Antimycobacterial activity generated by the amide coupling of (−)-fenchone derived aminoalcohol with cinnamic acids and analogues

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · SEPTEMBER 2014

Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2014.09.021

6 AUTHORS, INCLUDING:

Violeta Valcheva
The Stephan Angeloff Institute of Microbiol…
32 PUBLICATIONS **234** CITATIONS
SEE PROFILE

Iva Ugrinova
Bulgarian Academy of Sciences
35 PUBLICATIONS **536** CITATIONS
SEE PROFILE

Evdokia Pasheva
Bulgarian Academy of Sciences
26 PUBLICATIONS **388** CITATIONS
SEE PROFILE

Bioorganic & Medicinal Chemistry Letters 24 (2014) 5030–5033

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Antimycobacterial activity generated by the amide coupling of (−)-fenchone derived aminoalcohol with cinnamic acids and analogues

Ivaylo Slavchev [a], Georgi M. Dobrikov [a,*], Violeta Valcheva [b], Iva Ugrinova [c], Evdokia Pasheva [c], Vladimir Dimitrov [a,*]

[a] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, bl. 9, Acad. G. Bonchev str., Sofia 1113, Bulgaria
[b] Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, bl. 26, Acad. G. Bonchev str., Sofia 1113, Bulgaria
[c] Institute of Molecular Biology 'Roumen Tsanev', Bulgarian Academy of Sciences, bl. 21, Acad. G. Bonchev str., Sofia 1113, Bulgaria

ARTICLE INFO

Article history:
Received 18 June 2014
Revised 6 September 2014
Accepted 8 September 2014
Available online 16 September 2014

Keywords:
Fenchone
Amides
Cinnamic acids
Tuberculosis

ABSTRACT

Aminoethyl substituted 2-endo-fenchol prepared from (−)-fenchone was used as scaffold for the synthesis of series of 31 amide structures by N-acylation applying cinnamic acids and analogues. The evaluation of their in vitro activity against *Mycobacterium tuberculosis* $H_{37}Rv$ showed for some of them promising activity—up to 0.2 μg/ml, combined with relatively low cytotoxicity of the selected active compounds.

Tuberculosis (TB) is an infectious disease that remains a major global health problem. In 2012 alone it caused over 1.3 million deaths,[1] making its causative organism *Mycobacterium tuberculosis* (*Mtb*) one of the most lethal pathogens in the world. The vulnerability of the HIV positive patients and the emergence of resistant strains, which do not respond to the usual treatment, expand the problem seriously. The urgent need of new antimycobacterial agents is becoming more and more apparent.

Cinnamic acid and its derivatives were used in the earliest efforts for treating tuberculosis, starting from the late 19th century.[2–5] The potential of these compounds has been extensively examined for the past 20 years. Their synergic activity in combination with classic anti-TB drugs is well established.[6] Furthermore, the attachment of a cinnamyl moiety to the antibiotic rifamicin results in a product Rifacinna® (Fig. 1A), which is more active than rifamicin against susceptible Mtb strains, and also shows ability to overcome resistance.[7] Many natural[8–10] and synthetic[11–13] products, bearing the cinnamic moiety, have also been proven effective against Mtb. One of the most potent reported compounds of this type seems to be cinnamic hydrazide[13] (Fig. 1B), however, an alternative study[14] reports ca. 35 times lower activity of the same compound.

As a general rule, the presence of large lipophilic fragments within an active structure improves the anti-TB action. Especially promising results demonstrate compounds containing geranyl moiety.[13,15] In recent works[16,17] it was proven that compounds incorporating bicyclic monoterpene moiety also possess a significant antimycobacterial activity. A fenchone-based series (Fig. 1C), including unsubstituted cinnamamide (Fig. 1D), showed moderate ability to inhibit the growth of Mtb, and some examples even matched the activity of the classic anti-TB agent ethambutol (EMB).[18] The investigation of new compounds bearing bulk lipo-

