Bioorganic & Medicinal Chemistry Letters 23 (2013) 2404–2407

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of *trans* 6-methoxy-1,1-dimethyl-2-phenyl-3-aryl-2,3-dihydro-1*H*-inden-4-yloxyalkyl-amine derivatives against drug susceptible, non-replicating *M. tuberculosis* H37Rv and clinical multidrug resistant strains ☆

Shailesh Kumar [a,†], Atma P. Dwivedi [a], Vivek Kr. Kashyap [b], A. K. Saxena [a], A. K. Dwivedi [c], Ranjana Srivastava [b,*], Devi P. Sahu [a,*]

[a] *Medicinal and Process Chemistry Division, CSIR, Central Drug Research Institute, Lucknow 226001, India*
[b] *Microbiology Division, CSIR, Central Drug Research Institute, Lucknow 226001, India*
[c] *Pharmaceutics Division, CSIR, Central Drug Research Institute, Lucknow 226001, India*

ARTICLE INFO

Article history:
Received 16 October 2012
Revised 24 January 2013
Accepted 6 February 2013
Available online 15 February 2013

Keywords:
Antimycobacterial
MDR
M. tuberculosis H37Rv
trans 4-Aminoalkoxy-2-phenyl-3-aryl-2 3-Dihdro-1*H*-inden

ABSTRACT

Synthesis of a library of novel *trans* 6-methoxy-1,1-dimethyl-2-phenyl-3-aryl-2,3-dihydro-1*H*-inden-4-yloxy alkyl amines and their antimycobacterial activity against drug sensitive and multidrug resistant strains of *Mycobacterium tuberculosis* have been reported. All the new compounds in the series exhibited MIC between 1.56 and 6.25 μg/ml. Two compounds **1i** and **1j** with low MIC and low cytotoxicity showed significant reduction in CFU in infected mouse macrophages at 1× MIC concentration. The compound **1i** inhibited the growth of *M. tuberculosis* in mice at 100 mg/kg dose with 1.35 $\log_{10}$ reduction of CFU in lungs tissue and was active against non-replicating *Mycobacterium tuberculosis* under anaerobic condition.

Mycobacterium tuberculosis (*M. tb*), the causative agent for tuberculosis (TB) results in death of almost 3 million people each year and is positioned as the leading bacterial infectious agent.[1] Although an effective chemotherapy for tuberculosis is available, the frontline anti-tubercular drugs Isoniazid, Pyrazinamide and Rifamycin have been reported to have significant liver damaging effects. This along with the emergence of multi-drug resistance, reactivation of latent infection due to pathogenic synergy of the mycobacterial infection with the HIV infection[1,2] justifies the need for the design and development of new drug which would not only be active against multi-drug resistant TB (MDR-TB) and latent TB[3] but also, should be non-toxic and shorten the duration of current chemotherapy and be compatible with anti-HIV drugs.[4]

