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Exploring the diastereoselectivity for Fischer indolization of *L*-menthone under different conditions, spectral characterization, and biological activities of new (2*R*,4a*S*)-2,3,4,4a-tetrahydro-1*H*-carbazole analogs

Munisaa Younus [1], Marium Ahsan [1], Noor-ul-Huda [2], Maria Aqeel Khan [1,*], Saima Rasheed [2,*], Rabia Sadiq [3] and Fatima Zehra Basha [3]

[1] *Third World Center for Science and Technology, Husein Ebrahim Jamal Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, 75270, Pakistan*
[2] *Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, 75270, Pakistan*
[3] *Husein Ebrahim Jamal Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, 75270, Pakistan*

* *Corresponding author at: Third World Center for Science and Technology, Husein Ebrahim Jamal Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, 75270, Pakistan; Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, 75270, Pakistan.*
e-mail: drmaria.aqeel@iccs.edu (M.A. Khan), saima.rasheed@iccs.edu (S. Rasheed).

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ABSTRACT

Tetrahydrocarbazoles are important class of heterocycles that exhibit numerous biological properties. They are also found in several natural products. In the present study, Fischer indolization of *L*-menthone was investigated for diastereoselectivity using different reaction conditions. No appreciable diastereoselectivity was observed for the acids used except CuBr and boric acid at varying temperatures, where satisfactory results were obtained. In addition, a small library of new (2*R*,4a*S*)-2,3,4,4a-tetrahydro-1*H*-carbazole analogs was reported and structurally characterized using spectroscopic techniques herein. Additionally, the compounds were evaluated against different biological activities, such as carbonic anhydrase inhibitory, immunomodulatory, and anticancer activities and did not show any activity. As the synthesized library was found safe when tested against cytotoxicity in normal cell line, it will be explored for other biological activities in near future to identify its biological outcome.

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1. Introduction

Tetrahydrocarbazole is defined as an indole skeleton fused with a cyclohexane ring. This class is widely distributed in nature and has gained immense attention of medicinal chemists as found as the main skeleton in many bioactive compounds [1]. These compounds showed biological properties such as P-type ATPase inhibitory activity as antifungal [2], DNA biosynthesis inhibitory activity as antibacterial [3], antiviral [4], neuroprotective [5], β-3-adrenoceptor agonist [6], antitumor [7], *etc.* (Figure 1). Tetrahydrocarbazole exists in two isomeric forms: structure **I** with indole scaffold and structure **II** with indolenine skeleton.

4a-Substituted tetrahydrocarbazoles with an indolenine skeleton are found as important skeleton in several intricate alkaloidal compounds, such as tubifoline **III** [8], arboridinine **IV** [9], rhazinoline **V** [10], scholaricine A **VI** [11], *etc*. This skeleton has also been found to exhibit several intriguing biological activities, including monamine oxidase inhibitory activity by strictamine **VII** [12], anti-inflammatory and neurosteroid modulating activities by (-)-koumine **VIII** [13], and selective potentiator of *β*–lactams in Methicillin-resistant *Staphylococcus aureus* (MRSA) by compound **IX**. Based on large therapeutic index, our group has previously reported [14] synthesis of diastereomeric (2*R*,4a*R*)- and (2*R*,4a*S*)-4a-*iso*propyl-2-methyl-2,3,4,4a-tetrahydro-1*H*-carbazoles **X** and **XI** using Fischer indolization, which displayed selective cholinesterase inhibitory activity (Figure 2). Additionally, a library of seventeen (2*R*,4a*R*)-diastereomers and two derivatives of (2*R*,4a*S*)-isomers were previously reported [14].

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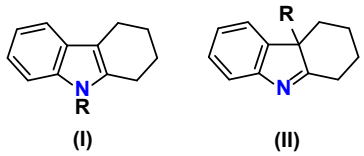


Figure 1. Isomeric forms of tetrahydrocarbazoles **I** and **II**.

