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Synthesis, Characterization and Antimicrobial Evaluation of Pyrimido Cyclohept[*B*] Indole Derivatives

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ABSTRACT

Some cyclohept[b]indole alkaloids were isolated from natural source and were mostly isolated from Ervatamia, Pandaca and Caulerpa that exhibit antimicrobial, anticancer, and anti-HIV activities. This work describes a strategic approach forthe synthesis ofefficient precursor 2-(4′-methyl)benzylidene-1-oxo-3,4,5,10-tetrahydrocyclohept[b]indole(3a)which can obtained by aldol condensation of the 7-methyl-1-oxo-2,3,4,5-tetra-hydrocyclohept[b]indole(1a) with 4-methyl benzaldehyde(2) under basic conditions. Hydroxypyrimido carbazoles were derived by the treatment of 2(4′-methyl)benzylidine-7-methyl-1-oxo-3,4,5,10-tetrahydrocyclohept[b]indole(3a) wastreatedwithurea and guanidine nitrate in sodium methoxide under proper condition.

Keywords: Pyrimido, Mixed aldol condensation, Antifungal, Antibacterial.

INTRODUCTION

On the basis of the interesting structures and biological ativities exhibited several heterocyclic systems possessing indole nucleus, we have chosen the indole nucleus fused with seven membered ring cyclohept[*b*]indole as the core subject matter for investigation in this synthetic study. In continuation of our study in 2-(4′-methyl)benzylidene-1-oxo-3,4,5,10-tetrahydrocyclohept[*b*]indole(3a)we became interested in developing novel methods to prepare some novel systems containing pyrazino-,isoxazolo-, pyrimido-, thiopyrimido-, aminopyrimido- and pyrano-rings annelated to carbazole skeleton. Our interest in such compounds has been further increased by the recent discovery of pyrido-carbazoles as an appropriate skeleton to design DNA intercalating drugs [1-3]. Besides this, many tetracyclic compounds have been synthesized by the replacement of pyridine ring by other heterocyclic rings and their effects on biological properties [4-12] were studied. Tetrahydrocarbazoles and its derivatives were examined as an antidepressant drugs [13]. Tetrahydrocyclohept[*b*]indole derivatives are associated with various potential biological activities such as anti-inflammatory, bactericidal, analgesic, antibiotic, insecticidal and fungicidal studies [14-19].

Nitrogen heterocycles bearing amino substituent are of broad pharmaceutical interest leading to continuous efforts in the study of their structure activity relationship. In particular, these compounds show remarkable antitumor characteristics and have good prospects for future medicinal uses. However, a major drawback in using these classes of compounds as drugs is their insolubility in water, which makes it extremely difficult to administer it in intravenous form, limiting its practical application. Incorporation of amino group into these systems was found to enhance the hydrophilicity and water solubility of these molecules. Amino carbazoles derivatives have showed potential activities to both Alzheimer's disease and cancer [20,21].

MATERIALS AND METHODS

Melting points were determined on a Boetius micro heating table or on a Raga heating block and are uncorrected. Thin Layer Chromatography [TLC] was performed using glass plates coated with silica gel G [incorporating $CaSO_4$ (13%) as binder]. Petroleum ether and ethyl acetate were used as irrigant. Spots were visualized with iodine. Purification of the crude samples was carried out using chromatographic columns packed with silica gel (60-120 mesh). IR Spectra were recorded on Shimadzu FT IR 8201(PC)S spectrometer model spectrometer, using KBR discmethod and the absorption frequencies quoted in reciprocal centimeters (cm^{-1}).^{1}H-NMR spectra were taken on BRUKER-400 (400 MHz), BRUKER-500 (500 MHz) spectrometer using trimethylsilane (TMS) as internal reference. The chemical shifts are quoted in parts per million (ppm) (s=singlet; d=doublet; t=triplet; q=quardret; bs=broad singlet and m=multiplet). Elemental analysis was performed on Vario EL III CHNS analyzer and Perkin-Elmer analyzer. Microanalysis was obtained (C, H, N, ± 0.4%).

