester 2 (0.41 g, 2.14 mmol) and DBU–LiBr (1:1, 10 mol%) as liquid, 0.40 g, in 43.9% yield. FT-IR (KBr, cm^{-1}) 3401, 1728, 1603; ^1H NMR (200 MHz, $CDCl_3$) δ 0.82–1.34 (m, 9H, OCH_2CH_3 and $C(CH_3)_2$), 1.84 (t, $J = 7.6$ Hz, 2H, CH_2), 2.73 (t, 2H, $J = 7.7$ Hz, CH_2), 3.98–4.06 (m, 2H), 4.24–4.32 (m, 2H, OCH_2), 4.67 (m, 1H, CH), 5.17 (d, 1H, $J = 2.6$ Hz, CH), 6.75 (d, 1H, $J = 8.2$ Hz, ArH), 6.96 (d, 2H, $J = 9.2$ Hz, ArH), 7.25–7.43 (m, 5H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.92, 14.68 (CH_3), 22.9 (CH_2), 27.3, (CH_3), 33.07 (CH_2), 61.93 (OCH_2), 68.04 (CH), 80.18 (C), 97.58 (CH), 118.44 (ArCH), 121.80 (ArC), 126.58 (ArCH), 128.44, 128.11 (ArC), 128.80, 129.09, 129.20 (ArCH), 130.01, 135.08, 139.09 (ArC), 154.31 (ArC), 171.8 (*C*OOEt); ESMS (m/z): 425 $[M + H]^+$.

Ethyl-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-(3-nitrophenyl)pyrrolidine-2-carboxylate **(9)**

It was obtained by the reaction of compound **6** (0.5 g, 2.14 mmol), [(3-nitrobenzylidene)-amino]-acetic acid ethyl ester **3** (0511 g, 2.14 mmol), and DBU–LiBr (1:1, 10 mol%) as liquid, 0.42 g, in 42.2% yield. FT-IR (KBr, cm^{-1}) 3422, 1727, 1609; ^1H NMR (200 MHz, $CDCl_3$) δ 1.26–1.34 (m, 9H, OCH_2CH_3 and $C(CH_3)_2$), 1.77 (t, $J = 6.6$ Hz, 2H, CH_2), 2.74 (t, 2H, $J = 6.6$ Hz, CH_2), 4.09–4.11 (m, 2H), 4.25–4.32 (m, 2H, OCH_2), 4.99 (d, 1H, CH), 5.24 (d, 1H, $J = 3.7$ Hz, CH), 6.74 (d, 1H, $J = 8.1$ Hz, ArH), 6.96 (m, 2H, ArH), 7.54–7.58 (d, 1H, $J = 7.9$ Hz, ArH), 7.70 (d, 1H, $J = 8.14$ Hz, ArH), 8.16 (d, 1H, $J = 8.17$ Hz, ArH), 8.28 (s, 1H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.61 (CH_3), 22.92 (CH_2), 27.30, 27.34 (CH_3), 33.03 (CH_2), 62.02 (OCH_2), 66.42, 67.33, 74.84 (CH), 96.76 (CH), 118.53 (ArCH), 121.94 (ArC), 122.67, 124.00, 126.55 (ArCH), 130.07, 132.78 (ArCH), 138.02, 148.70, 154.44 (ArC), 171.49 (*C*OOEt); ESMS (m/z): 470 $[M + H]^+$.

Ethyl-4-(4-methoxyphenyl)-3a-nitro-3-(4-methoxyphenyl)-1,2,3,3a,4,9b-hexahydrochromeno [3,4-c]pyrrole-1-carboxylate **(12)**

It was obtained by the reaction of compound **11** (0.5 g, 1.76 mmol), [(4-methoxybenzylidene) amino]-acetic acid ethyl ester (0.39 g, 1.76 mmol), and DBU–LiBr (1:1, 10 mol%) as white solid, m. p. 125°C, 0.54 g, in 60.8% yield. FT-IR (KBr, cm^{-1}) 3431, 1729, 1596; ^1H NMR (200 MHz, $CDCl_3$) δ 1.29 (t, 3H, $J = 7.2$ Hz, CH_3), 3.72–3.79 (m, 7H, OCH_3×2 and CH), 4.22–4.32 (q, 2H,

$J = 7.14$ Hz, OCH_2CH_3), 5.38–5.41 (m, 2H, CH×2), 6.01 (s, 1H, CH), 6.67–7.12 (m, 11H, ArH), 8.16 (d, $J = 8.0$ Hz, ArH); ESMS (m/z): 505 $[M + H]^+$.