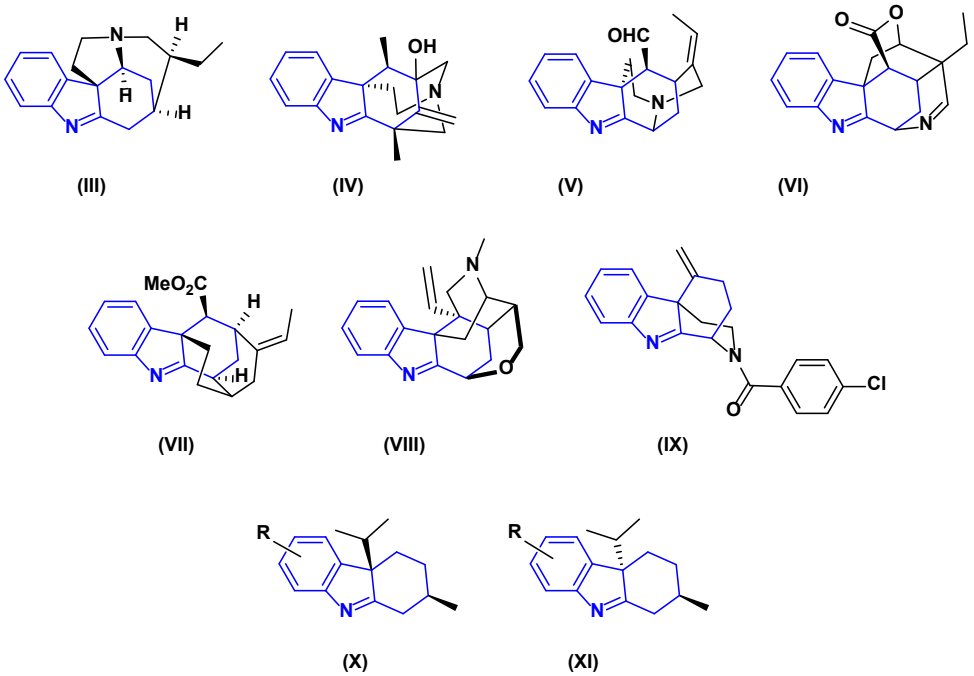


Figure 2. Some reported biologically important or complex alkaloids **I**-**IX** with a 4a-substituted 2,3,4,4a-tetrahydro-1*H*-carbazole moiety.

In this study, we reported six new (2*R*,4a*S*)-4a-*iso*propyl-2-methyl-2,3,4,4a-tetrahydro-1*H*-carbazole analogs to expand the library of aforementioned compounds as bioactive molecules. In addition, other acidic and thermal conditions were explored to investigate the diastereoselectivity of the reaction.

2. Experimental

2.1. Instrumentation

Chemicals (*L*-Menthone (96%) and substituted phenyl hydrazine hydrochlorides) were used for the synthesis and were purchased from Alfa Aesar, and Sigma Aldrich. Bovine carbonic anhydrase-II (Cat: C3934), 4-nitrophenylacetate (Cat: N8130), and acetazolamide (Cat: A6011) were purchased from Sigma-Aldrich. Tris-HCl was bought from Bio Basic (Item No: TB0103). Thin-layer chromatography (TLC) was developed on aluminum-supported precoated silica gel aluminum plates (Kieselgel 60F_{254}, E. Merck, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance spectrometers using 300 MHz and 400 MHz. EI-MS measurements were taken on a Finningan MAT-321A, Germany. IR spectra were obtained on a Shimadzu FT-IR 8900 spectrometer. UV spectra were taken on Hitachi U-3200 ND Thermoscientific Evolution 310 spectrophotometer. Optical activity was obtained with a P-2000 polarimeter.

2.2. Synthesis

L-Menthone (6.5 mmol) was taken in a round bottom flask and charged with substituted phenyl hydrazine hydrochlorides (6.8 mmol) in the corresponding acid. The reaction was heated for 30 minutes. Reaction mixture was then quenched with saturated solution of $NaHCO_3$ solution, and extracted with ethyl acetate (EtOAc) thrice. The combined organic layers were then washed with brine solution to remove inorganic impurities. The organic layer was then dried with $MgSO_4$ (anhydrous), filtered, and concentrated to obtain a diastereomeric mixture of products, which was purified using column chromatography (eluent: gradient mixture of *n*-hexane and EtOAc).

(2R, 4aR)-4a-Isopropyl-2-methyl-2, 3, 4, 4a-tetrahydro-1H-carbazole (**3**) [14]: Color: White. Yield: 40%. FT-IR (KBr, ν, cm^{-1}): 2960, 2927, 2855, 1619 (C=N), 1468 (C=C). ^{1}H NMR (300 MHz, $CDCl_3$, *δ*, ppm): 7.55 (d, $J_{8,7}$ = 7.8 Hz, 1H, H-8), 7.29 (m, 2H, H-5, H-7), 7.12 (td, $J_{6,(5,7)}$ = 7.5 Hz, $J_{6,8}$ = 0.6 Hz, 1H, H-6), 2.82 (ddd, $J_{1\text{-eq},1\text{-ax}}$ = 12.9 Hz, $J_{1\text{-eq},2}$ = 4.2 Hz, $J_{1\text{-eq},3\text{-eq}}$ = 1.5 Hz, 1H, 1-eq CH_2), 2.54 (dt, $J_{4\text{-eq},4\text{-ax}}$ = 13.8 Hz, $J_{4\text{-eq},(3\text{-ax},3\text{-eq})}$ = 2.7 Hz, 1H, 4-eq CH_2), 2.43 (sep, $J_{11,(12,13)}$ = 6.9 Hz, 1H, 11-CH), 2.20 (t, $J_{1\text{-ax},(1\text{-eq},2)}$ = 12.3 Hz, 1H,1-ax CH_2), 1.57 (m + qd, 3H, 2-CH/3-eq CH_2/3-ax CH_2), 1.21(d, $J_{12,11}$ = 6.9 Hz, 3H, 12-CH_3), 1.12 (d, $J_{10,2}$ = 6.3 Hz, 3H, 10-CH_3), 1.00 (td, $J_{4\text{-ax},(4\text{-eq},3\text{-ax})}$ = 13.4 Hz, $J_{4\text{-ax},3\text{-eq}}$ = 4.5 Hz, 1H, 4-ax CH_2), 0.25 (d, $J_{13,11}$ = 6.6 Hz, 3H, 13-CH_3). MS (EI, *m/z* (%)): 227 (M^+), 212, 185, 143. UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 250 (2.08), 219 (1.78).

