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Synthesis and Activity of Carbazole Derivatives Against *Mycobacterium tuberculosis***

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Infecting one-third of the world's inhabitants, *Mycobacterium tuberculosis* (MTB) is deemed a serious public health concern.[1] Failure to follow the current regimen along with the HIV pandemic have led to the emergence of multiple drug-resistant tuberculosis (MDR-TB) strains. The pursuit of more efficacious drugs and prophylaxis is warranted.[2,3] Findings of some naturally occurring carbazole alkaloids (Figure 1), exhibiting antituberculosis activity, have prompted us to explore further carbazole derivatives for structure–activity relationships.

Clausine K or clauszoline J, a natural product isolated independently from several sources,[4–6] was found to have weak antituberculosis activity (MIC of 100 $\mu g\,mL^{-1}$ against the $H_{37}Ra$ strain).[1,7] Ma et al. isolated micromeline from the stem bark of *Micromelum hirsutum* along with some known carbazole alkaloids, and found the MIC to be 31.5 $\mu g\,mL^{-1}$ against *M. tuberculosis* $H_{37}Rv$ (selectivity index >3).[8] Based on these findings, we screened a series of carbazole alkaloids and derivatives for their in vitro anti-TB activity to find out whether the carbazole framework represents a novel antituberculosis scaffold. The intention of the present study was to identify potent anti-TB active carbazoles and to establish preliminary structure–activity relationships (SAR).

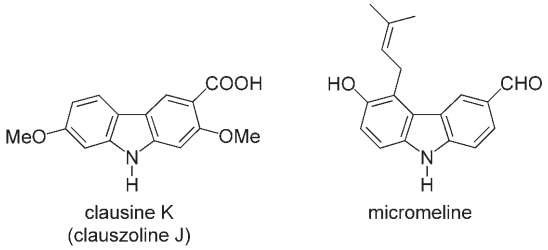
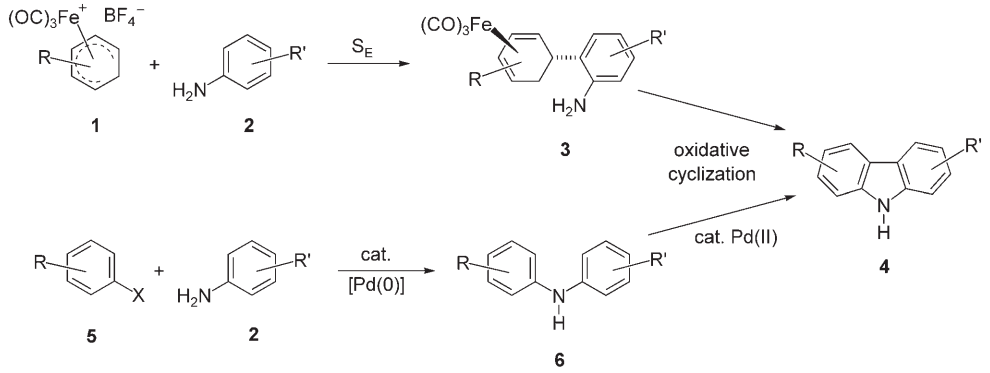


Figure 1. Naturally occurring carbazole alkaloids with anti-TB activity.

Compounds **4a–i** were purchased from Sigma Aldrich; **4j–v** and **8–15** were prepared using either the iron-mediated or the palladium-catalyzed approach previously developed by our group (Schemes 1 and 2, Table 1).[9] An electrophilic aromatic substitution of the tricarbonyliron-cyclohexadienylium salts **1** and the arylamines **2** affords functionalized tricarbonyliron complexes **3**. The oxidative cyclization of the tricarbonyliron complexes **3** furnishes substituted carbazole derivatives **4**. In another approach, the palladium(0)-catalyzed amination of aryl halides **5** by arylamines **2** leads to *N*,*N*-diarylamines **6**, which upon palladium(II)-catalyzed oxidative cyclization provide a simple route to functionalized carbazoles **4**. The 6-bromocarbazole **8a** represents a crucial intermediate for the total synthesis of the neuronal cell protecting carbazole alkaloids (±)-carquinostatin A and (±)-lavanduquinocin, and has been prepared by the iron-mediated approach.[17,18] Reaction of the 6-bromocarbazole **8a** with the dimeric π-allylnickel bromide complex, prepared in situ from β-cyclolavandulyl bromide **7** and an excess of tetracarbonylnickel greater than originally applied,[18] provides the hydrodebromination product **8b** along with recovered starting material **8a**, the lavanduquinocin precursor **9**, and the 6-acylcarbazole **10** (Scheme 2).[19] The latter product is formed by a nickel-mediated carbonylation reaction.