Ethyl-4-(4-methoxyphenyl)-3a-nitro-3-(4-nitrophenyl)-1,2,3,3a,4,9b-hexahydrochromeno[3,4-c]pyrrole-1-carboxylate **(13)**

It was obtained by the reaction of compound **11** (0.5 g, 1.76 mmol), [(4-nitrobenzylidene)amino]-acetic acid ethyl ester **4** (0.41 g, 1.76 mmol), and DBU–LiBr (1:1, 10 mol%) as white solid, m. p. 179–180°C, 0.49 g, in 54.5% yield. FT-IR (KBr, cm^{-1}) 3385, 1740, 1602, 1545; ^1H NMR (200 MHz, $CDCl_3$) δ 1.50 (t, 3H, $J = 7.1$ Hz, CH_3), 2.91 (m, 1H, NH), 3.70 (s, 3H, OCH_3), 4.06 (d, 1H, $J = 3.7$ Hz, CH), 4.35 (q, 2H, $J = 7.08$ Hz, OCH_2CH_3), 4.98 (s, 1H, CH), 5.41 (s, 1H, CH), 6.60 (d, 2H, $J = 8.6$ Hz, ArH), 6.76 (d, 1H, $J = 7.9$ Hz, ArH), 6.96–7.19 (m, 5H, ArH), 7.46–7.55 (m, 2H, ArH), 8.19 (d, 2H, $J = 8.6$ Hz, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.76 (CH_3), 45.34, 55.55, 55.54 (OCH_3), 62.69 (OCH_2), 68.95, 75.58 (CH), 114.40, 118.77 (ArCH), 123.82, 124.42, 128.65, 129.22, 129.56, 129.98 (ArCH), 142.42, 149.03, 150.30, 160.44 (ArC), 172.68 (*C*OOEt); ESMS (m/z): 520 $[M + H]^+$.

General procedure for preparation of compound (**14–18**)

A solution of alkyne (1.5 mmol), $Zn(OAc)_2{\cdot}2H_2O$ (0.1 mmol), aldehyde (1.0 mmol), and amine (1.3 mmol) in toluene was taken in a round-bottomed flask and inserted into a preheated oil bath (120°C bath temperature) and stirring continued for given time. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with aq NH_4Cl (2.5 ml) and stirred for 5 min. The aqueous layers were extracted with diethyl ether, dried over Na_2SO_4 and concentrated to give the crude product which was further purified by column chromatography on silica gel (ethylacetate/hexane = 1:6) to afford the corresponding pure propargylamine.

4-(1-(2,2-dimethylchroman-6-yl)-3-phenylprop-2-ynyl)morpholine **(14)**

It was obtained by the reaction of compound **5** (0.5 g, 2.63 mmol), morpholine (0.297 ml, 3.42 mmol), phenylacetylene (0.43 ml, 3.9 mmol), and $Zn(OAc)_2{\cdot}2H_2O$ (0.057 g, 0.263 mmol) as yellow liquid, 0.79 g, in 84.2% yield. FT-IR (KBr, cm^{-1}) 2927, 1697, 1618, 1533; ^1H NMR (200 MHz, $CDCl_3$) δ 1.33 (s, 6H, $C(CH_3)_2$), 1.83 (t, 2H, $J = 6.6$ Hz, CH_2), 2.57–2.65 (m, 4H, $–CH_2NCH_2–$), 2.75 (t, 2H, $J = 6.7$ Hz, CH_2), 3.69 (t, 4H, $–CH_2OCH_2–$), 4.65 (s, 1H, CH), 6.71 (d, 1H, $J = 8.2$ Hz, ArH), 7.25–7.32 (m, 5H, ArH), 7.46–7.51 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.00 (CH_2), 27.36 (CH_3), 32.24 (CH_2), 50.29 (NCH_2), 62.03 (CH), 67.52 (OCH_2), 86.07, 88.48 (C≡C), 117.38 (ArCH),120.71, 123.59 (ArC), 128.08, 128.64, 129.06, 129.96, 132.21 (ArCH), 154.04 (ArC); ESMS (m/z): 361 $[M + H]^+$.