General procedure for preparation of 2-(4′-methyl)benzylidine-1-oxo-3,4,5,10-tetrahydrocyclohept[*b*]indole (2a)

A mixture of the 1-oxo-2,3,4,5-tetrahydrocyclohept[*b*]indole (1a) (0.001 mol) and 4-methylbenzaldehyde (2)0.001 mol was treated with 4% alcholic potassium hydroxide and stirred for 24 h at room tempterature. The product 2-(4′-methyl)-benzylidine-1-oxo-3,4,5,10-tetrahydro-cyclohept[*b*]indole (3a-3d) precipitated as crystalline mass was filtered off and dried.

2-(4′-methyl)benzylidine-7-methyl-1-oxo-3,4,5,10-tetrahydrocyclohept[*b*]indole (3a)

Yellow solid, Yield: 0.182 g(85.44%), M.p: 202ºC; IR (KBr) [ν_{max}cm^{-1}]: 3326, 1620, 1943, 1435, 1350, 1234, 802, 709, 671 cm^{-1}; ^{1}H-NMR (CDC1$_3$) δ (ppm)=2.35(s, 3H,C$_7$-CH$_3$)2.38(s, 3H, C$_4$-CH$_3$), 2.79-3.12(m,6H, C$_4$-H$_2$, C$_5$-H$_2$ C$_6$-H$_2$),7.12- 7.54 (m, 8H, C$_6$-H, C$_8$-H,C$_9$-H, benzylic -H, C$_2$-H, C$_3$-H, C$_5$-H, C$_6$-H) and 11.28(s, 1H, -NH). Anal. calcd for $C_{22}H_{21}NO$:C, 83.778%, H,6.710%, N, 4.440%, Found: **C,**83.75%, H,6.70%, N, 4.40%.

2-(4′-methyl)benzylidine-8-methyl-1-oxo-3,4,5,10-tetrahydrocyclohept[*b*]indole (3b)

Yellow solid, Yield: 0.163 g (76.52%), M.p: 202ºC; IR (KBr) [ν_{max} cm^{-1}]: 3322,2918,1632,1560,1526,1432,1341,1271,1230,1182, 1160, 1034, 985, 911,873, 796, 761, 700 cm^{-1}; ^{1}H-NMR (CDC1$_3$) δ (ppm): 2.35(s, 3H,C$_7$-CH$_3$)2.38(s, 3H, C$_4$-CH$_3$), 2.79-3.12(m,6H, C$_4$-H$_2$, C$_5$-H$_2$ C$_6$-H$_2$),7.12-7.54 (m, 8H, C$_6$-H, C$_8$-H, C$_9$-H, benzylic -H, C$_2$-H, C$_3$-H, C$_5$-H, C$_6$-H) and11.28(s, 1H, -NH). Anal. calcd for $C_{22}H_{21}NO$:C, 83.778%, H,6.710%, N, 4.440%. Found: **C,**83.756%, H,6.65%, N, 4.340%.

2-(4′-methyl)benzylidine-9-methyl-1-oxo-3,4,5,10-tetrahydrocyclohep[*b*]indole (3c)

Yellow solid, Yield: 0.179 g (76.52%); M.p: 202ºC; IR (KBr) [ν_{max}cm^{-1}]: 3315, 2923, 2858, 1630, 1579,1529,1435, 1382, 1328,1252, 1160, 984, 910, 810,695 cm^{-1}; ^{1}H-NMR (CDC1$_3$) δ (ppm)=2.35(s, 3H,C$_7$-CH$_3$) 2.38(s, 3H, C$_4$-CH$_3$), 2.79-3.12(m,6H, C$_4$-H$_2$, C$_5$-H$_2$ C$_6$-$_2$),7.12- 7.54 (m, 8H, C$_6$-H, C$_8$-H,C$_9$-H, benzylic -H, C$_2$-H, C$_3$-H, C$_5$-H, C$_6$-H) and11.28(s, 1H, -NH). Anal. calcd for $C_{22}H_{21}NO$:C**,** 83.778%, H,6.710%, N, 4.440%. Found: **C,**83.776%, H,6.721%, N, 4.410%.