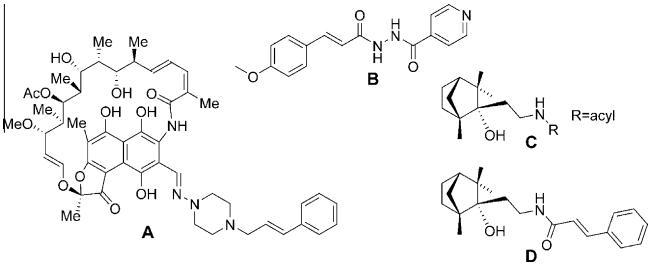
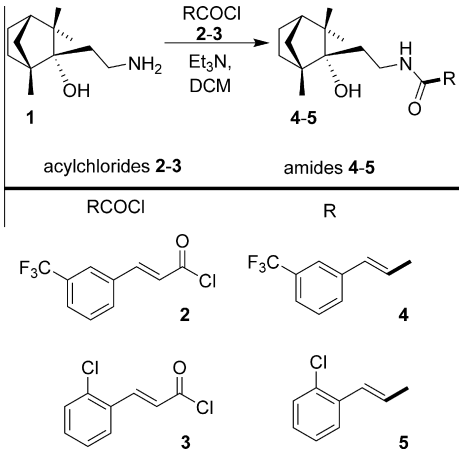


Figure 1.

* Corresponding authors. Tel.: +359 2 9606152; fax: +359 2 8700225.
E-mail address: gmdob@orgchm.bas.bg (G.M. Dobrikov).

http://dx.doi.org/10.1016/j.bmcl.2014.09.021
0960-894X/

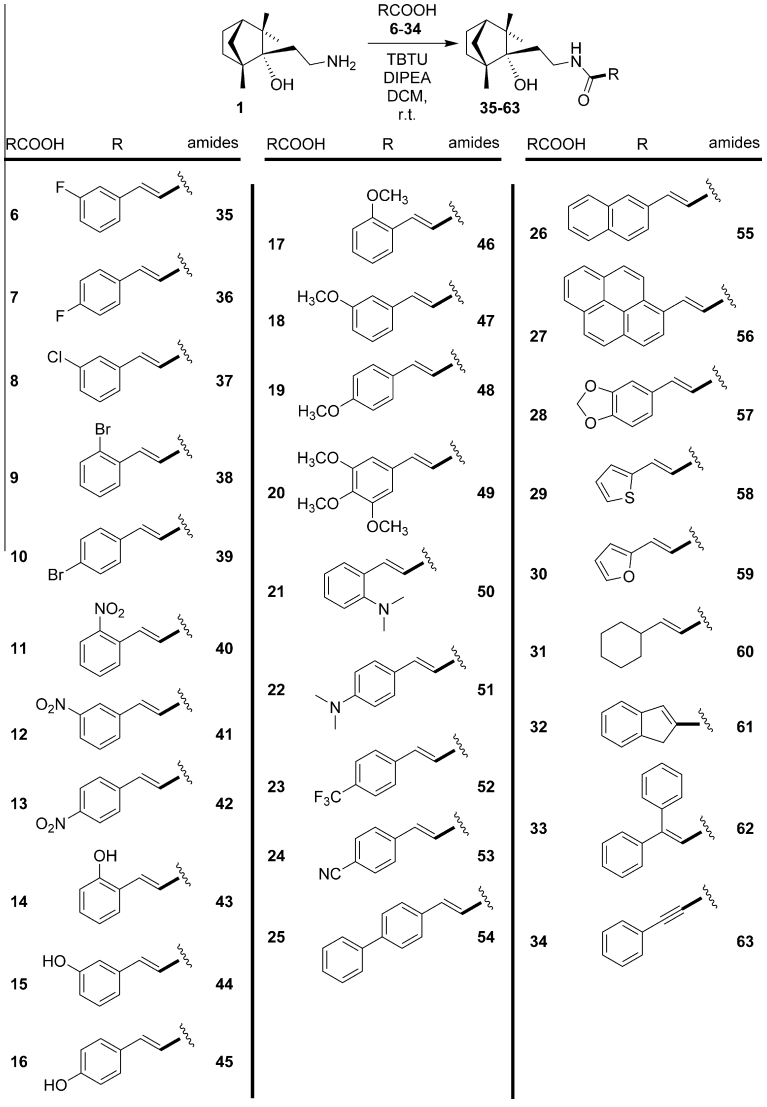


Scheme 1. Synthesis of compounds **4** and **5**.