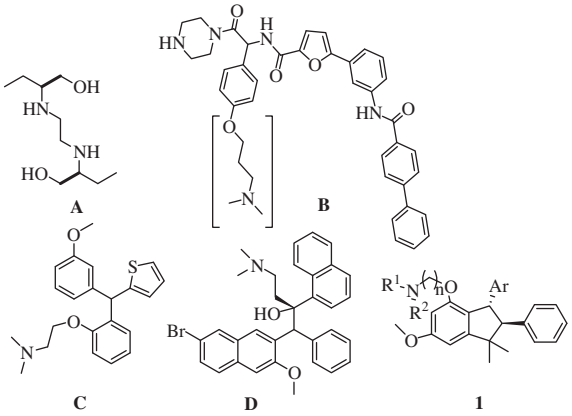


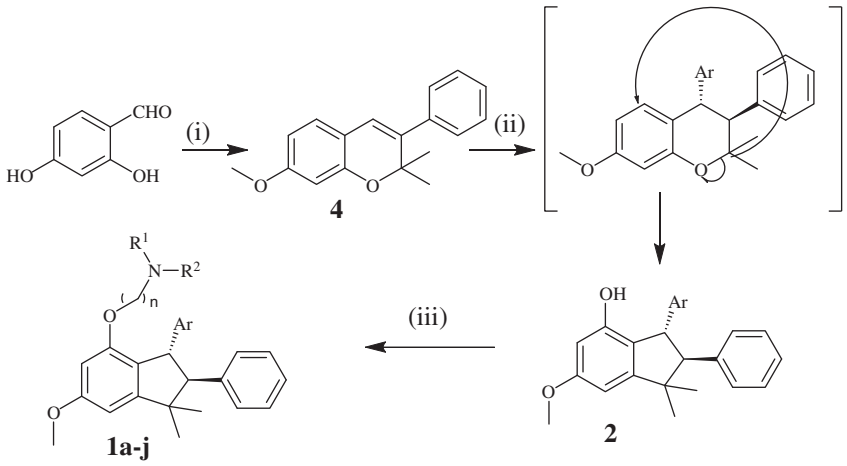
Figure 1.

Ethambutol **A** (Fig. 1), a well known tuberculostatic drug has 2-aminoethanol and 1,2-ethylenediamine as core structural features. A number of compounds such as **B**, **C**, and **D**[5–7] containing

☆ CDRI Communication No.: 86/2011/RS.

* Corresponding authors. Tel.: +91 522 2623411/18; fax: +91 522 2623405 (R.S.); tel.: +91 945 2586650 (D.P.S.).
E-mail addresses: drskum10@gmail.com (S. Kumar), ranjanasrivastava5@gmail.-com (R. Srivastava), dpsahuin@yahoo.com (D.P. Sahu).
† Present address: Dept. of Applied Chemistry, Babasaheb Bhimrao Ambedkar University, Lucknow 226025, India.

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.02.030



Scheme 1. Reagent and conditions: (i) (a) $PhCH_2COOH$, Ac_2O, NEt_3, 140–145 °C, 5 h, (b) $(CH_3)_2SO_4$, K_2CO_3, CH_3CN, 60 °C, 8 h, (c) Mg, CH_3I, THF, 50–55 °C, then HCl (5%), 20 °C, 5 h; (ii) ArH (**3**), $AlCl_3$, hexane/benzene (1:1), 0 °C, then 75–80°C, 6–10 h; (iii) substituted amino alkyl chloride, alkali, isopropanol, 50–55 °C, 3 h.

aminoalkoxy motif are shown to have anti-tubercular activity. Up to certain limit, compounds with higher lipophilicity have higher permeation across biological membranes (but lower aqueous solubility). Ethambutol (A) has lower lipophilicity than compounds **B**, **C** and **D**. It was envisaged novel 2, 3-dihydro-1*H*-inden, a ring isostere of important class of bio-active compounds such as chroman, flavones and isoflavone and their analogs should have drug like properties. We ventured to explore hitherto untraversed chemical space of 2,3-dihyro-1*H*-indens and here in disclose the preliminary result of anti-mycobacterial activity of the dialkyl amino alkoxy derivatives of *trans* 6-methoxy-1,1-dimethyl-2-phenyl-3-aryl-2,3-dihydro-1*H*-inden **1**.

The title compounds were synthesized starting from 2,2-dimethyl-3-phenyl-7-methoxy chromene **4** as shown in Scheme 1. Anhydrous aluminium chloride mediated tandem hydroarylation[8] of **4** with an arene **3** via rearrangement[9] of the intermediate chroman in a single pot afforded *trans* 6-methoxy-2-phenyl-3-aryl-4-hydroxy-2,3-dihydro-1*H*-inden derivative **2** exclusively in good yield. The transformation of **4**–**2** was might be proceeded by tandem hydroarylation of **4** with arene **3** to afford *trans* diarylchroman and subsequently ring contraction to *trans* diaryl 2,3-dihydro-1*H*-inden **2**. The exclusive formation of *trans* isomer is probably due to steric and thermodynamic factors. The chromene **4** could be conveniently synthesized from commercially available 2,4-dihydoxybenzaldehyde in three steps with following improved literature procedure.[10] Alkylation of **2** with appropriately substituted amino alkyl chloride in presence of an alkali (LiOH, KOH or NaOH) in a protic solvent followed by chromatographic purification furnished the titled compounds **1** in above 90% yield. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data and were supported by satisfactory micro-analytical data.