2-(4′-Methyl)benzylidine-1-oxo-3,4,5,10-tetrahydrocyclohept[*b*]indole (3d)

Yellow solid, Yield: 0.194 g (64.66%), M.p: 202ºC; IR (KBr) [ν_{max}cm^{-1}]: 3292, 2923, 1630, 1588, 1522, 1460, 1334, 1260, 1183, 981, 812, 744cm^{-1}; ^{1}H-NMR (CDC1$_3$) δ (ppm)=2.35(s, 3H,C$_7$-CH$_3$), 2.38(s, 3H, C$_4$-CH$_3$), 2.79-3.12(m,6H, C$_4$-H$_2$, C$_5$-H$_2$ C$_6$-$_2$), 7.12-7.54 (m, 8H, C$_6$-H, C$_8$-H,C$_9$-H, benzylic -H, C$_2$-H, C$_3$-H, C$_5$-H, C$_6$-H) and 11.28(s, 1H, -NH). Anal. calcd for $C_{21}H_{19}NO$:C**,** 83.778%, H,6.710%, N, 4.440%. Found: **C,**83.776%, H,6.712%, N, 4.420%.

General procedure for preparation of 2-hydroxy-5,6,7,12-tetrahydro-4(4′-ethyl)-phenyl-pyrimido[3′,4′:5,4]cyclo-hept-[*b*]indole (4a-4d)

A mixture of respective 2-(4′-methyl)benzylidine-1-oxo-3,4,5,10-tetrahydro cyclohept [*b*]indole(3a) (0.001 mol) and urea (0.001 mol) in presence of sodium methoxide in dry ethanol (10 ml) was refluxed on water bath for 18 h. The excess solvent was removed by evaporation and the reaction mixture was poured into an ice. The precipitate obtained was filtered off, dried and purified by passing through silica gel column and eluting with petroleum ether and ethyl acetate mixture (65:35) to yield the respective 2- hydroxy-5,6,7,12-tetrahydro-4(4′-methyl)phenylpyrimido [3′,4′:5,4]cyclohept[*b*]indole(4a).

***2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4(4′-methyl)phenylpyrimido[3′,4′:5,4] cyclohept [b]indole (4a*)**

Yield: 0.220 g [70%], Mp: 248ºC;IR (KBr) [ν_{max}cm^{-1}]: 3325,3757,2924,1620,1543,1435,1350,1237, 1141,918, 802, 763, 671, 524; ^{1}H-NMR (CDC1$_3$) δ (ppm): 2.38(s,3H, C$_9$CH$_3$) 2.50(s,3H, C$_4$-CH3) 2.07-3.13(m, 6H, C$_5$-H$_2$, C$_6$-H$_2$,C$_7$-H$_2$) 7.09-759 (m, 7H, C$_8$H,C$_{10}$-H, C$_{11}$-H C$_2$′-H, C$_3$′-H, C$_5$′-H, C$_6$-H) 7.37 (s, 1H, C$_2$-OH), 11.30(s, 1H indole NH). Anal. calcd for $C_{23}H_{21}N_3O$:C, 77.720%, H,5.955%; N, 11.82%.Found: **C,**77.70%, H,5.75%, N,11.62%.