philic polycyclic fragments is getting increasingly popular since the discovery of SQ109,[15] which now undergoes clinical trials and shows promising results.[19,20]

As part of our continuous program of developing new anti-TB agents[16–18,21] we designed a series of new hybrid structures. They consist of cinnamoyl-like motifs (containing condensed, substituted aryl and heteroaryl moieties), linked through an amide bond to functionalized fenchone-derived bicyclic system. Based on the argumentation presented above, it is expected for some of these molecular hybrids increased anti-TB activity. Herein, we are reporting the synthesis of 31 novel amidoalcohols and their screening for activity against drug sensitive ($H_{37}Rv$) strain of *Mtb*.

The chemistry was directed to the synthesis of structurally diverse compounds of the type **D** (Fig. 1) consisting of aminoethyl-fenchol scaffold and substituted cinnamoyl residue attached through amide bond. To the best of our knowledge, there is only limited number of studies concerning the investigation of antitubercular activity of differently substituted cinnamoyl derivatives.[8–13] The structures planned were designed in order to find more active cinnamamides through varying the type and positions of the substituents in the aromatic ring, as well as to ascertain possible structure–activity relationships (SAR). In addition, the pharmacophoric contribution of the cinnamoyl moiety itself was evaluated by replacement with different groups possessing structural similarity. In all cases the fenchane part of the molecules



Scheme 2. Synthesis of compounds **35–63**.

Table 1
In vitro screening data for antimycobacterial activity and cytotoxicity of synthesized compounds **4–5** and **35–63**

Compound	Yield (%)	Activity toward reference strain of *Mycobacterium tuberculosis* $H_{37}Rv$, MIC (μg/ml)	Cytotoxicity toward human embryonal kidney cell line 293T, IC_{50} (μg/ml)	Selectivity index, SI[a]	Log*P*[b]
4	82	7.0	NT	NT	5.10 ± 0.59
5	71	5.0	NT	NT	4.62 ± 0.40
35	81	5.0	NT	NT	4.36 ± 0.49
36	84	4.5	NT	NT	4.25 ± 0.50
37	78	5.0	NT	NT	4.85 ± 0.40
38	80	6.0	NT	NT	5.01 ± 0.44
39	87	>6.0	NT	NT	5.13 ± 0.53
40	80	2.5	94.2	37.7	3.79 ± 0.47
41	83	4.0	NT	NT	4.14 ± 0.40
42	73	6.0	NT	NT	4.02 ± 0.42
43	34	4.0	NT	NT	4.26 ± 0.55
44	57	6.0	NT	NT	3.65 ± 0.40
45	23	4.0	NT	NT	3.70 ± 0.40
46	87	6.5	NT	NT	4.25 ± 0.39
47	92	4.5	NT	NT	4.22 ± 0.39
48	86	3.5	NT	NT	4.19 ± 0.39
49	68	1.0	10.9	10.9	3.83 ± 0.41
50	89	5.0	NT	NT	4.35 ± 0.40
51	80	0.2	13.4	67.0	4.75 ± 0.48
52	76	6.0	NT	NT	5.20 ± 0.59
53	85	4.5	NT	NT	3.81 ± 0.50
54	80	4.5	NT	NT	6.00 ± 0.41
55	88	5.0	NT	NT	5.47 ± 0.39
56	73	4.0	NT	NT	7.19 ± 0.39
57	80	2.5	11.5	4.6	4.36 ± 0.48
58	78	5.0	NT	NT	4.06 ± 0.57
59	96	4.0	NT	NT	3.86 ± 0.40
60	90	6.0	NT	NT	4.69 ± 0.34
61	74	5.0	NT	NT	4.67 ± 0.39
62	82	5.0	NT	NT	6.40 ± 0.75
63	51	5.0	NT	NT	5.35 ± 0.60
EMB·2HCl	—	2.0	NT	NT	0.06[d]