Scheme 1. Synthesis of carbazoles **4** by iron-mediated or palladium-catalyzed oxidative cyclization.

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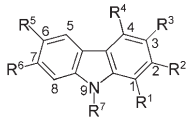
Table 1. Antituberculosis activity, vero cell cytotoxicity, and selectivity indices of the carbazole derivatives **4**.



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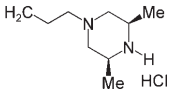
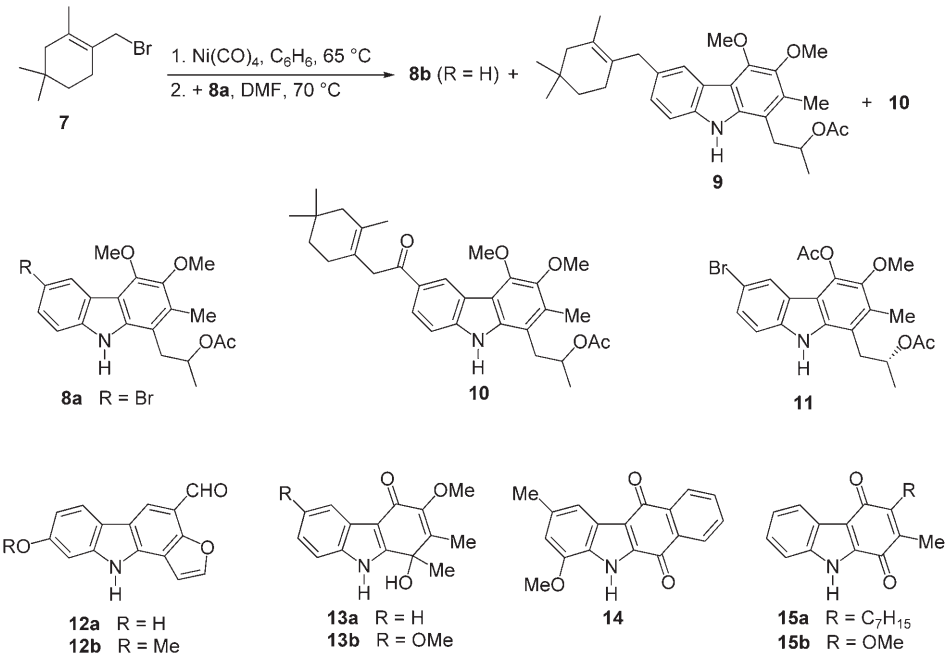
Compd	R^1	R^2	R^3	R^4	R^5	R^6	R^7	Ref.[a]	MIC[b]	IC_{50}[c]	SI[d]
4a	H	H	H	H	H	H	H	–	>128	109	<0.9
4b	H	H	H	H	H	H	Me	–	>128	>128	ND[e]
4c	H	H	H	H	H	H	Et	–	>128	>128	ND
4d	H	H	H	H	H	H	vinyl	–	>128	>128	ND
4e	H	H	CHO	H	H	H	Et	–	>128	116	<0.9
4f	H	H	NH_2	H	H	H	Et	–	55	62	1.1
4g	H	H	H	H	H	H		–	30	44	1.5
4h	H	H	Br	H	Br	H	H	–	>128	>128	ND
4i	H	OH	H	H	H	H	H	–	>128	>128	ND
4j	H	OH	Me	H	H	H	H	[10]	105	36	0.3
4k	OMe	H	Me	H	H	H	H	[11]	99	21	0.2
4l	OMe	H	CH_2OH	H	H	H	H	[11]	>128	>128	ND
4m	OMe	H	CHO	H	H	H	H	[11]	>128	>128	ND
4n	OMe	H	COOH	H	H	H	H	[11]	>128	>128	ND
4o	OMe	H	COOMe	H	H	H	H	[12]	69	>128	>1.9
4p	H	OMe	Me	H	H	OMe	H	[13]	>128	>128	ND
4q	H	OMe	CHO	H	H	OMe	H	[13]	>128	96	<0.8
4r	H	OMe	COOH	H	H	OMe	H	[13]	>128	25	<0.2
4s	H	OH	CHO	H	H	OH	H	[13]	89	114	1.3
4t	C_7H_{15}	Me	OMe	H	H	H	H	[14]	>128	30	<0.2
4u	Me	Me	OMe	H	OMe	H	H	[15]	>128	>128	ND
4v	C_7H_{15}	Me	OMe	OMe	H	H	H	[16]	11	40	3.6
RMP[f]	–	–	–	-	–	–	–	–	0.06	109	1817

[a] Reference for the synthetic method being applied. [b] Minimum inhibitory concentration in μM against $H_{37}Rv$ in MABA assay. Values are the mean of three replicate experiments. For experiment(s) that yielded a value higher than the maximum concentration used, >128 μM is denoted. [c] Concentration in μM effecting a 50% decrease in tetrazolium dye reduction by vero cells (African green monkey kidney cells). Values are means of three replicate experiments. For experiment(s) that yielded a value higher than the maximum concentration used, >128 μM is denoted. [d] Selectivity index: SI = IC_{50}/MIC. [e] Not determined. [f] Rifampicin (=rifampin) used as a positive control; solvent used as a negative control.