(2R, 4aS)-4a-Isopropyl-2-methyl-2, 3, 4, 4a-tetrahydro-1H-carbazole (**4**) [14]: Color: White. Yield: 39%. FT-IR (KBr, ν, cm^{-1}): 2963, 2934, 2875, 1617 (C=N), 1585, 1466. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.58 (d, $J_{8,7}$ = 7.5 Hz, 1H, H-8), 7.29 (m, 2H, H-5, H-7), 7.10 (t, $J_{6,(7,5)}$ = 6.9 Hz, 1H, H-6), 2.75 (dd, $J_{1\text{-eq},1\text{-ax}}$ = 13.1 Hz, $J_{1\text{-eq},2}$ = 6.3 Hz, 1H, 1-eq CH_2), 2.54 (m + dt, 2H, 1-ax CH_2/2-CH), 2.34 (m, 2H, 4-eq CH_2/11-CH), 2.02 (tt, $J_{3\text{-ax},(3\text{-eq},4\text{-ax})}$ = 14.1Hz, $J_{3\text{-ax},(2,4\text{-eq})}$ = 4.2 Hz, 1H, 3-ax CH_2), 1.35 (dm, 1H, 3-eq CH_2), 1.26 (td, $J_{4\text{-ax},(3\text{-ax},4\text{-eq})}$ = 14.1 Hz, $J_{4\text{-ax},3\text{-eq}}$ = 3.9 Hz, 1H, 4-ax

CH_2), 1.20 (d, $J_{12,11}$ = 6.9 Hz, 3H, 12-CH_3), 0.81 (d, $J_{10,2}$ = 7.2 Hz, 3H, 10-CH_3), 0.23 (d, $J_{13,11}$ = 6.6 Hz, 3H, 13-CH_3). MS (EI, *m/z* (%)): 227 (M^+), 212, 185, 143. UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 260 (2.02), 213 (1.80).

(2R, 4aS)-6-Fluoro-4a-isopropyl-2-methyl-2, 3, 4, 4a-tetra hydro-1H-carbazole (**5**): Color: Brown. Yield: 38%. FT-IR (KBr, ν, cm^{-1}): 3309, 2942, 2831, 2359, 1456 (C=C), 1113 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.52-7.47 (m, 3H, H-5, H-7, H-8), 2.77-2.60 (m, 1H, 1-eq CH_2), 2.58-2.57 (m, 2H, 1-ax CH_2/2-CH), 2.41-2.35 (m, 2H, 4-eq CH_2/11-CH), 2.03 (tt, $J_{3\text{-ax},(3\text{-eq},4\text{-ax})}$ = 15.4 Hz, $J_{3\text{-ax},(2\text{-eq},4\text{-eq})}$ = 5.0 Hz, 1H, 3-ax CH_2), 1.41 (dm, $J_{3\text{-eq},3\text{-ax}}$ = 14.2 Hz, 1H, 3-eq CH_2), 1.23 (d+m, $J_{12,11}$ = 6.4 Hz, 4H, 12-CH_3, 4-ax CH_2), 0.85 (d, $J_{10,2}$ = 7.2 Hz, 3H, 10-CH_3), 0.29 (d, $J_{13,11}$ = 6.6 Hz, 3H, 13-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 155.8 (C), 145.9 (C), 140.3 (C), 135.9/135.8 (d, C, *J* = 7.0 Hz), 113.2/112.9 (d, CH, *J* = 23.0 Hz), 111.8/111.6 (d, CH, *J* = 23.0 Hz), 110.9/110.8 (d, CH, *J* = 8.0 Hz), 65.0 (C), 40.7 (CH_2), 33.7 (CH), 30.8 (CH_2), 28.9 (CH), 24.9 (CH_2), 22.2 (CH_3), 17.8 (CH_3), 17.5 (CH_3). MS (EI, *m/z* (%)): 245 (M^+, 68), 230 (73), 203 (60), 188 (14), 174 (7), 161 (100), 148 (9), 133 (10), 95 (3). UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 260 (1.65), 249 (1.80). $[\alpha]_D^{25}$: -4.92 (*c* 0.26, CH_2Cl_2).