1-(1-(2,2-dimethylchroman-6-yl)-3-phenylprop-2-ynyl)piperidine **(15)**

It was obtained by the reaction of compound **5** (0.5 g, 2.63 mmol), piperidine (0.34 ml, 3.42 mmol), phenylacetylene (0.43 ml, 3.9 mmol), and $Zn(OAc)_2{\cdot}2H_2O$ (0.057 g, 0.263 mmol) as yellow liquid, 0.79 g, in 84.6% yield. FT-IR (KBr, cm^{-1}) 2972, 2932, 2854, 1588, 1492; ^1H NMR (200 MHz, $CDCl_3$) δ 1.32 (s, 6H, $C(CH_3)_2$), 1.40–1.48 (m, 6H, CH_2 piperidine ring) 1.75 (t, 2H, $J = 6.7$ Hz, CH_2), 2.53–2.55 (m, 4H, $–CH_2NCH_2–$), 2.74 (t, 2H, $J = 6.7$ Hz, CH_2), 4.66 (s, 1H, CH), 6.70 (d, 1H, $J = 8.3$ Hz, ArH), 7.24–7.31 (m, 5H, ArH), 7.46–7.51 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.00, 24.96, 26.61 (CH_2), 27.41 (CH_3), 33.33 (CH_2), 51.04 (NCH_2), 62.34 (CH), 74.44, 87.04, 87.91 (C≡C), 117.18 (ArCH), 120.47, 123.99 (ArC), 127.98, 128.25, 28.59, 129.84, 129.91,132.21 (ArCH), 153.79 (ArC); ESMS (m/z): 362 $[M + H]^+$.

1-(1-(2,2-dimethylchroman-6-yl)non-2-ynyl)piperidine **(16)**

It was obtained by the reaction of compound **5** (0.5 g, 2.63 mmol), piperidine (0.297 ml, 3.42 mmol), octyne (0.58 ml, 3.9 mmol), and $Zn(OAc)_2{\cdot}2H_2O$ (0.057 g, 0.263 mmol) as yellow liquid, 0.84 g, in 88.0% yield. FT-IR (KBr, cm^{-1}) 2931, 2856, 1656, 1584; ^1H NMR (200 MHz, $CDCl_3$) δ 0.86 (t, 3H, $J = 6.5$ Hz, CH_3), 1.32 (s, 6H, $C(CH_3)_2$), 1.38–1.45 (m, 14H), 1.75 (t, 2H, $J = 6.6$ Hz, CH_2), 2.26 (m, 2H, CH_2), 2.44 (m, 4H, $–CH_2NCH_2–$), 2.73 (t, 2H, $J = 6.6$ Hz, CH_2), 4.40 (s, 1H, CH), 6.66 (d, 1H, $J = 8.2$ Hz, ArH), 7.17–7.18 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.51 (CH_3), 19.27, 22.99, 23.04, 25.00, 29.02, 29.49, 31.79, 33.33, 50.86 (CH_2), 26.57 (CH_3), 61.91(CH), 74.35, 87.75 (C≡C), 116.98 (ArCH), 120.24 (ArC), 127.97, 129.81 (ArCH), 130.53, 153.56 (ArC). ESMS (m/z): 368 $[M + H]^+$.

4-(1-(2,2-dimethylchroman-6-yl)oct-2-ynyl)morpholine **(17)**

It was obtained by the reaction of compound **5** (0.5 g, 2.63 mmol), morpholine (0.297 ml, 3.42 mmol), heptyne (0.52 ml, 3.9 mmol), and $Zn(OAc)_2{\cdot}2H_2O$ (0.057 g, 0.263 mmol) as yellow liquid, 0.75 g, in 80.8% yield.

FT-IR (KBr, cm^{-1}) 2934, 2864, 1670, 1576; ^1H NMR (200 MHz, $CDCl_3$) δ 0.88 (t, 3H, $J = 7.0$ Hz, CH_3), 1.32–1.58 (m, 12H, $C(CH_3)_2$, $CH_2\times3$), 1.75 (t, 2H, $J = 6.6$ Hz, CH_2), 2.26 (m, 2H, CH_2), 2.49 (m, 4H, $–CH_2NCH_2–$), 2.73 (t, 2H, $J = 6.6$ Hz, CH_2), 3.66 (m, 4H, $–CH_2OCH_2$), 4.39 (s, 1H, CH), 6.67 (d, 1H, $J = 8.1$ Hz, ArH), 7.18–7.22 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.44 (CH_3), 19.19, 22.59, 22.97, 29.13, 30.09, 31.56, 33.28, 50.14 (CH_2), 27.35 (CH_3), 61.64 (CH), 67.47 (CH_2), 74.44, 76.37 (C≡C), 117.19 (ArCH), 120.47 (ArC), 128.00, 129.89 (ArCH), 153.86 (ArC). ESMS (m/z): 356 $[M + H]^+$.

