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Synthesis of Isoxazolo and Pyrazolino Annelated Carbazoles from 2-(4′-Methoxy)benzylidene-1-oxo-1,2,3,4-tetrahydrocarbazoles

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1-Oxo-1,2,3,4-tetrahydrocarbazole (**1**) on mixed aldol condensation with 4-methoxybenzaldehyde yielded 2-(4′-methoxy)benzylidine-1-oxo-1,2,3,4-tetrahydrocarbazole (**2**), which was further treated with hydrazine hydrate and hydroxylamine hydrochloride in separate reactions to afford 3,3a,4,5-tetrahydro-3-(4′-methoxy)-phenyl-2*H*-pyrazolino[3,4-a]carbazole (**3**) and 4,5-dihydro-3-(4′-methoxy)phenylisoxazolo[3,4-a]carbazole (**4**). The prepared compounds **3a–e** and **4a–e** were evaluated for their *in vitro* anti-bacterial and antifungal activities against certain pathogenic fungal and bacterial strains.

Key Words: Synthesis, Isoxazole, Pyrazoline, Annelated carbazoles, Anti-bacterial and antifungal activities.

INTRODUCTION

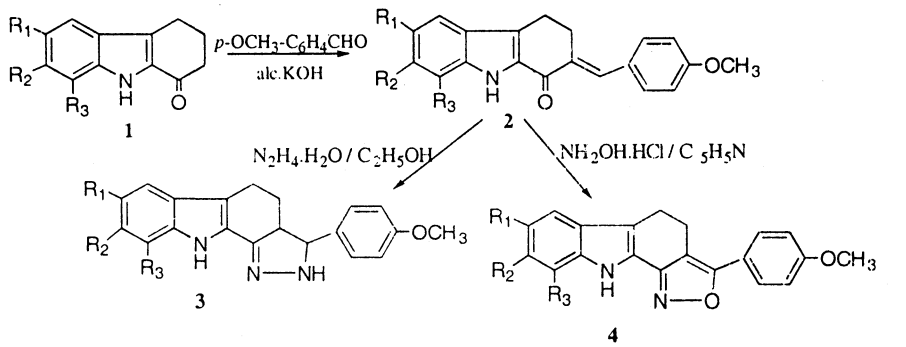
Carbazole alkaloids constitute an important class of naturally occurring compounds due to their biological activities mostly based on their special affinity towards DNA[1]. Therefore these compounds play a crucial role as potential leads for the discovery of an antitumor activity drugs using bioisosteric replacements[2]. However, the search for new methods for the simple and efficient construction of the indole ring system continues to be an important synthetic goal[3, 4], since pyrazole, isoxazole, pyrimidines and pyrans are useful heterocyclic moieties as they possess a broad spectrum of biological activities such as antiviral, CNS depressant, bactericidal, ulcer inhibitor, etc. In continuation of our efforts on the study of potential 1-oxo-1,2,3,4-tetrahydrocarbazole towards the construction of various fused carbazoles, in this work we describe the synthesis of some new pyrazolino and isoxazolo annelated carbazoles alongwith their activity against certain pathogenic fungi and bacteria.

RESULTS AND DISCUSSION

Mixed aldol reaction of 6-methyl-1-oxo-1,2,3,4-tetrahydrocarbazole (**1a**) with 4-methoxybenzaldehyde under basic condition gave 6-methyl-2-(4′-methoxy)-benzylidene-1-oxo-1,2,3,4-tetrahydrocarbazole (**2a**). The structure of **2a** was established on the basis of elemental analysis and spectral data. The IR spectrum

exhibited a sharp and strong absorption band at 1643 cm^{-1} characteristic of α,β-unsaturated carbonyl group and a band at 3240 cm^{-1} ascribable to —NH group. The disappearance of C_2 proton signal and appearance of benzylic proton signal as a singlet at δ 7.75 in its ^1H NMR spectrum proved the validity of mixed aldol reaction of **1a** with 4-methoxybenzaldehyde to give **2a.** Two sharp singlets appearing at δ 2.45 and δ 3.86 were due to C_6-CH_3 and C_4'-OCH_3 protons respectively. The C_3 and C_4 protons resonated as two multiplets at δ 3.03 and δ 3.26 respectively and a broad singlet at δ 8.89 was due to carbazole —NH. Further it exhibited a multiplet at δ 6.94–7.48 due to seven aromatic protons. The elemental analysis was compatible with the molecular formula $C_{21}H_{19}NO_2$. A series of similar compounds were realized with **1b–e** (**Scheme 1,** Tables 1 and 2).

