Bioorganic & Medicinal Chemistry 25 (2017) 6167–6174

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Anti-tuberculosis activity and structure–activity relationships of oxygenated tricyclic carbazole alkaloids and synthetic derivatives ☆

Carsten Börger [a], Christian Brütting [a], Konstanze K. Julich-Gruner [a], Ronny Hesse [a], V. Pavan Kumar [a], Sebastian K. Kutz [a], Marika Rönnefahrt [a], Claudia Thomas [a], Baojie Wan [b], Scott G. Franzblau [b], Hans-Joachim Knölker [a,*]

[a] Department Chemie, Technische Universität Dresden, Bergstraße 66, 01069 Dresden, Germany
[b] Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St., MC 964, Chicago, IL 60612-7231, USA

ARTICLE INFO

Article history:
Received 9 November 2016
Revised 13 December 2016
Accepted 23 December 2016
Available online 27 December 2016

Keywords:
Alkaloids
Anti-TB activity
Carbazoles
Natural products
Tuberculosis

ABSTRACT

A series of 49 oxygenated tricyclic carbazole derivatives has been tested for inhibition of the growth of *Mycobacterium tuberculosis* and a mammalian cell line (vero cells). From this series, twelve carbazoles showed a significant anti-TB activity. The four most active compounds were the naturally occurring carbazole alkaloids clauszoline-M (**45**), murrayaline-C (**41**), carbalexin-C (**27**), and the synthetic carbazole derivative **22** with MIC_{90} values ranging from 1.5 to 3.7 μM. The active compounds were virtually non-toxic for the mammalian cell line in the concentration range up to 50 μM.

1. Introduction

Tuberculosis (TB) is one of the most serious threats for global health. In the year 2015, >10 million people fell ill with TB and about 1.8 million people died. Most worrying is the increase of multidrug-resistant TB (MDR-TB) with about half a million new cases in 2015.[1] Therefore, the development of new and more efficacious drugs against *Mycobacterium tuberculosis* is urgently needed. Major initiatives have been started and many research projects towards this goal have been followed over the last decade.[2,3] Based on a few very preliminary reports on the weak antituberculosis (anti-TB) activity of some simple oxygenated tricyclic carbazole alkaloids like clausine-K (clauszoline-J)[4,5] and micromeline (Fig. 1),[6] we started the first investigations on the structure–activity relationships of carbazole derivatives.[7–9]

A broad variety of carbazole alkaloids with interesting structures and promising pharmacological activities has been isolated from diverse natural sources, like terrestrial plants, microorganisms and algae.[10] We have an ongoing program directed towards the total synthesis of biologically active carbazoles. Herein, we present a study describing the anti-TB activity of a series of oxygenated tricyclic carbazole alkaloids including a range of synthetic analogs. The carbazole derivatives have been prepared using either our iron-mediated (Scheme 1) or palladium-catalyzed synthesis (Scheme 2).[11,12]

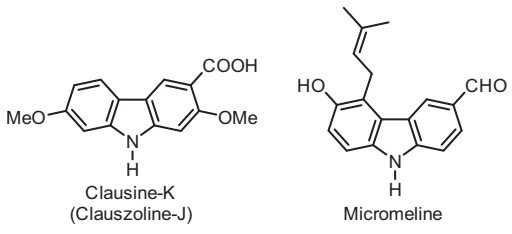
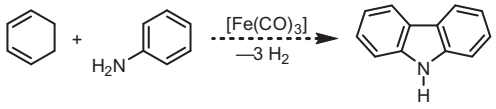


Fig. 1. Oxygenated tricyclic carbazole alkaloids.

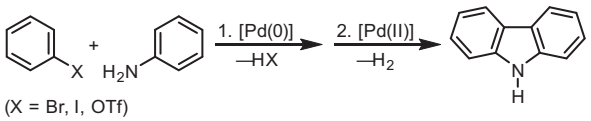


Scheme 1. Iron-mediated carbazole synthesis.

☆ Transition Metals in Organic Synthesis, Part 132. Part 131: Fritsche RF, Theumer G, Kataeva O, Knölker H-J. Angew Chem. 2017;129:564–568; Angew Chem Int Ed. 2017;56:549–553.