[a] Selectivity index: SI = IC_{50}/MIC; NT—not tested/calculated.
[b] Log*P*, octanol–water partitioning coefficient, was calculated using ACDLabs/ChemSketch 2012 (www.acdlabs.com).
[d] Log*P* of ethambutol dihydrochloride (EMB·2HCl) is known in the literature: N.R. Budha, R.E. Lee and B. Meibohm, *Curr. Med. Chem.* 2008, *15*, 809.

was introduced through aminoalcohol **1** by using relatively simple but efficient synthetic strategy.[18,22]

Initially the reaction of aminoalcohol **1** with the acid chlorides **2** and **3** was performed to obtain amidoalcohols **4** and **5** in very good yields. Standard conditions for acylation of **1** have been applied (0 °C and Et_3N in dry DCM) by using commercial acid chlorides (Scheme 1). The synthesized compounds were isolated in pure form applying column chromatography. The structures were confirmed by NMR and MS spectroscopy.

The reaction of aminoalcohol **1** and readily available organic acids offers more opportunities for obtaining structurally diverse series of compounds (Scheme 2). Amidoalcohols **35–63** were prepared through the coupling reaction between **1** and cinnamic acids **6–28**, cinnamic acid analogues **29–32** and 3-phenylpropiolic acid **34** respectively, in the presence of *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as activating agent. TBTU was chosen as one of the most effective and selective coupling reagent in peptide synthesis.[23] Some of the acids have been prepared by known procedures (**21**,[24] **27**[25] and **31**[26,27]), especially significantly improving the yield of **21** (70%, see Supplementary data). In the case of the hydroxyl substituted cinnamic acids **14–16** protection of the hydroxyl-functionality was not performed, which was the reason for moderate yields of the products **43–45** (34, 57 and 23%, respectively). Other desired products were isolated in high yields (68–96%) and all of them were obtained in pure form after column chromatography. The structure elucidation of the synthesized compounds was performed by mass-spectrometry and NMR spectroscopy (1D and 2D experiments).

Most of the compounds synthesized (except **54–56** and **62**) are in agreement with the formal Lipinski's rule of five (Table 1), but are practically insoluble in deionized water at 20 °C (solubility is significantly less 1 mg/ml). They were evaluated for in vitro activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (Table 1; results are presented in μg/ml) using the method of *Canetti*.[28–32]

As a whole, the compounds in this study show moderate level of antimycobacterial activity, which for the most of them varies between 30 and 70% of that of EMB (and 80–140% of that of **1**, respectively[18]). The MIC value of EMB has been determined by using pure commercially available product (see Table 1). In general, the presence of different substituents and the variation of their positions in the aromatic ring, as well as the introduction of heterocyclic or condensed aromatic rings of the cinnamoyl moiety, does not impact the activity significantly (Fig. 1D, MIC 18.96 μM).[18] More serious modification of the cinnamoyl moiety (triple instead of double bond; saturated instead of aromatic ring; another phenyl ring at β-position, etc.) causes the same weak effect. Compounds **40**, **49**, **51**, and **56–57** can be highlighted as most active (up to 10 times the activity of EMB). Interestingly, the activity of the most potent compound **51** (MIC 0.2 μg/ml) is much higher than the activity of its *ortho*-isomer **50** (MIC 5.0 μg/ml). The activity of the tri- and di-alkoxy-derivatives **49** and **57**, respectively, is higher than compared with the corresponding mono-methoxy analogues **46–48**. The somewhat better results for **56** could be due to its higher lipophilicity. According to the obtained results, some conclusions regarding SAR can be drawn. The modified fenchane moiety inherited from **1** is mainly responsible for the antimycobacterial activity, while the cinnamoyl moiety itself contributes to a lesser extent. Some N- and O-containing substituents at the aromatic ring can increase the activities (**40**, **49**, **51** and **57**). The presence of halogen or CF_3 substituents (compounds **35–39** and **52**) does not have any noticeable effect on the activity. Cinnamamide analogues bearing condensed aromatic rings (**55–56**) need further investigation.