(2R, 4aS)-8-Fluoro-4a-isopropyl-2-methyl-2, 3, 4, 4a-tetra hydro-1H-carbazole (**6**): Color: Brown. Yield: 34%. FT-IR (KBr, ν, cm^{-1}): 3309, 2942, 2830, 2358, 2342, 1456 (C=C), 1115 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 7.11-7.10 (m, 2H, H-5, H-6), 7.07-7.01 (m, 1H, H-7), 2.79-2.70 (m, 2H, 1-ax CH_2, 1-eq CH_2), 2.55-2.50 (m, 1H, 2-CH), 2.42-2.33 (m, 2H, 4-eq CH_2/11-CH), 2.03 (tt, $J_{3\text{-ax},(3\text{-eq},4\text{-ax})}$ = 14.1 Hz, $J_{3\text{-ax},(2,4\text{-eq})}$ = 4.2 Hz, 1H, 3-ax CH_2), 1.40 (dm, $J_{3\text{-eq},3\text{-ax}}$ = 15.0 Hz, 1H, 3-eq CH_2), 1.27 (td, $J_{4\text{-ax},(3\text{-ax},4\text{-eq})}$ = 14.1 Hz, $J_{4\text{-ax},3\text{-eq}}$ = 3.8 Hz, 1H, 4-ax CH_2), 1.22 (d, $J_{12,11}$ = 6.9 Hz, 3H, 12-CH_3), 0.84 (d, $J_{10,2}$ = 7.2 Hz, 3H, 10-CH_3), 0.27 (d, $J_{13,11}$ = 6.7 Hz, 3H, 13-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 154.9 (C), 146.4/146.3 (d, C, *J* = 2.5 Hz), 141.8/141.7 (d, C, *J* = 12.5 Hz), 125.4/125.3 (d, CH, *J* = 12.5 Hz), 124.2 (C), 118.8/118.7 (d, CH, *J* = 2.5 Hz), 114.7/114.5 (d, C, *J* = 25.0 Hz), 62.3 (C), 35.6 (CH_2), 31.7 (CH_2), 29.4 (CH), 28.6 (CH), 26.1 (CH_2), 17.9 (CH_3), 16.9 (CH_3), 16.4 (CH_3). MS (EI, *m/z* (%)): 245 (M^+). UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 261 (1.80). $[\alpha]_D^{25}$: +3.306 (*c* 0.75, CH_2Cl_2).

(2R, 4aS)-8-Chloro-4a-isopropyl-2-methyl-2, 3, 4, 4a-tetra hydro-1H-carbazole (**7**): Color: Brown. Yield: 30%. FT-IR (KBr, ν, cm^{-1}): 2366, 2844, 2341, 1653 (C=N), 1583, 1456 (C=C), 1056 (C-N), 1032, 823 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 7.31 (d, $J_{7,8}$ = 7.4 Hz, 1H, H-7), 7.21 (d, $J_{5,6}$ = 7.1 Hz, 1H, H-5), 7.06 (t, $J_{6,(5,7)}$ = 7.6 Hz, 1H, H-6), 2.74-2.73 (m, 2H, 1-ax CH_2, 1-eq CH_2), 2.53-2.50 (m, 1H, 2-CH), 2.38-2.31 (m, 2H, 4-eq CH_2/11-CH), 2.03 (tt, $J_{3\text{-ax},(3\text{-eq},4\text{-ax})}$ = 13.9 Hz, $J_{3\text{-ax},(2,4\text{-eq})}$ = 4.1 Hz, 1H, 3-ax CH_2), 1.39 (dm, $J_{3\text{-eq},3\text{-ax}}$ = 14.0 Hz, 1H, 3-eq CH_2), 1.26 (td, $J_{4\text{-ax},(3\text{-ax},4\text{-eq})}$ = 14.2 Hz, $J_{4\text{-ax},3\text{-eq}}$ = 4.0 Hz, 1H, 4-ax CH_2), 1.21 (d, $J_{12,11}$ = 6.8 Hz, 3H, 12-CH_3), 0.84 (d, $J_{10,2}$ = 7.2 Hz, 3H, 10-CH_3), 0.26 (d, $J_{13,11}$ = 6.6 Hz, 3H, 13-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 189.6 (C), 153.5 (C), 144.6 (C), 127.6 (CH), 123.6 (CH), 120.7 (C), 110.5 (CH), 61.9 (C), 35.6 (CH_2), 33.6 (CH), 31.7 (CH_2), 29.3 (CH), 26.2 (CH_2), 17.9 (CH_3), 16.9 (CH_3), 16.4 (CH_3). MS (EI, *m/z* (%)): 265 (M^++2), 263 (M^+). UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 261 (2.12), 253 (1.98). $[\alpha]_D^{25}$: +2.81 (*c* 1.08, CH_2Cl_2).

(2R, 4aS)-4a-Isopropyl-2,6-dimethyl-2, 3, 4, 4a-tetrahydro-1H-carbazole (**8**): Color: Brown. Yield: 49%. FT-IR (KBr, ν, cm^{-1}): 3313, 2942, 2831, 2359, 2341, 1456 (C=C), 1114 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 7.48 (d, $J_{8,7}$ = 7.7 Hz, 1H, H-8), 7.11-7.09 (s+d, $J_{7,8}$ = 10.0 Hz, 2H, H-5, H-7), 2.74 (dd, $J_{1\text{-ax},1\text{-eq}}$ = 13.0 Hz, $J_{1\text{-ax},2}$ = 6.1 Hz, 1H, 1-eq CH_2), 2.61 (d, $J_{1\text{-eq},2}$ = 13.0 Hz, 1H, 1-eq CH_2), 2.49-2.48 (m, 1H, 2-CH), 2.36 (s, 3H, 14-CH_3), 2.34-2.29 (m, 2H, 4-eq CH_2/11-CH), 2.07 (tt, $J_{3\text{-ax},(3\text{-eq},4\text{-ax})}$ = 14.1 Hz, $J_{3\text{-ax},(2\text{-eq},4\text{-eq})}$ = 4.0 Hz, 1H, 3-ax CH_2), 1.34 (dm, $J_{3\text{-eq},3\text{-ax}}$ = 14.1 Hz, 1H, 3-eq CH_2), 1.24 (td, $J_{4\text{-ax},(3\text{-ax},4\text{-eq})}$ = 14.1 Hz, $J_{4\text{-ax},3\text{-eq}}$ = 3.7 Hz, 1H, 4-ax CH_2), 1.21 (d, $J_{12,11}$ = 6.8 Hz, 3H, 12-CH_3), 0.81 (d, $J_{10,2}$ = 7.2 Hz, 3H, 10-CH_3), 0.25 (d, $J_{13,11}$ = 6.7 Hz, 3H, 13-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 187.8 (C), 152.9 (C), 143.0 (C), 128.0 (CH), 124.8 (C), 124.0 (CH), 119.4 (CH), 61.2 (C), 35.5 (CH_2), 31.7 (CH), 29.4 (CH_2), 28.5 (CH), 26.3 (CH_2), 21.4 (CH_3), 18.1 (CH_3), 17.0 (CH_3), 16.5 (CH_3). MS (EI, *m/z* (%)): 241 (M^+, 78), 226 (70), 184 (13), 182 (18), 168 (10), 157 (100), 144 (8), 128 (12), 115 (12), 91 (3), 89 (3). UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 261 (2.09). $[\alpha]_D^{25}$: +2.90 (*c* 0.93, CH_2Cl_2).