* Corresponding author.
E-mail address: hans-joachim.knoelker@tu.dresden.de (H.-J. Knölker).

http://dx.doi.org/10.1016/j.bmc.2016.12.038
0968-0896/



Scheme 2. Palladium-catalyzed carbazole synthesis.

2. Results and discussion

We have screened a broad range of oxygenated tricyclic carbazole derivatives for their anti-TB activity (Table 1). The minimum concentrations effecting a 90% inhibition of growth (MIC) of *M. tuberculosis* strain $H_{37}Rv$ were determined by the microplate alamar blue assay (MABA).[13,14] The *in vitro* cytotoxicity towards mammalian (vero) cells was examined as previously reported.[13,15] The carbazoles shown in Table 1 have been arranged according to their oxygenation pattern. Carbazoles mono-oxygenated at position 1 were already screened in our previous study and thus have not been considered here.[7] From the present study, the results can be summarized as follows. Among the 1,6-dioxygenated carbazoles **1**–**4**, the alkaloid clausine-I (**4**) and among the 1,7-dioxygenated carbazoles **5**–**10**, the synthetic compound **10** exhibit a significant inhibitory activity. The trioxygenated carbazole alkaloid murrayastine (**11**) shows only a very minor effect. For the 2-oxygenated carbazoles **12**–**23**, a moderate activity was found for mukoenine-A (girinimbilol) (**13**) and a very strong activity for the pityriazole derivative **22**. Compound **22** represents a precursor for our total synthesis of the carbazole alkaloid pityriazole (**23**).[23] It is noteworthy that the natural product itself (compound **23**) was completely inactive, at least in the concentration range which has been tested (up to 50 μM). The 2,5-dioxygenated carbazole **24** showed a moderate effect. The carbazole alkaloid carbalexin-C (**27**) exhibited the strongest inhibition towards *M. tuberculosis* among the 2,6-dioxygenated carbazoles **25**–**30** and also among all 49 carbazole derivatives of the present investigation. In the large group of 2,7-dioxygenated carbazoles **31**–**41**, we found two compounds with a moderate inhibiting activity, the synthetic derivative **34** and isomurrayafoline-B (**37**), and also one compound with a very strong activity, murrayaline-C (**41**). Among the 2,8-dioxygenated carbazoles **42**–**45**, we found a moderate activity for the synthetic derivative **43** and a very strong activity for the carbazole alkaloid clauszoline-M (**45**). The 3,4-dioxygenated carbazole **46** showed a significant inhibition, whereas neocarazostatin-B (**47**) had only a very diminished activity. From the tricyclic carbazole derivatives investigated in this study, it appears that their inhibiting activity towards *M. tuberculosis* is primarily dependent on the oxygenation pattern, the oxidation state of carbon substituents as well as the presence and position of additional functional groups. The presence of lipophilic prenyl or geranyl groups at a carbazole framework with a specific substitution pattern has either no (compare **6** with **7**, **8** and **32** with **38**) or only a minor effect on the inhibiting activity (compare **12** with **13** and **31** with **37**). To confirm this observation, we have tested 3-prenylcarbazole (**48**) and the isomeric 3-*tert*-prenylcarbazole (**49**) which in fact both exhibited only a moderate activity. 3-Methoxy-2-methylcarbazole-1,4-quinone (**50**) was identified previously as a very active compound and has been included in the present study as an internal reference in order to be able to compare the results of the different investigations.[7–9]

In conclusion, twelve out of the 49 oxygenated tricyclic carbazole derivatives which have been tested exhibited significant activities with selectivity indices ranging from 4.2 to >33. Among these, the compounds **13**, **24**, **34**, **37** and **49** show a moderate activity (SI ranging from >4.2 to >5.4). The compounds **4**, **10** and **46** show a strong activity (SI ranging from >7.2 to >8.1). The most active compounds were clauszoline-M (**45**) (MIC_{90} = 3.7 μM, SI > 13), the protected pityriazole **22** (MIC_{90} = 2.9 μM, SI > 17.2), murrayaline-C (**41**) (MIC_{90} = 2.8 μM, SI = 16.8) and carbalexin-C (**27**) (MIC_{90} = 1.5 μM, SI > 33). The fact that most of the compounds were virtually nontoxic for the mammalian cell line indicates that further improvement of the anti-TB potencies by structural variation may lead to carbazole-based tuberculosis drug candidates.