Table 1
trans-1-[2-(6-Methoxy-1,1-dimethyl-2-phenyl-3-aryl-2,3-dihydro-1*H*-inden-4-yloxy alkyl amine **1a**–**j**

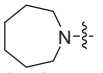
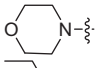
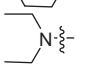
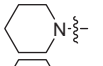
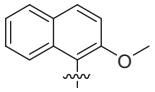
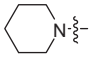
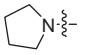
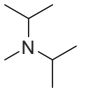
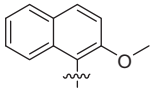
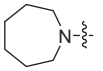
Entry	Ar	*n* =	R^1R^2N– =
1a	Ph	2	
1b	Ph	3	$(CH_3)_2$-N–
1c	Ph	2	
1d	Ph	2	
1e	Ph	2	$(CH_3)_2$-N–
1f	Ph	2	
1g		2	
1h	Ph	2	
1i	Ph	2	
1j		2	

Table 2
In vitro activity against *M. tuberculosis* H37Rv, cytotoxicity and selective index of indene derivatives **1a**–**j**

S. No.	Compound	Log*P*	MIC (μg/ml)	CC_{50} (μg/ml)	SI value
1	**1a**	7.66	3.125	8.11	2.6
2	**1b**	6.62	3.125	4.59	1.4
3	**1c**	6.11	3.125	17.48	5.6
4	**1d**	7.19	3.125	3.74	1.19
5	**1e**	7.24	6.25	2.96	0.47
6	**1f**	6.57	1.56	2.41	1.54
7	**1g**	8.11	1.56	5.52	3.53
8	**1h**	6.83	3.125	7.49	2.39
9	**1i**	7.82	3.125	49.16	15.73
10	**1j**	8.53	1.56	23.98	15.37
11	ETB	0.14	0.5	38.51	77.02
12	RFM	3.71	0.39	62.46	160.1

All compounds were tested in parallel for MIC and cytotoxicity.

A total of 10 novel compounds were synthesized (Table 1) and evaluated for their anti-TB activity by BACTEC radiometric method[11] against *M. tuberculosis* H37Rv. Table 2 summarizes the anti-mycobacterial activity (minimum inhibitory concentration, MIC) of the synthesized compounds. The MIC of the compounds ranged from 1.56 to 6.25 μg/ml (Table 2). The substituents on the aminoalkoxy side chain as well as the length of the spacer have limited effect on the in vitro activity.

The MIC of the compounds **1f**, **1g** and **1j** having piperidine or azepine substituent were found to be active at 1.56 μg/ml whereas the MIC for the compounds having morpholine (**1c**), pyrrolidine (**1h**), dimethylamino (**1e**) substituent were found to be active at higher value (3.125 μg/ml).

The in vitro activity of compound having two carbons spacer was found to be favorable over those having three carbons spacer (Table 1).

Table 2 summarizes the anti-mycobacterial activity, cytotoxicity and lipophilicity of all the compounds. The compounds **1i** and **1j** with low MIC and low cytotoxicity are better with respect to lipophilicity.