(2R,4aS)-4a-Isopropyl-2,8-dimethyl-2,3,4,4a-tetrahydro-1H-carbazole (**9**): Color: Brown. Yield: 36%. FT-IR (KBr, ν, cm^{-1}): 3312, 2942, 2830, 2359, 2342, 1456 (C=C), 1115 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 7.14 (d, $J_{5,6}$ = 7.4 Hz, 1H, H-5), 7.09 (d, $J_{7,8}$ = 7.4 Hz, 1H, H-7), 7.01 (t, $J_{6,(5,7)}$ = 7.5 Hz, 1H, H-6), 2.75 (dd, $J_{1\text{-eq},1\text{-ax}}$ = 13.2 Hz, $J_{1\text{-eq},2}$ = 6.1 Hz, 1H, 1-eq CH_2), 2.64-2.60 (m, 1H, 1-ax CH_2), 2.58 (s, 3H, 14-CH_3), 2.50-2.48 (m, 1H, 2-CH), 2.35-2.31 (m, 2H, 4-eq CH_2/11-CH), 2.02 (tt, $J_{3\text{-ax},(3\text{-eq},4\text{-ax})}$ = 14.1 Hz, $J_{3\text{-ax},(2,4\text{-eq})}$ = 4.2 Hz, 1H, 3-ax CH_2), 1.35 (dm, $J_{3\text{-eq},3\text{-ax}}$ = 13.9 Hz, 1H, 3-eq CH_2), 1.22 (td, $J_{4\text{-ax},(3\text{-ax},4\text{-eq})}$ = 14.1 Hz, $J_{4\text{-ax},3\text{-eq}}$ = 3.9 Hz, 1H, 4-ax CH_2), 1.19 (d, $J_{12,11}$ = 6.8 Hz, 3H, 12-CH_3), 0.83 (d, $J_{10,2}$ = 7.2 Hz, 3H, 10-CH_3), 0.23 (d, $J_{13,11}$ = 6.7 Hz, 3H, 13-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 187.7 (C), 153.7 (C), 142.8 (C), 128.8 (CH), 123.9 (CH), 121.6 (C), 120.6 (CH), 61.4 (C), 35.6 (CH_2), 31.7 (CH), 29.4 (CH_2), 28.6 (CH), 26.4 (CH_2),18.2 (CH_3), 17.3 (CH_3), 16.9 (CH_3), 16.5 (CH_3). MS (EI, *m/z* (%)): 241 (M^+, 32), 226 (33), 216 (54), 198 (40), 184 (28), 174 (46), 159 (100), 146 (25), 130 (22), 115 (12), 91 (7). UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 261 (2.01), 254 (2.02). $[\alpha]_D^{25}$: +3.87 (*c* 0.8, CH_2Cl_2).