Four of compounds synthesized, which have shown activities higher that of EMB, were selected for further evaluation. The cytotoxic activity of the tested compounds was investigated onto two different human embryonic cell lines from kidney (HEK293) and from umbilical vein/vascular endothelium (HUVEC) (data not shown) by using the MTT dye reduction assay.[33,34] The corresponding IC_{50} values of the tested compounds were calculated using non-linear regression analysis. As the calculated values for both tested cell lines were quite similar, only these for HEK293 are summarized and present in Table 1. It could be seen that three (**49**, **51** and **57**) of the four tested compounds have demonstrated relatively high cytotoxicity with IC_{50} in the range 10.9–13.4 μg/ml. However one of them (**51**) has shown high selectivity index 67 due to its MIC value (10 times higher activity than that of EMB) that makes it a promising hit structure for further optimization. The last of the tested compounds **40** demonstrated MIC similar to EMB and very low cytotoxic effect of 94.2 μg/ml, which also results in a good selectivity index (37.7). Consequently this structure could be used for further optimization trough synthetic variations.

In conclusion, the facile synthesis of 31 new diastereoisomerically pure derivatives of (−)-fenchone bearing amidoalcohol functionality has been accomplished. All synthesized amidoalcohols were evaluated for their in vitro activity against *Mycobacterium tuberculosis* $H_{37}Rv$. It was found that compounds **40**, **49** and **57** possess activity comparable with that of the reference EMB. The most

active substance **51** showed MIC = 0.2 μg/ml. The cytotoxic activity of selected active compounds toward human embryonic cell lines from kidney (HEK293) revealed that amidoalcohols **40** and **51** have good selectivity indexes (37.7 and 67, respectively). Thus, both substances can be outlined as most promising in this series and could be used as inspiration for further structural optimization.

Acknowledgments

The financial support of the Bulgarian Science Fund for the purchase of Bruker Avance II+ 600 NMR spectrometer in the framework of the Program 'Promotion of the Research Potential through Unique Scientific Equipment'–project UNA-17/2005 is gratefully acknowledged.

Supplementary data

Supplementary data (experimental procedures, characterization of final compounds, biological assays protocols and NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.09.021.