When 6-methyl-2-(4′-methoxy)benzylidene-1-oxo-1,2,3,4-tetrahydrocarbazole (**2a**) was treated with hydrazine hydrate in ethanol, it afforded the expected 7-methyl-3,3a,4,5-tetrahydro-3-(4′-methoxy) phenyl-2*H*-pyrazolino[3,4-*a*]carbazole (**3a**) in 80% yield. Its IR spectrum revealed the formation of >C=N (1612 cm^{-1}) thereby indicating the absence of carbonyl absorption. The ^1H NMR spectrum of **3a** in $CDCl_3$ showed a multiplet at δ 2.78–2.89 for C_4-H_2 and C_5-H_2 and two multiplets centred at δ 4.39 and δ 4.83 for C_{3a} and C_3 protons respectively; two sharp singlets at δ 2.43 and δ 3.83 for C_7-CH_3 and C_4'-OCH_3 protons respectively and a broad singlet at δ 4.56 accountable for pyrazolino —NH proton. The seven aromatic protons resonated between δ 6.81–7.46 as a multiplet. Carbazole —NH appeared as a broad singlet at δ 8.78. Further, the elemental analysis agreed well with the molecular formula $C_{21}H_{21}N_3O$. On the basis of the aforesaid data, the product was attested to be 7-methyl-3,3a,4,5-tetrahydro-3-(4′-methoxy)phenyl-2*H*-pyrazolino[3,4-a]carbazole (**3a**). Extension of the above reaction to be **2b–e** yielded to corresponding pyrazolino[3,4-a]carbazoles (**3b–e**) (**Scheme 1,** Tables 1 and 2).



1 - 4 **a**: $R_1 = CH_3$, $R_2 = R_3 = H$
b: $R_2 = CH_3$, $R_1 = R_3 = H$
c: $R_3 = CH_3$, $R_1 = R_2 = H$
d: $R_1 = R_2 = R_3 = H$
e: $R_1 = Cl$, $R_2 = R_3 = H$

Scheme-1

TABLE-1
PHYSICAL AND IR SPECTRAL DATA OF COMPOUNDS **2a–e**, **3a–e** AND **4a–e**

Compd.	m.p. (°C)	Yield (%)	IR (ν_{max})	m.f. (m.w.)	Calcd. (Found) %		
					C	H	N
2a	197	93	3240 1643	$C_{21}H_{19}NO_2$ (317.386)	79.47 (79.31)	06.03 (06.20)	04.41 (04.30)
2b	200	89	3262 1641	$C_{21}H_{19}NO_2$ (317.386)	79.47 (79.34)	06.03 (06.10)	04.41 (04.27)
2c	191	90	3236 1645	$C_{21}H_{19}NO_2$ (317.386)	79.47 (79.52)	06.03 (06.15)	04.41 (04.39)
2d	185	87	3236 1641	$C_{20}H_{17}NO_2$ (303.356)	79.18 (79.31)	05.64 (06.59)	04.61 (04.70)
2e	185	85	3226 1641	$C_{20}H_{16}NO_2Cl$ (337.804)	71.11 (71.25)	04.77 (04.62)	04.14 (04.29)
3a	180	80	3651 3200	$C_{21}H_{21}N_3O$ (331.416)	76.10 (76.30)	06.38 (06.51)	12.67 (12.71)
3b	195	85	3440 3311	$C_{21}H_{21}N_3O$ (331.416)	76.10 (76.21)	06.38 (06.23)	12.67 (12.75)
3c	175	82	3450 3236	$C_{21}H_{21}N_3O$ (331.416)	76.10 (76.27)	06.38 (06.40)	12.67 (12.56)
3d	179	79	3470 3180	$C_{20}H_{19}N_3O$ (317.390)	75.68 (75.51)	06.03 (06.00)	13.23 (13.34)
3e	170	60	3649 3250	$C_{20}H_{18}N_3OCl$ (351.835)	68.27 (68.11)	05.15 (05.29)	11.94 (11.80)
4a	170	65	3232 1610	$C_{21}H_{18}N_2O_2$ (330.385)	76.34 (76.21)	05.49 (05.60)	08.47 (08.55)
4b	171	70	3219 1610	$C_{21}H_{18}N_2O_2$ (330.385)	76.34 (76.40)	05.49 (05.34)	08.47 (08.51)
4c	179	62	3455 2950	$C_{21}H_{18}N_2O_2$ (330.385)	76.34 (76.49)	05.49 (05.36)	08.47 (08.50)
4d	164	72	3451 1619	$C_{20}H_{16}N_2O_2$ (316.385)	75.93 (75.80)	05.09 (06.20)	08.85 (04.79)
4e	185	85	3421 1603	$C_{20}H_{15}NO_2Cl$ (350.803)	68.27 (68.11)	05.15 (05.20)	11.94 (11.72)