All the compounds were tested for in vitro cytotoxicity. The cytotoxicity was determined with Vero monkey kidney cells (ATCC CCL-81) using Resazurin assay.[11a] The indene derivatives **1** with low MIC exhibited moderate to high degree of cytotoxicity. The compounds **1i** and **1j** attained selective index above 15.5 which is the unique feature proving the higher efficacy and low toxicity of the above two compounds. Similar to in vitro activity, the cytotoxicity of the compounds were marginally affected by the substituents on nitrogen of aminoalkoxy side chain. The compound with piperidine sustistuent has moderate whereas the compound containing azepane, morpholine have lower cytototoxicity. Two compounds **1i** and **1j** with SI value >15 were selected for in vitro activity against MDR strains of *M. tuberculosis* and their efficacy in mouse macrophages. In vitro activity of compounds **1i** and **1j** were evaluated against MDR (multidrug resistant) strains of *M. tuberculosis* (clinical isolates from patients) which exhibited resistance to Rifampicin and Isoniazid.[11a] The activity was evaluated by agar dilution method and confirmed by BACTEC radiometric method. Both compounds **1i** and **1j** were active against MDR strains of TB at 6.25 and 3.125 μg/ml (Table 3).

The compounds **1i** and **1j** were tested for ex vivo efficacy in mouse macrophage cell line J774A1 at 1× and 2× MIC concentrations as described in Supplementary data section. Lysates from 0 and 6 days of control and test samples were plated on MB7H10 agar containing OADC and CFU/ml were determined (Fig. 2).

As compared to control compounds **1i** and **1j** caused effective inhibition of intracellular replication of *M. tuberculosis* within mouse macrophages at 1× and 2× MIC concentrations. However, in case of compound **1i**, as compared to control, more than 90% inhibition of growth of *M. tuberculosis* H37Rv was obtained following 6 day treatment at 1× MIC concentration (Fig. 2). The compound **1i** was selected for in vivo activity evaluation in mice and in vitro against non-replicating *M. tb*.

The in vivo efficacy of the selected compound **1i** was evaluated in murine TB model for acute infection.[11b] The mouse (18–20 g) were infected iv with *M. tuberculosis* $H37R_v$ (10^6 CFU) and their chemotherapy was initiated 2 days later and continued for 30 days (6 days/week). The mice treated with INH (25 mg/kg) were positive control. Infected untreated mouse served as negative control. The polyvinylpyrrolidone (PVP) complex of the compound was prepared which was soluble in water and was administered by oral gavage at a dose of 100 mg/kg. By the end of therapy, six mice for every group were sacrificed, and 10-fold dilutions of lung homogenates were plated on MB7H10-OADC agar for CFU determination. The experimental protocols were approved by the animal ethics committee of the Institute.

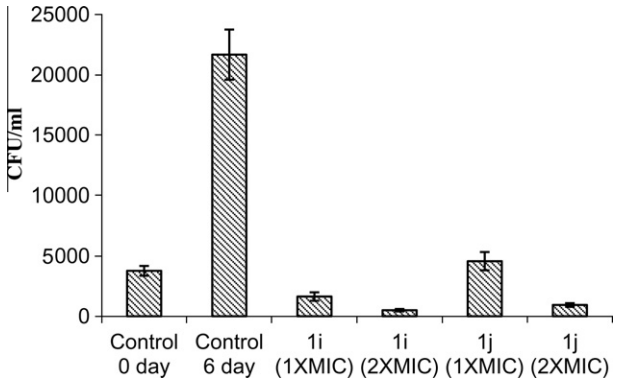


Figure 2. Efficacy of selected compounds in macrophages. Macrophages were lysed and viable counts were determined at 0 and 6 day time points at 1× and 2× MIC concentration.

