Bioorganic & Medicinal Chemistry Letters 21 (2011) 1997–1999

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and antimycobacterial activity of cinnamide derivatives: A molecular hybridization approach

Manoj D. Kakwani [a], Prashant Suryavanshi [c], Muktikant Ray [b], M. G. R. Rajan [b], Sharmila Majee [c], Abdul Samad [c], Padma Devarajan [a], Mariam S. Degani [a,*]

[a] Department of Pharmaceutical Sciences & Technology, Institute of Chemical Technology, N. P. Marg, Matunga, Mumbai 400 019, India
[b] Radiation Medicine Centre, Tata Memorial Hospital, BARC, Parel, Mumbai 400 012, India
[c] Department of Veterinary Microbiology, Bombay Veterinary College, Parel, Mumbai 400 012, India

ARTICLE INFO

Article history:
Received 20 September 2010
Revised 25 January 2011
Accepted 7 February 2011
Available online 12 February 2011

Keywords:
Diamine
Cinnamic acid
Hybridization
Mycobacterium tuberculosis (Mtb)
Antimycobacterial activity
Synergy

ABSTRACT

A series of cinnamide derivatives was designed as potential antimycobacterial agents using molecular hybridization approach. The diamine moiety, a key feature of ethambutol and its other analogs, and certain structural features of cerulenin and cinnamic acid were hybridized to obtain cinnamide derivatives. The minimum inhibitory concentration (MIC) of all synthesized compounds was determined against *M. tuberculosis* $H_{37}R_v$ using Resazurin Microtitre plate Assay (REMA) method. The synthesized molecules showed good to moderate activity with MIC in the range of 5–150 μM and good safety profile. Additionally, the most potent compound **1a**, having MIC 5.1 μM exhibited synergy with rifampicin.

Tuberculosis (TB) is an airborne, contagious disease caused by *Mycobacterium tuberculosis* (Mtb) in humans. WHO reported nearly 8–9 million new cases of TB in 2009 including three million deaths which represent the largest number of incidence of human deaths attributable to a single etiological agent.[1] In particular, the current concern is the massive problem resulting from extensively drug-resistant tuberculosis, XDR-TB.[2]

Ethambutol, a clinically used first line drug for tuberculosis, has 1,2-ethylenediamine as a core structural feature. This scaffold has been explored extensively, and one of the candidates with this scaffold, SQ109, has advanced into clinical trials.[3] Studies have shown that replacing the basic scaffold of 1,2-ethylenediamine by other similar diamine like piperazine or homopiperazine (e.g., SQ786 and SQ775), still retains useful activity (Fig. 1).

On the other hand, cerulenin and trans-cinnamic acid (Fig. 2) are traditionally known to have antimycobacterial activity and also proven to have synergistic action when tested along with known clinically used drugs.[4] Additionally, cinnamyl derivative of rifampicin showed improved intracellular and in vivo activities than by rifampicin per se.[5]

In our continuous program in the search for new, potent antimycobacterial agents,[6] we have designed a new class of cinnamide derivatives. The design strategy incorporated the diamine feature of ethambutol and its analogs along with the

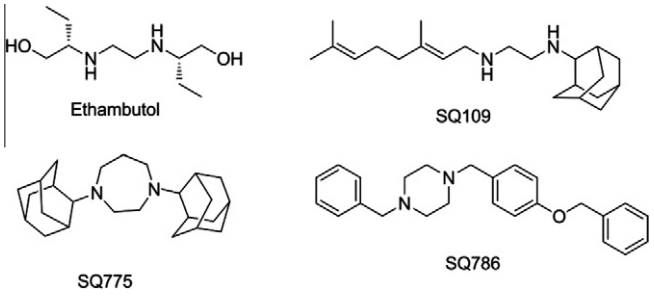


Figure 1. Diamine based antimycobacterials.

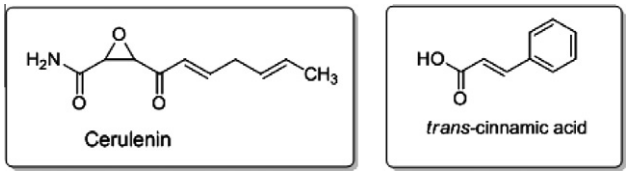


Figure 2. Traditionally known antimycobacterials having synergistic action.

* Corresponding author. Tel.: +91 22 3361 2213; fax: +91 22 3361 1020.
E-mail address: ms.degani@ictmumbai.edu.in (M.S. Degani).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.02.022

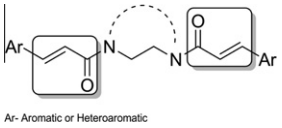


Figure 3. Design concept for cinnamide derivatives.