4-(1-(2,2-dimethylchroman-6-yl)non-2-ynyl)morpholine **(18)**

It was obtained by the reaction of compound **5** (0.5 g, 2.63 mmol), morpholine (0.297 ml, 3.42 mmol), octyne (0.58 ml, 3.9 mmol), and $Zn(OAc)_2{\cdot}2H_2O$ (0.057 g, 0.263 mmol) as yellow liquid, 0.74 g, in 76.5% yield. FT-IR (KBr, cm^{-1}) 2931, 2857, 1585, 1494; ^1H NMR (200 MHz, $CDCl_3$) δ 0.86 (t, 3H, $J = 6.6$ Hz, CH_3), 1.32–1.57 (m, 14H, $C(CH_3)_2$, $CH_2\times4$), 1.75 (t, 2H, $J = 6.6$ Hz, CH_2), 2.26 (m, 2H, CH_2), 2.49 (m, 4H, $–CH_2NCH_2–$), 2.73 (t, 2H, $J = 6.6$ Hz, CH_2), 3.66 (t, 4H, $J = 4.22$ Hz, $–CH_2OCH_2–$), 4.39 (s, 1H, CH), 6.67 (d, 1H, $J = 8.2$ Hz, ArH), 7.17–7.18 (m, 2H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.51 (CH_3), 19.24, 23.00, 29.04, 29.41, 31.75, 33.27, 50.14 (CH_2), 27.35 (CH_3), 61.63 (CH), 67.47 (CH_2), 74.35 (C≡C), 117.19, 128.01; ESMS (m/z): 370 $[M + H]^+$.

Biological activity

Activity against *M. tuberculosis* H37Ra strain

All of the synthesized compounds were evaluated for their efficacy against *M. tuberculosis* H37Ra at active concentration ranging from 50 μg/ml to MIC using twofold dilutions in the initial screen. Log phase culture of *M. tuberculosis* H37Ra is diluted so as to give final OD 550 nm of 0.05 in Sauton's medium. In 96-well white plates, 190 ml of culture is dispensed in each well. A dimethyl sulfoxide (DMSO) solution of test compounds is dispensed into 96-well plates so as to make final test concentration of 25 mg/ml (5 mg test compound is dispensed into 10 ml of DMSO). Then, the plate is incubated at 37°C/5% CO_2 for 5 days. On 5th day, 15 ml Alamar Blue solution is added to each well of the plate. The plate is again incubated overnight at 37°C/5% CO_2 incubator. The fluorescence is read on BMG polar star with excitation frequency at 544 nm and emission frequency at 590 nm. The compounds, which were found to be active (>90% inhibition as compared with control) at this concentration are then tested at six serial dilutions starting from 50 to 1.56 μg/ml.

Activity against *M. tuberculosis* H37Rv strain

Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H37Rv were performed by agar microdilution method where twofold dilutions of each test compound were added into 7H10 agar supplemented with OADC and organism. A culture of *M. tuberculosis* H37Rv growing on L–J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of 1 mg/ml concentration of extracts/compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 Middle Brook's medium (containing 1.7 ml medium and 0.2 ml OADC supplement) at different concentrations of compound keeping the volume constant, i.e., 0.1 ml. Medium was allowed to cool by keeping the tubes in slanting position. These tubes were then incubated at 37°C for 24 h followed by streaking of *M. tuberculosis* H37Rv ($5–10^4$ bacilli per tube). These tubes were then incubated at 37°C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.

Acknowledgments Surendra, Vivek, and Sarvesh are thankful to CSIR New Delhi for award of SRF and RA, respectively. We sincerely acknowledge the financial assistance from DRDO New Delhi. This is CDRI communication No. 7853.

References

Ahluwalia VK, Arora KK (1981) Nuclear isoprenylation of polyhydroxy acetophenones: acid catalysed condensation with isoprene. Tetrahedron 37:1437–1439

Annunziata R, Clinquini M, Cozzi F, Raimondi L, Pilati T (1991) 1,3-dipolar cycloaddition reactions of azomethine ylides on enantiomerically pure (*E*)-γ-alkoxy-α,β-unsaturated esters. Tetrahedron Asymmetry 2:1329–1342

Babu KS, Raju BC, Praveen B, Kishore KH, Murty US, Rao JM (2003) Microwave assisted synthesis and antimicrobial activity of 2,2-dimethylchromenes. Heterocycl Commun 9:519–526