2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4(4'-methyl)phenylpyrimido[3',4':5,4] cyclohept [b]indole (4b)

Yield: 0.204 g [65%], Mp: 250ºC; IR (KBr) [ν_{max}cm^{-1}]: 3412, 2923, 1706, 1620, 1517, 1443, 1331, 1230, 802; ^{1}H-NMR (CDC1$_3$) δ (ppm)=2.26 s, 3H, C$_{10}$ -CH$_3$) 2.10 (s, 3H, C$_4$-CH3) 2.05-2.99 (m, 6H, C$_5$-H$_2$, C$_6$-H$_2$, C$_7$-H$_2$) 6.96-7.52 (m, 7H, C$_8$H,C$_9$-H, C$_{11}$-H C$_2$′-H, C$_3$′-H, C$_5$′-H, C$_6$′-H) 7.260 (s, 1H, C$_2$-OH), 8.78 (s, 1H indole NH). Anal. calcd for $C_{23}H_{21}N_3O$: C, 77.720%, H, 5.955%, N, 11.82%. Found: C, 77.70%, H, 5.75%, N, 11.52%.

2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4(4'-methyl)phenylpyrimido[3',4':5,4] cyclohept [b]indole (4c)

Yield:0.208 g [65%], Mp: 248ºC; IR (KBr) [ν_{max}cm^{-1}]: 3447, 2924, 1621, 1444, 1330, 1211, 802, 743, 620; ^{1}H-NMR (CDC1$_3$) δ (ppm)=2.35 (s,3H, C$_{11}$ -CH$_3$) 2.20 (s,3H, C$_4$-CH3) 2.10-2.99 (m, 6H, C$_5$-H$_2$, C$_6$-H$_2$, C$_7$-H$_2$) 7.02-7.86 (m, 7H, C$_8$H, C$_9$-H, C$_{10}$-H C$_2$′-H, C$_3$′-H, C$_5$′-H, C$_6$′-H) 6.310 (s,1H, C$_2$-OH), 9.22 (s, 1H indole NH). Anal. calcd for $C_{23}H_{21}N_3O$: C, 77.720%, H, 5.955%, N, 11.82%. Found: C, 77.70%, H, 5.75%, N, 11.62%.

2-hydroxy-4(4′-methyl)phenyl-5,6,7,12-tetrohydropyrimidocyclohept[b]indole (4d)

Yield:0.205 g [65%], Mp: 252ºC; IR (KBr) [ν_{max}cm^{-1}]: 3308, 3152, 2289, 1617, 1545, 1445, 1328, 1206, 1051, 984, 802, 582, ^{1}H-NMR (CDC1$_3$) δ (ppm)=2.38 (s, 3H, C$_4$CH$_3$) 2.163-2.98 (m, 6H, C$_5$-H$_2$, C$_6$-H$_2$, C$_7$-H$_2$) 6.27 (s, 1H, C$_2$-OH), 6.91-7.64, (m, 7H, C$_8$-H, C$_9$-H, C$_{10}$-H, C$_{11}$-H,C$_2$′-H, C$_3$′-H, C$_5$′-H, C$_6$′-H) 8.23 (s, 1H indole NH). Anal. calcd for $C_{23}H_{19}N_3O$, C, 77.720%, H, 5.955%, N, 11.82%. Found: C, 77.70%, H, 5.75%, N, 11.62%.

General procedure for preparation of 2-amino-5,6,7,12-tetra-hydro-4(4′-methyl)phenyl-pyrimido-[3′,4′:5,4]-cyclohept [*b*]indole (5a-5d)

A mixture of respective 2-(4′-methyl)benzylidine-1-oxo-3,4,5,10-tetrahydrocyclohept[*b*]indole (0.001 mol) and guanidine nitrate (0.001 mol) in presence of sodium methoxide in dry ethanol was refluxed on water bath for 18 hours. The excess solvent was removed by evaporation and the reaction mixture was poured into anice. The precipitate obtained was filtered, dried and purified by column, eluting with petroleum ether and ethyl acetate mixture (65: 35) to yield the respective 2-amino-5,6,7,12-tetrahydro-4-(4′-methyl)phenylpyrimido[3′,4′:5,4] cyclohept[*b*]indole(5a-5d).