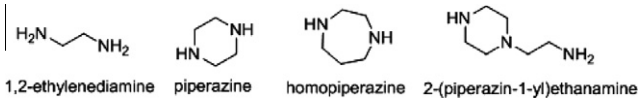


Figure 4. Different diamine linkers used for synthesis.

aromatic α,β-unsaturated carbonyl moiety, to obtain cinnamide compounds (Fig. 3). Earlier reports have used similar strategies where cinnamic acid has been coupled to isoniazid in the search of potential antimycobacterial agents.[7] Also according to recent reports, the designed cinnamide derivatives may act on the biosynthesis of mycolic acids and more specifically on FAS-II, which uses α,β-enoyl systems as substrates.[8] Moreover, these molecules could also have synergy with rifampicin, similar to that exhibited by cerulenin and trans-cinnamic acid.

Initially, molecules with different diamine linkers were synthesized, that is, 1,2-ethylenediamine, piperazine, and homopiperazine (Fig. 4). In addition, 2-(piperazin-1-yl) ethanamine as a novel diamine scaffold was envisaged. The target compounds, that is, cinnamide derivatives **1a**–**1d** were synthesized according to Scheme 1 using the reaction between trans-cinnamic acid and diamine via a mixed anhydride intermediate, formed using methyl chloroformate in presence of triethylamine.[9] Some of the synthesized derivatives are already reported as glycogen phosphorylase inhibitors[10] and hence their spectral data was matched and found to be in accordance with reported data.

The antimycobacterial activity of all the synthesized compounds was tested against *M. tuberculosis* $H_{37}R_v$ to determine the minimum inhibitor concentration (MIC) using REMA (Resazurin Microtitre Assay) method.[11] Ethambutol was used as the reference drug. The biological results (Table 1) show that the compound having a 1,2-ethylenediamine **1a** linkage was the most active amongst the synthesized molecules, that is, **1a**–**1d** in this series. Further, to optimize the potency of the compound derived from 1,2-ethylenediamine **1a**, the conventional Topliss method was applied.[12]

Table 1
Cinnamide derivatives with their antimycobacterial activity and toxicity

Compound	MIC[a] (μM)	CC_{50}[b] (μM)	SI
1a	5.1	618	121
1b	9.0	855	95
1c	17.3	708	41
1d	16.7	480	29

SI—selective index.
[a] Compounds were tested against Mtb $H_{37}Rv$ strains using REMA method.
[b] Cytotoxicity studies were done on VERO cell line C1008 using MTT assay.

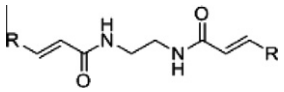
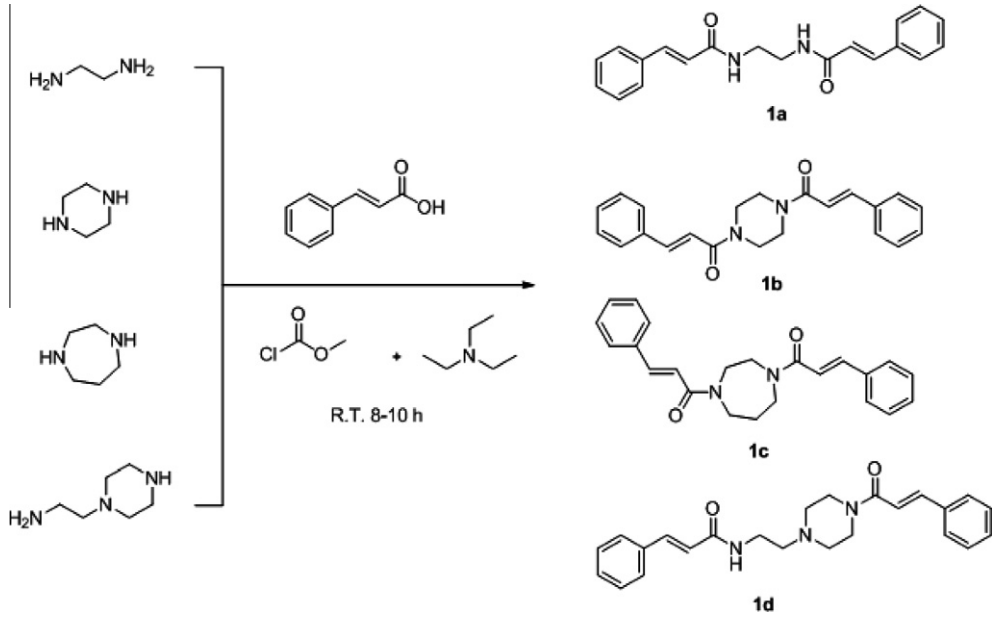


Figure 5. General structure of cinnamide derivatives.