3. Experimental

3.1. General

All reactions were carried out in oven-dried glassware using anhydrous solvents under an argon atmosphere, unless stated otherwise. CH_2Cl_2, Et_2O, and THF were dried using a solvent purification system (MBraun-SPS). Petroleum ether refers to a hydrocarbon mixture with a boiling range of 40–65 °C. All other chemicals were used as received from commercial sources. Flash chromatography was performed using silica gel from Acros Organics (0.035–0.070 mm) occasionally on a Büchi Sepacore system equipped with an UV monitor. TLC was performed with TLC plates from Merck (60 F254) using UV light for visualization. Melting points were measured on a Gallenkamp MPD 350 melting point apparatus. Ultraviolet spectra were recorded on a PerkinElmer 25 UV/Vis spectrometer. Fluorescence spectra were obtained using a Varian Cary Eclipse spectrometer. IR spectra were recorded on a Thermo Nicolet Avatar 360 FT-IR spectrometer using the ATR method (Attenuated Total Reflectance). NMR spectra were recorded on Bruker DRX 500 and Avance III 600 spectrometers. ACD/NMR Processor Academic Edition and Bruker TopSpin V2.1 were used for processing. Chemical shifts δ are reported in parts per million with the solvent signal as internal standard. Standard abbreviations were used to denote the multiplicities of the signals. Mass spectra were recorded on a Finnigan MAT-95 spectrometer (electron impact, 70 eV) or by GC/MS-coupling using an Agilent Technologies 6890 N GC System equipped with a 5973 Mass Selective Detector (electron impact, 70 eV). ESI-MS spectra were recorded on an Esquire LC mass spectrometer with an ion trap detector from Bruker. Positive and negative ions were detected. Elemental analyses were measured on a EuroVector EuroEA3000 elemental analyzer.

3.2. 6-Methyl-8-(tosyloxy)carbazol-2-yl 3,3-dimethylacrylate (*10*)

7-Hydroxy-3-methyl-1-(tosyloxy)carbazole (**6**)[17] (50.0 mg, 0.136 mmol) and aluminum chloride (12.6 mg, 94.7 μmol) were added sequentially at room temperature to a solution of 3,3-dimethylacryloyl chloride (17.6 mg, 0.149 mmol) in diethyl ether (2 mL). The mixture was stirred at room temperature for 4 h. Water and hydrochloric acid (2 M) were added, the mixture was extracted with ethyl acetate, the organic layer was dried with magnesium sulfate, and the solvent was evaporated. Purification of the residue by column chromatography on silica gel (isohexane–ethyl acetate, 5:1) provided compound **10** (39.3 mg, 87.5 μmol, 64%) as a light yellow solid, mp: 149.5–151.0 °C. UV (MeOH): λ = 232, 257 (sh), 297, 339 nm. Fluorescence (MeOH): λ_{ex} = 232 nm; λ_{em} = 342 nm. IR (ATR): ν = 3379, 2918, 2856, 1733, 1629, 1585, 1492, 1440, 1364, 1303, 1260, 1214, 1174, 1140, 1117, 1078, 1064, 973, 882, 849, 809, 774, 733, 700, 659 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 2.01 (d, J = 0.8 Hz, 3H), 2.26 (d, J = 0.8 Hz, 3H), 2.36 (s, 3H), 2.41 (s, 3H), 5.97 (br s, 1H), 6.66 (s, 1H), 6.94 (dd, J = 8.4, 1.7 Hz, 1H), 7.13 (d, J = 1.7 Hz, 1H), 7.28 (d, J = 8.2 Hz, 2H), 7.63 (s, 1H), 7.73 (d, J = 8.2 Hz, 2H), 7.90 (d, J = 8.4 Hz, 1H), 8.36 (s, 1H). ^{13}C NMR and DEPT (150 MHz, $CDCl_3$): δ = 20.52 (CH_3), 21.14 (CH_3), 21.68 (CH_3), 27.68 (CH_3), 104.55 (CH), 114.16 (CH), 115.23 (CH),