Brown CW, Shengquan L, Klucik J, Berlin KD, Brennan PJ, Kaur D, Benbrook DM (2004) Novel heteroarotinoids as potential antagonists of *Mycobacterium bovis* BCG. J Med Chem 47:1008–1017

Cegielski JP, Chin DP, Espinal MA, Frieden TR, Rodriquez Cruz R, Talbot EA, Weil DE, Zaleskis R, Raviglione MC (2002) The global tuberculosis situation: progress and problems in the 20th century, prospects for the 21st century. Infect Dis Clin N Am 16:1–58

Cheng P, Chen JJ, Huang N, Wang RR, Zheng YT, Liang YZ (2009) Synthesis and anti-human immunodeficiency virus type 1

activity of (*E*)-N-phenylstyryl-*N*-alkylacetamide derivatives. Molecules 14:3176–3186

Collins LA, Franzblan SG (1997) Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob Agents Chemother 41:1004–1009

Dwivedi N, Dube D, Kukshal V, Srivastava SK, Ramachandran R, Tripathi RP (2008) NAD+ dependent DNA ligase (Rv3014c) from *M tuberculosis*: strategies for inhibitor design. Med Chem Res 17:189–198

Fukushima T, Tanaka M, Gohara M (2000) Benzophenone derivatives, their manufacture with exserohilum & their agricultural uses. US Patent JP 63, 376, see Chem Abstr 2000, 132, 179673r

Galley G, Liebscher J, Pätzel M (1995) Polyfuntionalized pyrrolidines by stereoslective 1,3-dipolar cycloaddition of azomethine ylides to chiral enones. J Org Chem 60:5005–5010

Morphy R, Kay C, Rankovic Z (2004) From magic bullets to designed multiple ligands. Drug Discov Today 9:641–651

Nicolaou KC, Roecker AJ, Barluenga S, Pfefferkorn JA, Cao GQ (2001) Discovery of novel antibacterial agents active against methicillin-resistant *Staphylococcus aureus* from combinatorial benzopyran libraries. Chem Bio Chem 2:460–465

Pätzel M, Galley G, Jones PG, Charapkowsky A (1993) Synthesis of enantiomerically pure pyrrolidines by stereospecific cycloaddition of azomethine ylides with enones. Tetrahedron Lett 34:5707–5710

Pandey J, Dwivedi N, Singh N, Srivastava AK, Tamarkar A, Tripathi RP (2007) Diastereoselective synthesis of glycosylated prolines as α-glucosidase inhibitors and organocatalyst in asymmetric aldol reaction. Bioorg Med Chem Lett 17:1321–1325

Prado S, Ledeit H, Michel S, Koch M, Darbord JC, Cole ST, Tillequin F, Brodin P (2006) Benzofuro[3,2-f][1]benzopyrans: a new class of antitubercular agents. Bioorg Med Chem 14:5423–5428

Prado S, Janin YL, Joanis BS, Brodin P, Michel S, Koch M, Cole ST, Tillequinc F, Bost PE (2007) Synthesis and antimycobacterial evaluation of benzofurobenzopyran analogues. Bioorg Med Chem 15:2177–2186

Ramu E, Varala R, Sreelatha N, Adapaa SR (2007) $Zn(OAc)_2{\cdot}2H_2O$: a versatile catalyst for the one-pot synthesis of propargylamines. Tetrahedron Lett 48:7184–7190

Saito H, Tomioka H, Sato K, Emori M, Yamane T, Yamashita K (1991) In vitro antimycobacterial activities of newly synthesized benzoxazinorifamycins. Antimicrob Agents Chemother 35: 542–547

Spigelman MK (2007) New tuberculosis therapeutics: a growing pipeline. J Infect Dis 196:S28–S34

Tripathi RP, Tewari N, Dwivedi N, Tiwari VK (2005) Fightingtuberculosis: an old disease with new challenges. Med Res Rev 25:93–131

Tsou EL, Chen SY, Yang MH, Wang SC, Cheng TRR, Cheng WC (2008) Synthesis and biological evaluation of a 2-aryl polyhydroxylated pyrrolidine alkaloid-based library. Bioorg Med Chem 16:10198–10204

Viranyi A, Marth G, Dancso A, Blasko G, TTke L, Nyerges M (2006) 3-Nitrochromene derivatives as 2p components in 1,3-dipolar cycloadditions of azomethine ylides. Tetrahedron 62:8720–8730

Xin He et al (2006) Pyrrolidine carboxamides as a novel class of inhibitors of enoyl acyl carrier protein reductase from *Mycobacterium tuberculosis*. J Med Chem 49:6308–6323