Table 2
Cinnamide derivatives with their antimycobacterial activity and toxicity

Compound	R	MIC[a] (μM)	CC_{50}[b] (μM)	SI
1a	Phenyl	5.1	618	121
1e	4-CH_3 phenyl	143.7	565	4
1f	4-OCH_3 phenyl	131.6	680	5
1g	4-Cl phenyl	128.9	788	6
1h	3,4-diCl phenyl	54.8	653	12
1i	2-Cl phenyl	64.4	725	11
1j	3-NO_2 phenyl	68.5	615	9
1k	2-OCH_3 phenyl	65.8	695	11
1l	2,6-diCl phenyl	54.8	653	12
1m	Furan	10.8	683	63
1n	Thiophene	9.41	692	74
EMB	—	15.3	1470	96

SI—selective index.
EMB—Ethambutol.
[a] Compounds were tested against Mtb $H_{37}Rv$ strains using REMA method.
[b] Cytotoxicity studies were done on VERO cell line C1008 using MTT assay.



Scheme 1. Synthetic scheme for cinnamide derivatives.

The biological results of all synthesized molecules are as shown in (Fig. 5, Table 2). The MIC values of the compounds having substitution at *para*-position on the aromatic ring **1e**–**1h**, showed decrease in antimycobacterial activity relative to the unsubstituted compound **1a**. Next, the molecules with substitution at *ortho*- or *meta*-position of aromatic ring **1i**–**1l** were synthesized. However these molecules also did not exhibit increased potency. Further, heteroaromatic ring system was incorporated as in **1m** and **1n** instead of phenyl ring. Both these derivatives showed better activity than substituted aromatic compounds; however they exhibit less potency than unsubstituted compound **1a**.

All the synthesized compounds were tested for cytotoxicity against mammalian VERO cell line (C1008) using MTT assay.[13] All the compounds had low cytotoxicity in this assay, and the potent compounds had a good selective index.

The most potent cinnamide derivative **1a**, was further tested for its synergistic activity with rifampicin.[14] The MIC of rifampicin and **1a** was found to be 0.125 and 1.625 μg/mL, respectively. In combination, MIC of rifampicin was reduced to 0.0078 μg/mL and of compound **1a** to 0.406 μg/mL, resulting in 16-fold and 4-fold reduction in MIC of rifampicin and **1a**, respectively, which represents synergism.

In summary, a small library of cinnamide derivatives has been synthesized using molecular hybridization approach. Among the synthesized molecules, compound **1a** shows promising results as an antimycobacterial agent. Thus this strategy could prove useful in design of antimycobacterial agents with inherent activity as well as synergy with rifampicin.

Acknowledgments

M.K. and P.S. are thankful to Department of Biotechnology (DBT), India for financial support. This work is funded by Department of Biotechnology, India; Grant No. BT/PR7858/Med/14/1142/2006.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.022.

References and notes

1. http://www.who.int/tb/publications/global_report/2009/en/index.html.
2. Daniel, T. M.; Bates, J. H.; Downes, K. A. *Tuberculosis: Pathogenesis, Protection, and Control*; ASM Press: Washington, DC, 1994. pp 13–24.
3. Lee, R. E.; Protopopova, M.; Crooks, E., et al. *J. Comb. Chem.* **2003**, *5*, 172.
4. Rastogi, N.; Goh, K. S.; Horgen, L.; Barrow, W. W. *FEMS Immunol. Med. Microbiol.* **1998**, *21*, 149.
5. Reddy, V. M.; Nadadhur, G.; Daneluzzi, D.; Dimova, V.; Gangadharam, P. R. *J. Antimicrob. Agents Chemother.* **1995**, *39*, 2320.
6. Bairwa, R.; Kakwani, M.; Tawari, N. R.; Lalchandani, J.; Ray, M. K.; Rajan, M. G. R.; Degani, M. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1623.
7. Carvalho, S. A.; da Silva, E. F.; de Souza, M. V. N.; Lourenco, M. C. S.; Vicente, F. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 538.
8. Yoya, G. K.; Belval, B. F.; Constant, P.; Duran, H.; Daffe, M.; Baltas, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 341.
9. Guan, L. P.; Wei, C. X.; Deng, Q. X.; Sui, X.; Piao, H. R.; Quan, Z. S. *Eur. J. Med. Chem.* **2009**, *44*, 3654.
10. (a) Onda, K.; Shiraki, R.; Yonetoku, Y.; Momose, K.; Katayama, N.; Orita, M.; Yamaguchi, T.; Ohta, M.; Tsukamoto, S. *Bioorg. Med. Chem. Lett.* **2008**, *16*, 8627; (b) Hill, T.; Odell, L. R.; Edwards, J. K.; Graham, M. E.; Mcgeachie, A. B.; Rusak, J.; Quan, A.; Abagyan, R.; Scott, J. L.; Robinson, P. J.; McCluskey, A. *J. Med. Chem.* **2005**, *48*, 7781.
11. Palomino, J. C.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F. *Antimicrob. Agents Chemother.* **2002**, *46*, 2720.
12. Topliss, J. G. *J. Med. Chem.* **1977**, *20*, 463.
13. Freshney, R. L. *Culture of Animal Cells: A Manual of Basic Techniques*, 4th ed.; Willy-LISS, 2000. p 329.
14. Moody, J. A. *Clinical Microbiology Procedures Handbook*; American Society for Microbiology: Washington, DC, 1992. pp 5.18.1–28.