Table 4
Tissue bacillary load (CFU) in lungs of BALB/c mice infected with *M. tuberculosis* H37Rv after 30-day drug treatment

Treatment regimen	Dose (mg/kg)	$\log_{10}$ CFU ± SE in lungs
Untreated		7.61 ± 0.37
INH	25	4.83 ± 0.07
1i	100	6.25 ± 0.31

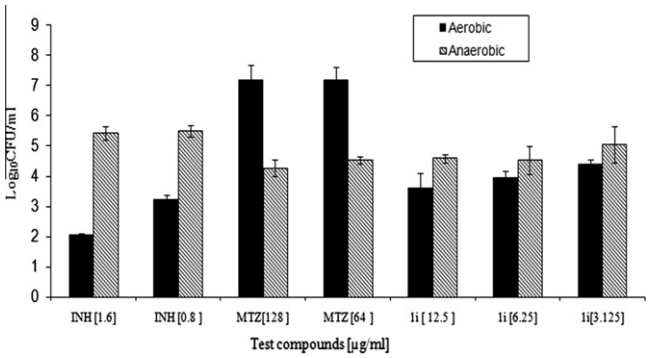


Figure 3. Comparison of growth of hypoxic *M. tb* bacilli treated with INH (Isonizid), MTZ (Metronidazole) and test compound. Viability was determined as $\log_{10}$ CFU/ml as described in Supplementary data. Concentration of compound is given as μg/ml.

As shown in Table 4, a reduction of bacterial CFU in lungs of mouse as a result of chemotherapy using compound **1i** was observed. In the murine model of TB infection, the **1i** showed 1.35 $\log_{10}$ reductions of the CFUs in the lungs of treated mouse at 100 mg/kg body weight dose compared to 2.8 $\log_{10}$ reductions of the same by INH which is indicative of anti-TB activity

Table 3
In vitro activity of compounds **1i** and **1j** against clinical drug susceptible and MDR strains of *M. tuberculosis*

M. tuberculosis strains	Susceptibility or resistance to antitubercular drugs	MIC (μg/ml)
Strain 2643 (MDR) [a]	Rif^r INH^r	6.25 (**1i**) 3.125 (**1j**)
Strain 1678/05[a] (MDR)	Rif^r INH^r	6.25 (**1i**) 3.125 (**1j**)
Strain 2280[a]	Drug sensitive	3.125 (**1i**) 1.56 (**1j**)

[a] The strains were obtained from Professor Sarnam Singh, AIIMS, New Delhi.

(tuberculosis antimicrobial acquisition and coordinating facility, http://www.tacf.org).

For evaluation of activity against non-replicating *M. tb*, cells were grown in hypoxia, that is, oxygen depletion condition.[12a,b,c] Depletion of oxygen triggers the shift down of *M. tuberculosis* to a state of dormancy. Metronidazole (active only on anaerobically grown organisms) and isoniazid (acting only under aerobic conditions) were used as controls. Cell viability in control and after drug/compound addition was determined by CFU determination as described in Supplementary data. The compound **1i** was found to be active against non-replicating hypoxic *M. tuberculosis* at 6.25 μg/ml which was twofold higher than MIC reported in replicating phase (Fig. 3).

In conclusion, the preliminary in vitro antimycobacterial activity evaluation of all the compounds except **1e**, library of novel 4-aminoalkoxy indane **1** exhibited MIC below 6.25 μg/ml. The compound **1i** was selected as lead compound on basis of MIC, selective index >**15**, susceptibility to drug sensitive, non-replicating *M. tuberculosis* H37Rv and clinical MDR strains of *M. tuberculosis*. In the mouse model of TB infection, the compound **1i** showed 1.35 $\log_{10}$ reduction of the CFUs in the lungs of treated mice which is indicative of anti-TB activity. Further lead search is in progress.

Acknowledgments

We thank Director, CDRI for support and facilities. S.K. and V.K.K. are thankful to the University Grants Commission, New Delhi for the financial support and to the Sophisticated Analytical Instrument Facility, CDRI, and Lucknow for providing spectroscopic data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.030.