Scheme 2. Carbazole derivatives **8**–**15**.

The MIC values of the carbazoles against the MTB $H_{37}Rv$ strain were determined using the microplate alamar blue assay (MABA).[25,26] The in vitro cytotoxicity was performed as previously described.[3,26] From our initial screening of carbazoles (Tables 1 and 2) the following conclusions could be drawn. Carbazole **4a** was inactive. Substituents at the carbazole nitrogen atom (**4b**–**4e**) did not contribute to antitubercular activity. However, the presence of a nitrogen-containing group (in **4f** and **4g**) increased the activity substantially. Little to no activity was found for 3,6-dibromocarbazole **4h**, 2-hydroxycarbazole **4i**, 2-hydroxy-3-methylcarbazole **4j**, or the 1-methoxy-

Table 2. Antituberculosis activity, vero cell cytotoxicity, and selectivity indices of the carbazoles **10**–**15**.

Compd	Ref.[a]	MIC[b]	IC_{50}[c]	SI[d]
10	[17, 19]	8	>128	>16
11	[20]	55	25	0.5
12a	[21]	51	40	0.8
12b	[21]	>128	27	<0.2
13a	[22]	>128	>128	ND[e]
13b	[22]	>128	>128	ND
14	[23]	>128	>128	ND
15a	[24]	117	>128	>1.1
15b	[24]	9	>128	>14

[a] Reference for the synthetic method being applied. [b] Minimum inhibitory concentration in μM against H_{37}Rv in MABA assay. Values are the mean of three replicate experiments. For experiment(s) that yielded a value higher than the maximum concentration used, >128 μM is denoted. [c] Concentration in μM effecting a 50% decrease in tetrazolium dye reduction by vero cells (African green monkey kidney cells). Values are the mean of three replicate experiments. For experiment(s) that yielded a value higher than the maximum concentration used, >128 μM is denoted. [d] SI = IC_{50}/MIC. [e] Not determined.

carbazole alkaloids (murrayafoline A (**4k**), koenoline (**4L**), murrayanine (**4m**), and mukoeic acid (**4n**)). However, the presence of a methoxycarbonyl group, as for mukonine (**4o**), increased the activity by at least twofold. The 2,7-dioxygenated carbazole alkaloids (2,7-dimethoxy-3-methylcarbazole (**4p**), 7-methoxy-*O*-methylmukonal (**4q**), clausine K (**4r**), and clausine O (**4s**)) showed little or no inhibitory activity against MTB. Clausine K (or clauszoline J, **4r**) was previously reported to exhibit activity against the H_{37}Ra strain.[7] The contrary result reported herein may be explained by the different highest concentration tested (128 μM in this study versus 200 μg mL^{-1} or 737 μM in the original study). The tricyclic 9*H*-carbazoles, which are 3-oxygenated or 3,6-dioxygenated (*O*-methylcarazostatin (**4t**) and 4-deoxycarbazomycin C (**4u**)), were inactive, whereas the 3,4-dioxygenated carbazole derivatives **4v**, **10**, and **11** exhibited significant anti-TB activity. Among this group of analogues there is a general trend toward lower anti-TB activity with fewer substitutions.

The furo[3,2-*a*]carbazole alkaloid furoclausine A (**12a**) was also found to show anti-TB activity, whereas its corresponding methyl ether **12b** was inactive. Finally, we investigated carbazolequinols and carbazolequinones. Tran and colleagues screened quinone analogues against mycobacteria and reported a few active compounds with low to moderate toxicity.[27] The carbazole-1,4-quinol alkaloids, carbazomycin G (**13a**) and carbazomycin H (**13b**), and the benzo[*b*]carbazolequinone **14** were inactive against TB, as was the carbazole-1,4-quinone **15a**, whereas the carbazol-1,4-quinone **15b** exhibited one of the lowest MIC values. The fact that compound **15b** is a 3,4-dioxygenated carbazole derivative, as are **4v**, **10**, and **11** described above, is noteworthy.