References and notes

1. WHO Global Tuberculosis Report 2013. Available at: http://www.who.int/tb/publications/global_report/en/.
2. Landerer, A. *Anweisung zur Behandlung der Tuberculose mit Zimmtsäure*; F. C. W. Vogel: Leipzig, 1893.
3. Jacobson, M. J. *Bulletins et Mémoires de la Société Médicale des Hôpitaux de Paris* **1919**, *35*, 322.
4. Corper, H. J.; Gauss, H.; Gekler, W. A. *Colo. Am. Rev. Tuberculosis* **1920**, *4*, 464.
5. Gainsborough, H. *Lancet* **1928**, *211*, 908.
6. Rastogi, N.; Goh, K. S.; Horgen, L.; Barrow, W. W. *FEMS Immunol. Med. Microbiol.* **1998**, *21*, 149.
7. Cegielski, J. P. *Clin. Infect. Dis.* **2010**, *50*, S195.
8. Mata, R.; Morales, I.; Pérez, O.; Rivero-Cruz, I.; Acevedo, L.; Enriquez-Mendoza, I.; Bye, R.; Franzblau, S.; Timmermann, B. *J. Nat. Prod.* **2004**, *67*, 1961.
9. Barnes, C. C.; Smalley, M. K.; Manfredi, K. P.; Kindscher, K.; Loring, H.; Sheele, D. M. *J. Nat. Prod.* **2003**, *66*, 1457.
10. Lekphrom, R.; Kanokmedhakul, S.; Kanokmedhakul, K. *J. Ethnopharmacol.* **2009**, *125*, 47.
11. Yoya, G. K.; Bedos-Belval, F.; Constant, P.; Duran, H.; Daffé, M.; Baltas, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 341.
12. Kakwani, M. D.; Suryavanshi, P.; Ray, M.; Rajan, M. G. R.; Majee, S.; Samad, A.; Devarajan, P.; Degani, M. S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1997.
13. De, P.; Yoya, G. K.; Constant, P.; Bedos-Belval, F.; Duran, H.; Saffon, N.; Daffé, M.; Baltas, M. *J. Med. Chem.* **2011**, *54*, 1449.
14. Carvalho, S. A.; da Silva, E. F.; de Souza, M. V. N.; Lourenço, M. C. S.; Vicente, F. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 538.
15. Lee, R. E.; Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M.; Barry, C. E., III *J. Comb. Chem.* **2003**, *5*, 172.
16. Stavrakov, G.; Philipova, I.; Valcheva, V.; Momekov, G. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 165.
17. Stavrakov, G.; Philipova, I.; Valcheva, V.; Doytchinova, I. *Eur. J. Med. Chem.* **2013**, *70*, 372.
18. Dobrikov, G. M.; Valcheva, V.; Nikolova, Y.; Ugrinova, I.; Pasheva, E.; Dimitrov, V. *Eur. J. Med. Chem.* **2014**, *77*, 243.
19. Rawat, B.; Rawat, D. S. *Med. Res. Rev.* **2013**, *33*, 693.
20. Barot, K. P.; Nikolova, S.; Ivanov, I.; Ghate, M. D. *Mini-Rev. Med. Chem.* **2013**, *13*, 1664.
21. Petkova, Zh.; Valcheva, V.; Momekov, G.; Petrov, P.; Dimitrov, V.; Doytchinova, I.; Stavrakov, G.; Stoyanova, M. *Eur. J. Med. Chem.* **2014**, *81*, 150.
22. Dimitrov, V.; Dobrikov, G.; Genov, M. *Tetrahedron: Asymmetry* **2001**, *12*, 1323.
23. Valeur, E.; Bradley, M. *Chem. Soc. Rev.* **2009**, *38*, 606.
24. Tsou, H.-R.; Overbeek-Klumpers, E. G.; Hallett, W. A.; Reich, M. F.; Floyd, M. B.; Johnson, B. D.; Michalak, R. S.; Nilakantan, R.; Discafani, C.; Golas, J.; Rabindran, S. K.; Shen, R.; Shi, X.; Wang, Y.-F.; Upeslacis, J.; Wissner, A. *J. Med. Chem.* **2005**, *48*, 1107.
25. Bergmann, E.; Bograchov, E. *J. Am. Chem. Soc.* **1940**, *62*, 3016.
26. Šmejkal, T.; Breit, B. *Angew. Chem., Int. Ed.* **2008**, *47*, 3946.
27. Šmejkal, T.; Breit, B. *Angew. Chem.* **2008**, *120*, 4010.
28. Canetti, G.; Rist, N.; Grosset, J. *Rev. Tuberc. Pneumol.* **1963**, *27*, 217.
29. Canetti, G.; Froman, S.; Grosset, J.; Hauduroy, P.; Langerova, M.; Mahler, H. T.; Meissner, G.; Mitchison, D. A.; Sula, L. *Bull. WHO* **1963**, *29*, 565.
30. Canetti, G.; Fox, W.; Khomenko, A.; Mahler, H. T.; Menon, N. K.; Mitchinson, D. A.; Rist, N.; Smelev, N. A. *Bull. Org. Mond. Sante* **1969**, *41*, 21.
31. Heifets, L. Conventional methods for antimicrobial susceptibility testing of Mycobacterium tuberculosis. In *Multidrug-resistant Tuberculosis*; Bastian, I., Portaels, F., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000.
32. Cell Titer 96 Non-Radioactive Cell proliferation assay, Technical Bulletin #TB112, Promega Corporation USA. Revised 12/99.
33. Dobrikov, G. M.; Valcheva, V.; Nikolova, Y.; Ugrinova, I.; Pasheva, E.; Dimitrov, V. *Eur. J. Med. Chem.* **2013**, *63*, 468.
34. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.