In another experiment 6-methyl-2-(4′-methoxy)benzylidene-1-oxo-1,2,3,4-tetrahydrocarbazole **(2a)** was condensed with hydroxylamine hydrochloride in dry pyridine. This reaction mixture after workup afforded a solid mass which was purified by column chromatography. The IR spectrum of this compound exhibited two absorptions at 3232 and 1610 cm^{-1} which were ascribable for —NH and —C=N stretching vibrations respectively. The ^1H NMR spectrum registered two singlets for three protons at δ 2.44 and δ 3.91 for methyl and methoxy groups respectively and a couple of multiplets at δ 2.80–2.82 and δ 3.10–3.12 corresponding to C_4 and C_5 protons, in addition to an aromatic cluster between δ 7.00-7.98 for seven aromatic protons and a broad singlet at δ 8.96 for —NH

TABLE-2
^{1}H NMR DATA OF COMPOUNDS **2a–e**, **3a–e** AND **4a–e**

Compd.	^{1}H NMR (δ, ppm)
2a	2.45 (s, 3H, C_6-CH_3), 3.03 (m, 2H, C_3-H_2), 3.26 (m, 2H, C_4-H_2), 3.86 (s, 3H, C_4'-OCH_3), 6.94–7.48 (m, 7H, aromatic-H), 7.75 (s, 1H, benzylic-H), 8.89 (bs, 1H, C_9-NH)
2b	2.48 (s, 3H, C_7-CH_3), 2.56 (m, 2H, C_3-H_2), 3.46 (m, 2H, C_4-H_2), 3.86 (s, 3H, C_4'-OCH_3), 7.24–7.43 (m, 7H, aromatic-H), 7.76 (s, 1H, benzylic-H), 9.10 (bs, 1H, C_9-NH)
2c	2.53 (s, 3H, C_8-CH_3), 3.07 (m, 2H, C_3-H_2), 3.28 (m, 2H, C_4-H_2), 3.86 (s, 3H, C_4'-OCH_3), 6.95–7.52 (m, 7H, aromatic-H), 7.77 (s, 1H, benzylic-H), 9.11 (bs, 1H, C_9-NH)
2d	3.08 (m, 2H, C_3-H_2), 3.28 (m, 2H, C_4-H_2), 3.86 (s, 3H, C_4'-OCH_3), 6.8—7.67 (m, 8H, aromatic-H), 7.77 (s, 1H, benzylic-H), 9.03 (bs, 1H, C_9-NH)
2e	3.01 (m, 2H, C_3-H_2), 3.28 (m, 2H, C_4-H_2), 3.86 (s, 3H, C_4'-OCH_3), 6.95–7.63 (m, 7H, aromatic-H), 7.77 (s, 1H, benzylic-H), 9.12 (bs, 1H, C_9-NH)
3a	2.43 (s, 3H, C_7-CH_3), 2.78–2.89 (m, 4H, C_4-H_2 and C_5-H_2), 3.83 (s, 3H, C_4'-OCH_3), 4.56 (bs, 1H, pyrazolino-NH), 4.39 (m, 1H, C_{3a}-H), 4.83 (m, 1H, C_3-H), 6.81–7.46 (m, 7H, aromatic-H), 8.78 (bs, 1H, carbazole-NH)