(2R, 4aS)-6-Ethyl-4a-isopropyl-2-methyl-2, 3, 4, 4a-tetra hydro-1H-carbazole (**10**): Color: Brown. Yield: 35%. FT-IR (KBr, ν, cm^{-1}): 3315, 2942, 2831, 2361, 2342, 1456 (C=C), 1113 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 7.16-7.14 (s+d, $J_{7,8}$ = 7.5 Hz, 2H, H-5, H-7), 7.06 (*app.* d, $J_{8,7}$ = 7.4 Hz, 1H, H-8), 3.01 (q, $J_{14,15}$ = 7.4 Hz, 2H, 14-CH_2), 2.75 (dd, $J_{1\text{-eq},1\text{-ax}}$ = 13.2 Hz, $J_{1\text{-eq},2}$ = 6.1 Hz, 1H, 1-eq CH_2), 2.66 (d, $J_{1\text{-ax},2}$ = 13.0 Hz, 1H, 1-ax CH_2), 2.50-2.48 (m, 1H, 2-CH), 2.36-2.29 (m, 2H, 4-eq CH_2/11-CH), 2.01 (tt, $J_{3\text{-ax},(3\text{-eq},4\text{-ax})}$ = 13.9 Hz, $J_{3\text{-ax},(2\text{-eq},4\text{-eq})}$ = 3.9 Hz, 1H, 3-ax CH_2), 1.37 (dm, $J_{3\text{-eq},3\text{-ax}}$ = 13.8 Hz, 1H, 3-eq CH_2), 1.29 (t, $J_{15,14}$ = 7.6 Hz, 3H, 15-CH_3), 1.25-1.22 (m, 1H, 4-ax CH_2), 1.20 (d, $J_{12,11}$ = 6.9 Hz, 3H, 12-CH_3), 0.82 (d, $J_{10,2}$ = 7.2 Hz, 3H, 10-CH_3), 0.24 (d, $J_{13,11}$ = 6.7 Hz, 3H, 13-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 187.7 (C), 142.8 (C), 135.8 (C), 127.0 (CH), 124.2 (CH), 120.6 (CH), 115.3 (C), 61.3 (C), 35.6 (CH_2), 31.8 (CH), 29.4 (CH_2), 28.7 (CH), 26.4 (CH_2), 24.4 (CH_2), 18.2 (CH_3), 17.0 (CH_3), 16.5 (CH_3), 15.3 (CH_3). MS (EI, *m/z* (%)): 255 (M^+, 84), 240 (62), 213 (69), 198 (17), 171 (100), 156 (16), 143 (7), 128 (6), 115 (7), 91 (2). UV/Vis ($CHCl_3$, λ_{max}, nm, (ε)): 332 (0.55), 325 (0.56), 260 (1.50), 249 (1.61). $[\alpha]_D^{25}$: +99.6 (*c* 0.51, CH_2Cl_2).

2.3. Biological activities

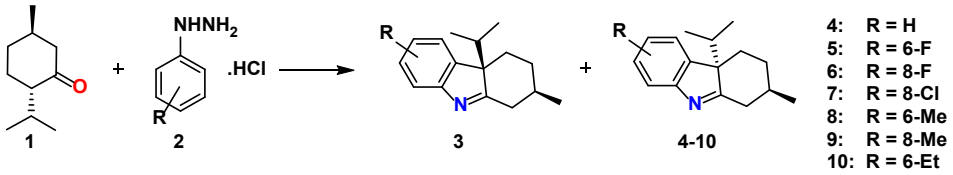
2.3.1. Carbonic anhydrase inhibitory activity

The carbonic anhydrase inhibition assay was performed following the method reported by Shank *et al*. with a slight modification. Reaction was carried out in a 96-well plate and the total reaction volume was 200 µL per well. Each well comprised of 20 µL of test compound (prepared in DMSO), 140 µL of tris-HCl buffer (0.05 M, pH = 7.4), 20 µL of purified bovine CA-II (0.15 mg/mL; prepared in deionized water) and 20 µL of a substrate, *i.e*., 4-nitrophenyl acetate (4-NPA) (0.7 mM; dissolved in 95% ethanol). 20 µL of test compounds were incubated with enzyme and buffer for 15 min in 96-well flat bottom plates. After incubation, absorbance was recorded at 400 nm (pre-read). The final rate of product formation was monitored with the addition of 20 µL of 4-NPA as substrate at 25 °C for 30 min with regular intervals of 1 min, by using microplate readers (Multiskan GO Spectrophotometer, Thermo Scientific, USA). The reaction was carried out in triplicates for each concentration of the compound, and the results were recorded as standard error of mean (SEM) of the triplicates [15,16].

Table 1. Investigation of diastereoselectivity for Fischer indolization*.

Sample no	Acid source	Diastereomeric ratio (*d.r.*) for (2*R*,4a*R*)- and (2*R*,4a*S*)-isomers	
		70 °C	120 °C
1	Uncatalyzed	1:1	1:1
2	AcOH	1:1	1:1
3	CuBr	1:2	1:1.1
4	CuI	1:1.5	1.2:1
5	$CuSO_4 \cdot 5H_2O$	1:1	-
6	$MgSO_4$	1:1	1.2:1
7	Boric acid	2:1	2:1
8	4-Chlorobenzoic acid	1.5:1	1.2:1
9	D-Camphoric acid	1:1	1:1
10	Iso-ascorbic acid	1:1	1.2:1
11	L-Tartaric acid	1.5:1	1.2:1

* The diastereomeric ratio (*d.r.*) was explored using ^{1}H NMR studies.



Scheme 1. Fischer indolization of *L*-menthone.

2.3.2. Immunomodulatory activity

Luminol-enhanced chemiluminescence protocol was adopted to perform immunomodulatory activity. In this method, test compounds were mixed with whole blood HBSS^{++} (containing magnesium and calcium chloride) and incubated in white half area 96-well plates for 15 min. Then, luminol and serum-opsonized zymozan (SOZ) were added to the plates except the blank. Reactive oxygen species (ROS) levels were measured using a luminometer [17].