References and notes

1. (a) Tuberculosis, HIV/Aids and Malaria, The Status and Impact of the Three Diseases. WHO, Geneva, 2005.; (b) Raviglione, M. C. *Tuberculosis* **2003**, *83*, 4; (c) Espinal, M. A. *Tuberculosis* **2003**, *83*, 44; (d) Girling, D. J. *Tubercle* **1978**, *59*, 13.
2. (a) World Health Organization, The Global MDR-TB & XDR-TB Response Plan 2007–2008 Geneva, Switzerland, 2007.; (b) Young, D. B.; Duncan, K. *Ann. Rev. Microbiol.* **1995**, *49*, 641; (c) Schaeffer, M. L.; Khoo, K. H.; Besra, G. S.; Chatterjee, D.; Brennan, P. J.; Belisle, J. T.; Inamine, J. M. *J. Biol. Chem.* **1999**, *274*, 31625; (d) Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004; (e) Saito, H.; Tomioka, H.; Sato, K.; Emori, M.; Yamane, T.; Yamashita, K.; Hosoe, K.; Hidaka, T. *Antimicrob. Agents Chemother.* **1991**, *35*, 542.
3. Pedro, E.; Silva, A. D.; Ramosa, D. F.; Bonacorso, H. G.; Iglesia, A. I.; Oliveira, M. R.; Coelho, T.; Navarini, J.; Morbidoni, H. R.; Zanatta, N.; Martins, M. A. P. *Int. J. Antimicrob. Agents* **2008**, *32*, 139.
4. Duncan, K. *Tuberculosis* **2003**, *83*, 201.
5. Jefferson, E. A.; Seth, P. P.; Robinson, D. E.; Winter, D. K.; Miyaji, A.; Risen, L. M.; Osgood, S. A.; Bertrand, M.; Swayze, E. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5257.
6. Parai, M. K.; Panda, G.; Chaturvedi, V.; Manju, Y. K.; Sinha, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 289.
7. (a) Nayyar, A.; Malde, A.; Coutinho, E.; Jain, R. *Bioorg. Med. Chem.* **2006**, *14*, 7302; (b) Prado, S. C.; Janin, Y. L.; Saint-Joanis, B.; Brodin, P.; Michel, S.; Koch, M.; Cole, S. T.; Tillequin, F.; Bost, P. *Bioorg. Med. Chem.* **2007**, *15*, 2177.
8. (a) Rueping, M.; Nachtsheim, B. J.; Scheidt, T. *Org. Lett.* **2006**, *8*, 3717; (b) Kischel, J.; Jovel, I.; Mertins, K.; Zapf, A.; Beller, M. *Org. Lett.* **2006**, *8*, 19.
9. Loudon, J. D.; Razdan, R. K. *J. Chem. Soc.* **1954**, 4299.
10. Xiao, S.; Wang, X.; Yan, J. *J. Chin. Pharm. Sci.* **1998**, *7*, 69.
11. (a) Saquib, M.; Husain, I.; Sharma, S.; Yadav, G.; Singh, V. K.; Sharma, S. K.; Shah, P.; Siddiqi, M. I.; Kumar, B.; Lal, J.; Jain, G. K.; Srivastava, B. S.; Srivastava, R.; Shaw, A. K. *Eur. J. Med. Chem.* **2011**, *46*, 2217; (b) Kelly, B. P.; Furney, S. K.; Jessen, M. T.; Orme, I. M. *Antimicrob. Agents Chemother.* **1996**, *40*, 2809.
12. (a) Wayne, L. G.; Hayes, L. G. *Infect. Immun.* **1996**, *64*, 206; (b) Lenaerts, A. J.; Gruppo, V.; Marietta, K. S.; Johnson, C. M.; Driscoll, K.; Tompkins, M.; Jerry, D. R.; Reynolds, R. C.; Orme, I. M. *Antimicrob. Agents Chemother.* **2005**, *49*, 2294; (c) Saxena, A.; Srivastava, V.; Srivastava, R.; Srivastava, B. S. *Tuberculosis* **2008**, *88*, 518.