3b	2.45 (s, 3H, C_8-CH_3), 3.00 (m, 2H, C_4-H_2), 3.10 (m, 2H, C_5-H_2), 3.83 (s, 3H, C_4'-OCH_3), 4.38–4.41 (m, 3H, pyrazolino-NH , C_{3a}-H and C_3-H), 6.80–7.47 (m, 7H, aromatic-H), 8.73 (bs, 1H, carbazole-NH)
3c	2.47 (s, 3H, C_9-CH_3), 2.82 (m, 2H, C_4-H_2), 3.05 (m, 2H, C_5-H_2), 3.83 (s, 3H, C_4'-OCH_3), 4.35 (m, 1H, C_{3a}-H), 4.57 (bs, 1H, pyrazolino-NH), 4.83 (m, 1H, C_3-H), 6.92-7.52 (m, 7H, aromatic-H), 9.03 (bs, 1H, carbazole-NH).
3d	2.76 (m, 2H, C_4-H_2), 3.07 (m, 2H, C_5-H_2), 3.76 (s, 3H, C_4'-OCH_3), 4.42 (m, 1H, C_{3a}-H), 4.56 (s, 1H, pyrazolino-NH), 4.84 (m, 1H, C_3-H), 6.76–7.54 (m, 8H, aromatic-H), 8.80 (bs, 1H, carbazole-NH)
3e	2.73 (m, 2H, C_4-H_2), 3.12 (m, 2H, C_5-H_2), 3.76 (s, 3H, C_4'-OCH_3), 4.39 (m, 1H, C_{3a}-H), 4.56 (s, 1H, pyrazolino-NH), 4.86 (m, 1H, C_3-H), 6.79–7.59 (m, 7H, aromatic-H), 9.28 (bs, 1H, carbazole-NH)
4a	2.44 (s, 3H, C_7-CH_3), 2.81 (m, 2H, C_4-H_2), 3.11 (m, 2H, C_5-H_2), 3.91 (s, 3H, C_4'-OCH_3). 7.00–7.98 (m, 7H, aromatic-H), 8.69 (bs, 1H, carbazole-NH)
4b	2.48 (s, 3H, C_9-CH_3), 2.65 (m, 2H, C_4-H_2), 3.00 (m, 2H, C_5-H_2), 3.89 (s, 3H, C_4'-OCH_3), 7.00–7.55 (m, 7H, aromatic-H), 8.78 (bs, 1H, carbazole-NH)
4c	2.47 (s, 3H, C_9-CH_3), 3.31 (m, 2H, C_4-H_2), 3.11 (m, 2H, C_5-H_2), 3.83 (s. 3H, C_4'-OCH_3), 6.98–7.48 (m, 7H, aromatic-H), 8.03 (bs, 1H, carbazole-NH).
4d	2.67 (m, 2H, C_4-H_2), 2.72 (m, 2H, C_5-H_2), 3.76 (s, 3H, C_4'-OCH_3), 6.93–7.50 (m, 8H, aromatic-H), 8.02 (bs, 1H, carbazole-NH)
4e	2.67 (m, 2H, C_4-H_2), 2.70 (m, 2H, C_5-H_2), 3.88 (s, 3H, C_4'-OCH_3), 7.09–7.57 (m, 7H, aromatic-H), 8.04 (bs, 1H, carbazole-NH)

proton. The elemental analysis agreed well with the molecular formula $C_{21}H_{18}N_2O_2$. Based on the above mentioned spectral data the structure of product was assigned to be 7-methyl-4,5-dihydro-3-(4′-methoxy)phenylisoxazolo[3,4*a*] carbazole **(4a).** Similarly following the above condition, compounds **2b–e** afforded the corresponding isoxazolo[3,4-a]carbazoles **(4b–e)** (**Scheme 1,** Tables 1 and 2).

Antibacterial and antifungal activities of compounds 3a–e and 4a–e.