2.3.3. Anti-cancer or cytotoxicity activity

Anticancer or cell cytotoxicity of the synthesized library was performed using a MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay. In 96-well microplates, 3×10^5 HeLa (Cervical cancer) or normal 3T3 (Mouse fibroblast) cells were cultivated. Minimum Essential Medium or Dulbecco's Modified Eagle Medium were the medium used, respectively. Penicillin, streptomycin, and fetal bovine serum (FBS) (5%) were then added to the medium and left for incubation at 37 °C. Cells were then diluted to 6×10^4 cells/mL concentration, followed by MTT addition. Upon completion of the reaction, it was examined for absorbance measurement at 570 nm under microplate reader (Spectra Max plus, Molecular Devices, USA). Doxorubicin was used as a standard for anticancer activity, while cycloheximide was the reference used for cytotoxicity [18,19]. Soft-Max Pro software was used to process the results. Inhibition (%) was calculated using Equation (1),

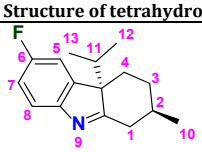
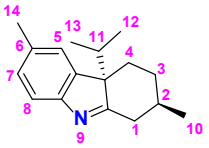
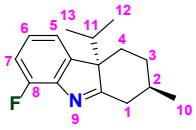
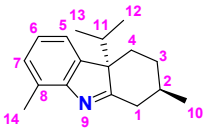
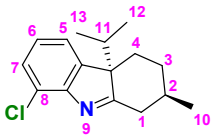
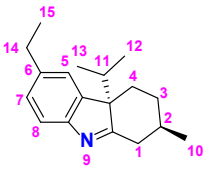
% Inhibition = [100 – (mean of O.D. of test compound - mean of O.D. of negative control)/ (mean of O.D. of positive control - mean of O.D. - of negative control)] × 100 (1)

3. Results and discussion

Fischer indolization of *L*-menthone with phenyl hydrazine was used to investigate the synthesis of diastereomeric (2*R*,4a*R*)- and (2*R*,4a*S*)-4a-*iso*propyl-2-methyl-2,3,4,4a-tetra hydro-1*H*-carbazoles under different acidic and thermal conditions. This reaction was previously reported by our group using AcOH as an acid source at refluxing conditions [14], which regioselectively gave 4a-substituted tetrahydrocarbazole *via* installing indolenine skeleton. However, epimerization led to an equimolar mixture of two diastereoisomers: (2*R*,4a*R*)- and (2*R*,4a*S*)-isomers. Initially, the reaction was carried out in the absence of an acid source at two different temperatures, *i.e.*, 70 and 120 °C. Both conditions showed 1:1 diastereomeric ratio (*d.r.*) for (2*R*,4a*R*)- and (2*R*,4a*S*)-isomers (**3** and **4**), respectively. Same results (*d.r.* 1:1) were obtained for reactions conducted in AcOH at 70 and 120 °C. The reaction was then explored using Lewis acids, such as $CuSO_4 \cdot 5H_2O$, CuBr, CuI, and $MgSO_4$ at 70 and 120 °C. For $CuSO_4 \cdot 5H_2O$, diastereomeric ratio (*d.r.*) was found to be 1:1 at 70 °C, while the reaction was decomposed at 120 °C. Reaction when conducted at 70 °C in the presence of CuBr, *d.r.* was found to be 1:2, while at 120 °C, *d.r.* was 1:1.1. Thereby CuBr when used at 70 °C, showed slightly better diastereoselectivity for (2*R*,4a*S*)-isomer. On the contrary, when CuI and $MgSO_4$ were used as acid sources, no appreciable selectivity was observed. For CuI, the *d.r.* was found to be 1:1.5 at 70 °C and 1.2:1 at 120 °C. While, in the presence of $MgSO_4$, *d.r.* was 1:1 at 70 °C and 1.2:1 at 120 °C. The reaction was then explored using 4-chlorobenzoic acid as an organic acid source, which revealed *d.r.* as 1.5:1 and 1.2:1 at 70 and 120 °C, respectively. Reaction was also explored in the presence of chiral organic acids including D-camphoric acid, *iso*-ascorbic acid, and L-tartaric acid however no appreciable diastereoselectivity was observed, as illustrated in Table 1. Comparatively, boric acid showed slightly better diastereoselectivity for (2*R*,4a*R*)-isomer than other acids, *i.e.,* 2:1 at both 70 °C and 120 °C temperature.

A library of (2*R*,4a*S*)-isomers was prepared by treating *L*-menthone with phenyl hydrazine hydrochlorides in the presence of acetic acid (Scheme 1) using our previously reported protocol [14]. Six new (2*R*,4a*S*)-4a-*iso*propyl-2-methyl-2,3,4,4a-tetrahydro-1*H*-carbazole analogs with varying substitutions on the aromatic ring were reported as shown in Table 2, and characterized using spectroscopic techniques. Compound **7** was taken as a representative of the series for the explanation of spectral data. The compound was obtained as a brown liquid with the molecular ion peak (M^+) at *m/z* 263. ^{1}H NMR spectrum showed characteristic three doublets at δ 1.21, 0.26 and 0.84 ppm for *iso*propyl methyls CH_3-12, CH_3-13, and isolated methyl CH_3-10, respectively. Diastereotopic methylenes at C-3 appeared at δ 1.39 and 2.03 ppm as a doublet of multiplets and a triplet of triplet, respectively.

Table 2. Library of (2*R*,4a*S*)-4a-*iso*propyl-2-methyl-2,3,4,4a-tetrahydro-1*H*-carbazole analogs.