It is generally accepted that in vitro efficacy alone is insufficient to advance potential lead structures to druglike candidates. Lipinski's rule of five is commonly utilized to separate druglike from non-druglike candidates.[28] Most of the 31 carbazole derivatives tested in the present study are compliant with the rule of five. Compound **10** exceeds the Lipinski log*P* and molecular weight cutoffs, the same two parameters found to be higher for oral antimicrobial agents than for oral drugs used to treat other indications.[29] Thus, it is concluded that with further structural modification to improve anti-TB potency, carbazoles may be developed as anti-TB drug candidates. Overall, the carbazole derivatives **4g**, **4v**, **10**, and **15b** exhibited the best MIC values. Among these, **10** and **15b** were most active with MIC values of 8 μM (4.0 μg mL^{-1}) and 9 μM (2.2 μg mL^{-1}), respectively, and were selective (SI > 10). The MIC of compound **10** for a second strain, *M. tuberculosis* Erdmann, was within a twofold dilution of that for *M. tuberculosis* H_{37}Rv. The synthesis of novel functionalized carbazoles and their structure–activity studies are currently underway in our research groups.

Acknowledgements

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Keywords: carbazoles · natural products · structure–activity relationships · tuberculosis

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[19] Under argon atmosphere, a solution of $Ni(CO)_4$ (4.3 mL, 33.1 mmol) in degassed dry benzene (30 mL) was heated at 65 °C. β-Cyclolavandulyl bromide **7** (1.44 g, 6.62 mmol) was added, and the mixture was stirred for 3.5 h at 65 °C under a light stream of argon. Heating was discontinued and all volatile components of the red solution were evaporated under vacuum. A solution of the 6-bromocarbazole **8a** (1.39 g, 3.31 mmol) in degassed dry DMF (20 mL) was added to the red residue, and the resulting dark red solution was stirred for 16 h at 70 °C under argon. After cooling to room temperature, the reaction mixture was poured into a solution of HCl (conc., 2 mL) and H_2O (60 mL). The aqueous layer was extracted twice with Et_2O (50 mL). The combined organic

layers were washed with 5% HCl (40 mL), then with H_2O (40 mL), and dried over Na_2SO_4. Filtration, removal of the solvent, and flash chromatography of the residue on silica gel (hexane/EtOAc, 4:1) afforded the 6-(β-cyclolavandulyl)carbazole **9** (yield: 695 mg, 44%) as colorless crystals, the carbazole **8b** (yield: 45 mg, 4%) as light-yellow crystals, re-isolated 6-bromocarbazole **8a** (yield: 460 mg, 33%) as colorless crystals, and the 6-acylcarbazole **10**: yield: 220 mg (13%), colorless crystals; mp: 58–60 °C; UV/Vis (MeOH): $\lambda = 216$, 238, 249 (sh), 276, 288 (sh), 332 nm; IR (DRIFT): $\tilde{\nu} = 3321$, 2946, 1738, 1713, 1674, 1660, 1607, 1503, 1447, 1396, 1373, 1310, 1259, 1111, 1057, 1008 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 0.87$ (s, 6H), 1.31 (d, $J = 6.3$ Hz, 3H), 1.34 (t, $J = 6.4$ Hz, 2H), 1.74 (s, 3H), 1.82 (s, 2H), 2.03 (m, 2H), 2.18 (s, 3H), 2.41 (s, 3H), 3.03 (dd, $J = 13.8$, 10.2 Hz, 1H), 3.25 (dd, $J = 13.8$, 2.6 Hz, 1H), 3.84 (s, 2H), 3.88 (s, 3H), 4.14 (s, 3H), 5.00 (m, 1H), 7.48 (d, $J = 8.5$ Hz, 1H), 8.08 (dd, $J = 8.5$, 1.6 Hz, 1H), 8.88 (d, $J = 1.6$, 1H), 10.04 ppm (br s, 1H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): $\delta = 12.84$ (CH_3), 19.32 (CH_3), 19.89 (CH_3), 21.51 (CH_3), 28.15 (CH_2), 28.22 (2 CH_3), 29.27 (C), 35.00 (CH_2), 35.85 (CH_2), 43.27 (CH_2), 46.09 (CH_2), 60.40 (CH_3), 60.89 (CH_3), 72.09 (CH), 110.37 (CH), 113.80 (C), 114.83 (C), 122.00 (C), 123.31 (C), 123.88 (CH), 125.78 (CH), 128.26 (C), 129.19 (C), 129.54 (C), 137.60 (C), 142.48 (C), 144.72 (C), 147.03 (C), 172.82 (C=O), 198.53 ppm (C=O); MS (150 °C): *m/z* (%) = 505 [M^+] (15), 368 (100): HRMS: *m/z* calcd. for $C_{31}H_{39}NO_5$ [M^+]: 505.2828, found: 505.2817, Anal. calcd. for $C_{31}H_{39}NO_5$: C 73.63, H 7.77, N 2.77; found: C 73.42, H 7.70, N 2.81.

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