2-amino-9-methyl-5,6,7,12-tetrahydro-4(4′-methyl)phenylpyrimido[3′,4′:5,4]cyclohept [b]indole (5a)

Yield:0.225g [65%], Mp: 248ºC;IR (KBr) [ν_{max}cm^{-1}]: 3325,3324,1620,1541,1427,1257, 802; ^{1}H-NMR (CDC1$_3$) δ (ppm)= 2.38(s,3H, C$_9$-CH$_3$), 2.35(s,3H, C$_4$′-CH$_3$), 2.08-3.13 (m, 6H, C$_5$-H$_2$, C$_6$-H$_2$, C$_7$-H$_2$) 7.08-7.59(m, 7H, C$_8$-H, C$_{10}$-H, C$_{11}$-H,C$_2$′-H, C$_3$′-H, C$_5$′-H, C$_6$′-H) 7.26 (s, 2H,C$_2$-NH$_2$) 11.31 (s, 1H indole NH).Anal. calcd for $C_{23}H_{22}N_4$: **C,** 77.965%, H, 6.2604%, N, 15.817%. Found: **C,** 15.817%, H,6.10%, N, 15.71%.

2-amino-10-methyl-5,6,7,12-tetrahydro-4(4'-methyl)phenylpyrimido[3',4':5,4]cyclohept [b]indole (5b)

Yield: 0.225 g [62%], Mp: 246ºC;IR (KBr) [ν_{max} cm^{-1}]: 3441,2920,1617,1544,1443,133, 1213,1040, 803. ^{1}H-NMR ($CDC1_3$) δ (ppm)=2.38 (s,3H,$C_{10}CH_3$) 2.73 (s, 3H, C_4-CH_3) 2.81-2.87(m, 2H, C_5-H_2,) 3.11-316(m, 2H, C_6-H_2),3.42-347 (m,2H, C_7-H_2)6.79-7.48(m, 7H, C_8-H, C_9-H, C_{11}-H, C_2-H, C_3-H, C_5-H, C_6-H),7.26 (s, 2H, C_2-NH_2),9.57(s, 1H-C_{12}, indole NH). Anal. calcd for $C_{23}H_{22}N_4$: C,77.965%, H, 6.2604%, N, 15.817%. Found: **C,** 15.817%, H, 6.10%, N, 15.71%.

2-amino-11-methyl-5,6,7,12-tetrahydro-4(4'-methyl)phenylpyrimido[3',4':5,4]cyclohept [b]indole (5c)

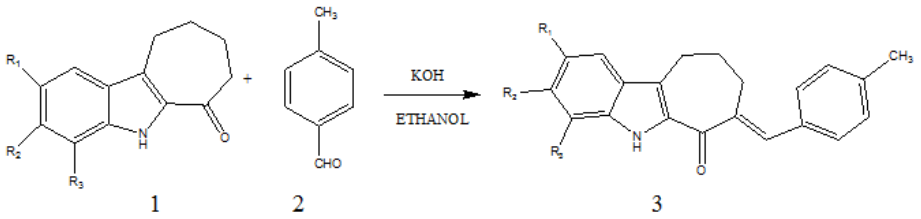
Yield: 0.225 g [62%], Mp: 244ºC; IR (KBr) [$\nu_{max}$$cm^{-1}$]: 3432,2920,1617,1542,1444, 1333, 1201,1038,939,819,739,576;^{1}H-NMR ($CDC1_3$) δ (ppm)=22.42(s,3H, $C_{11}CH_3$), 2.57(s,3H, C_4-CH_3), 2.02(m, 2H, C_5-H_2), 2.85-2.89(m, 2H,C_6-H_2),3.142-318(m, 2H, C_7-H_2),7.26 (s, 2H, C_2-NH_2), 7.01-7.61(m, 7H, C_8-H, C_9-H, C_{11}-H, C_2'-H, C_3'-H, C_5'-H, C_6'-H), 9.52 (s, 1H, C_{12} indole NH). Anal. calcd for $C_{23}H_{22}N_4$: C,77.965%, H, 6.2604%, N, 15.817%. Found: **C,** 77.817%, H, 6.10%\;N, 15.761%.