Compound	Structure of tetrahydrocarbazoles	Yield (%)	Compound	Structure of tetrahydrocarbazoles	Yield (%)
5		38	8		49
6		34	9		36
7		30	10		35

The signals at δ 1.26 ppm (triplet of doublet) and δ 2.38-2.31 ppm (multiplet) corresponds to diastereotopic methylene protons at C-4. Diastereotopic methylenes at the 1-position appeared as a multiplet at δ 2.74-2.73 ppm. *Iso*propyl methine (CH-11) appeared as a merged multiplet signal at δ 2.38-2.31 ppm, while the methine of the cyclohexyl ring (CH-2) showed a multiplet at *δ* 2.53-2.50 ppm. The aromatic methine protons H-5, H-7, and H-8 appeared at δ 7.21 (doublet), 7.31 (doublet), and 7.06 ppm (triplet), respectively. The ^{13}C NMR spectrum showed a characteristic indolenine pattern. Three methyl carbons appeared at δ 16.4, 16.9, and 17.9 ppm, and three methylenes appeared at δ 26.2, 31.6, and 36.8 ppm. Aliphatic methine carbons showed signals at δ 29.3 and 35.1 ppm, while aromatic methines appeared at δ 120.6, 123.6, and 127.6 ppm. Quaternary carbon, C-9a appeared at δ 189.5 ppm, which is characteristic of indolenine, whereas rest of the quaternary carbons showed signals at δ 62.9, 127.6, 144.6 and 153.5 ppm.

Synthesized tetrahydrocarbazole compounds were investigated for different biological activities such as carbonic anhydrase inhibitory, anticancer, and cytotoxicity activities. The results revealed that the synthesized compounds (**4**-**10**) when checked for their inhibitory potency against bovine carbonic anhydrase-II (bCA-II), showed less than 50% inhibition against acetazolamide (IC_{50} = 0.13±0.01 µM) as a standard drug, therefore considered inactive. Tetrahydrocarbazole analogs were also found inactive against immune-modulatory activity using ibuprofen (IC_{50}= 11.2±1.9 µM) as a reference drug. In addition, the synthesized compounds were explored for anticancer activity against HeLa cell lines using doxorubicin (IC_{50} = 0.90±0.14 µM) as a reference, but none of them was found active. Compounds were also found safe when tested for their cytotoxicity against 3T3 (mouse fibroblast) normal cell line using cycloheximide (IC_{50} = 0.61±0.17 µM) as a standard.

4. Conclusions

In summary, Fischer indolization of *L*-menthone was investigated using different inorganic, organic and chiral acids. Slightly satisfactory diastereoselectivity for (2*R*,4a*S*)-isomer was observed in the case of CuBr as a catalyst at 70 °C. Comparatively, boric acid showed slightly better diastereoselectivity for (2*R*,4a*R*)-isomer at both temperature conditions (70 °C and 120 °C). In contrast, no appreciable results were obtained using chiral organic acids. In addition, a small library of new (2*R*,4a*S*)-4a-*iso*propyl-2-methyl-2,3,4,4a-tetrahydro-1*H*-carbazole analogs was reported, which was investigated against different biological activities. The results showed no potency for carbonic anhydrase inhibitory, immune-modulatory, anticancer, and cytotoxicity activities. Thus, the synthesized compounds will be explored for other bioactivities to explore their bioefficacy in the near future.

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Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Fatima Zehra Basha, Maria Aqeel Khan; Methodology: Munisaa Younus, Marium Ahsan, Noor-ul-Huda; Software: Munisaa Younus, Marium Ahsan, Noor-ul-Huda; Validation: Munisaa Younus, Marium Ahsan, Noor-ul-Huda;Formal Analysis: Munisaa Younus, Marium Ahsan, Noor-ul-Huda; Investigation: Munisaa Younus, Marium Ahsan, Noor-ul-Huda; Resources: Maria Aqeel Khan, Saima Rasheed; Data Curation: Munisaa Younus, Marium Ahsan, Noor-ul-Huda; Writing - Original Draft: Maria Aqeel Khan, Munisaa Younus; Writing - Review and Editing: Fatima Zehra Basha, Maria Aqeel Khan, Rabia Sadiq; Visualization: Munisaa Younus, Marium Ahsan, Noor-ul-Huda;Funding acquisition: Maria Aqeel Khan; Supervision: Maria Aqeel Khan, Saima Rasheed; Project Administration: Maria Aqeel Khan.

ORCID and Email

Munisaa Younus
monisayounus19@gmail.com
https://orcid.org/0000-0001-7297-5526
Marium Ahsan
mariumahsan8@gmail.com
https://orcid.org/0000-0002-1106-8882
Noor-ul-Huda
huda.imran2017@gmail.com
https://orcid.org/0000-0003-0790-0143
Maria Aqeel Khan
drmaria.aqeel@iccs.edu
markhan883@gmail.com
https://orcid.org/0000-0002-8072-4775

Saima Rasheed
saima.rasheed@iccs.edu
https://orcid.org/0000-0002-6742-3003
Rabia Sadiq
sadiqrabia@ymail.com
https://orcid.org/0000-0003-2493-4898
Fatima Zehra Basha
bashafz@gmail.com
https://orcid.org/0000-0001-8092-7639

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