The newly synthesized compounds **(3a–d), (4a–d)** were tested for their *in vitro* antibacterial and antifungal activities by agar-well diffusion method[5] against pathogenic bacteria such as *Salmonella typhi* and *Shigella dysentatiae* and fungal strains *Aspergillus flavus, Rizoctonia solani* and *Fusarium ocysporium.* Bacteria were cultured in nutrient agar medium and used as inoculum for study. Bacteria cells were swabbed on nutrient agar medium [peptone (0.5 g), beef extract (0.5 g), agar (2.0 g) in 100 mL distilled water; pH = (7.5 ± 0.2)] in petri-plates. Fungi were cultured in sabouraud dextrose medium and used as inoculum for study. Fungus cells were swabbed on sabouraud dextrose medium [peptone (1.0 g), dextrose (2.0 g), agar (2.0 g) in 100 mL distilled water; pH = (7.5 ± 0.2)] in petri plates. The compounds were dissolved in DMSO to a final concentration of 0.5% and tested against bacteria and fungi. The compound was placed in the culture medium in petri plates and incubated at 24–30 h at 37°C for bacteria and 144–168 h at 28°C for fungi. The inhibition of bacteria and fungal growth expressed in percentage (%), was determined by comparing the fungal growth in the test plates with that in the respective control plates as given by Vincent equation[6], and results are listed in Table-3. The activity of the compounds has been compared with the commercial fungicide *Bavistin* and bactericide *Streptomycin.* All compounds exhibited good activity against both the species of bacteria and fungi.

TABLE-3
ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF COMPOUNDS **3a–e** AND **4a–e**

Compd.	% of inhibition				
	Salmonella typhi	*Shigella dysentatiae*	*Aspergillus flavus*	*Rizoctonia solani*	*Fusarium ocysporium*
3a	18	13	16	20	19
3b	15	14	18	17	26
3c	15	14	18	17	26
3d	20	19	13	21	16
3e	23	22	26	23	24
4a	21	20	22	28	20
4b	19	24	22	15	22
4c	22	19	25	25	21
4d	23	17	20	22	20
4e	27	26	27	25	29

EXPERIMENTAL

Melting points were determined by Mettler FP-5 apparatus and were uncorrected. The reactions were monitored by thin-layer chromatography. Column chromatographic separations were done using silica gel. IR spectra were recorded in KBr pellets on Perkin-Elmer model 1600 FT-IR instrument. ^{1}H NMR spectra (400 MHz) were recorded on Varian AMX 400 spectrometer using TMS as an internal standard. Elemental analyses were carried out on Carlo-Erba 1108 model elemental analyzer. Electron impact (EI) mass spectra were recorded in Jeol (D)-300 EI mass spectrometer.

Preparation of 2-(4′-methoxy)benzylidene-1-oxo-1,2,3,4-tetrahydrocarbazoles (2): A mixture of the respective 1-oxo-1,2,3,4-tetrahydrocarbazole (**1**, 4 mmol) and 4-methoxybenzaldehyde (4 mmol) was treated with 4% alc. KOH (15 mL) and the mixture was stirred for 6 h at room temperature. The precipitated crystalline product was filtered off and washed with rectified spirit. A further crop of condensation product was obtained on neutralization with acetic acid and dilution with water. Finally the products **2** were recrystallized from methanol.

Preparation of 3,3a,4,5-tetrahydro-3-(4′-methoxy)phenyl-2*H*-pyrazolino-[3,4-a]carbazoles (3): The solution of 2-(4′-methoxy)benzylidene-1-oxo-1,2,3,4-tetrahydrocarbazole (**2**, 1 mmol) was dissolved in absolute ethanol (20 mL); hydrazine hydrate (0.5 mL, 10 mmol) was added and this mixture was refluxed for 4 h at 100°C. The excess solvent was removed under reduced pressure; then the crude reaction mixture was poured into ice-cold water and extracted with chloroform (3 × 15 mL). The combined organic layers were dried over anhydrous sodium sulphate. Evaporation of the solvent followed by crystallization with petroleum ether yielded the corresponding 3,3a,4,5-tetrahydro-3-(4′-methoxy)phenyl-2*H*-pyrazolino[3,4-a]carbazole (**3**) as colourless prism.

Preparation of 4,5-dihydro-3-(4′-methoxy)phenylisoxazolo[3,4-a]carbazoles (4): A mixture of 2-(4′-methoxy)benzylidene-1-oxo-1,2,3,4-tetrahydrocarbazole (**2**, 1 mmol) with hydroxylamine hydrochloride (1 g, 4 mmol) was refluxed in pyridine (5 mL) at 130°C for 10 h. The reaction mixture was then poured into crushed ice, the resulting semi-solid separated was extracted with chloroform, subsequently washed with dilute hydrochloric acid and water successively. The combined organic layers were dried over anhydrous sodium sulphate. Removal of solvent yielded the crude product which was purified by column chromatographic technique using petroleum ether-ethyl acetate as a solvent system over silica gel column. The products **4** were recrystallized from the same solvent system.

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