2-amino-4(4'-methyl)phenyl-5,6,7,12-tetrohydropyrimidocyclohept[b] indole (5d)

Yield: 0.260 g [63%], Mp: 243ºC;IR (KBr) [$\nu_{max}$$cm^{-1}$]: 3291,2929,1618,1541,1444,1329, 1203,1100,1041,802,740,628,584; ^{1}H-NMR ($CDC1_3$) δ (ppm)=2.42(s,3H, C_4'-CH3),2.03-2.10 (m,2H, C_5-H_2),2.86-2.90(m, 2H, C_6-H_2),3.15-3.19(m,2H, C_7-H_2),7.26 (s 2H, C_2NH_2)7.09-7.61 (m,7H, C_8-H, C_9-H,C_{10}-H, C_{11}-H C_2'-H, C_3'-H, C_5'-H,C_6'-H) 9.51(s, 1H,C_{12} indole NH). Anal. calcd for $C_{22}H_{20}N_4$: C, 77.620%, H, 5.9291%, N, 16.458%. Found:C, 77.52%, H, 5.89%, N, 16.34%.

RESULTS AND DISCUSSION

1-oxo-1,2,3,8 tetrahydrocyclohept [*b*] indoles [1] was considered to be an efficient precursor for the synthesis of many novel heterocyclic fused cyclohepta[*b*]indole derivatives. Mixed aldol reaction [22] of 7-methyl-1-oxo-1,2,3,4-tetra-hydrocyclohept[*b*]indole(1a) with 4-methylbenzaldehyde(2) under basic condition led to the formation of 2-(4-methyl)benzylidine-1-oxo-1,2,3,8-tetrahydrocyclohept[*b*]indole in 90% yield. The synthon 2-(4′-methyl)benzylidene-7-methyl-1-oxo-3,4,5,10-tetra-hydrocyclohept[*b*]indole(3a)was prepared by stirring an equimolar mixture of 7-methyl-1-oxo-2,3,4,5-tetrahydrocyclohept[*b*]indole(1a)and 4-methyl benzaldehyde(2) in ethanolic KOH for 48 hours. IR spectrum of (3a)showed strong absorptions at3326 cm^{-1} and 1620 cm^{-1} assigned to -NH and >C=O stretchingvibrations respectively. The H^1-NMR spectrum of (3a) exhibited a broad singlet at δ11.28 corresponds to an indole -NH. Two three proton singlet appeared at δ2.35 and δ2.38 were assigned to C_7-CH_3 and C_4-CH_3. C_3-H_2, C_4-H_2 and C_5-H_2 protons appeared as a multiplet ranging from δ2.79 - δ 3.12. A cluster of multipletfor eight protons appeared in region δ7.12-7.54 was due to C_6-H, C_8-H, C_9-H, benzylic-H, C_2'-H, C_3'-H,C_5'-H, C_6'-H protons. Mass spectra and elemental analysis was also in accordance with the molecular formula. Mixed aldol condensation of 1a-1d with 4-methylbenzaldehyde (2) results the corresponding 2-(4′-methyl)benzylidine-1-oxo-3,4,5,10 tetrahydrocyclohept[*b*]indole 3a-3d in good yield showed in Scheme 1 ($C_{22}H_{21}NO$).

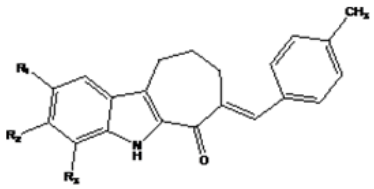


1,3 a : R_2= R_3= H, R_1= CH_3

b : R_1 = R_3= H, R_2= CH_3

c : R_1= R_2= H, R_3=CH_3

d : R_1=R_2= R_3= H

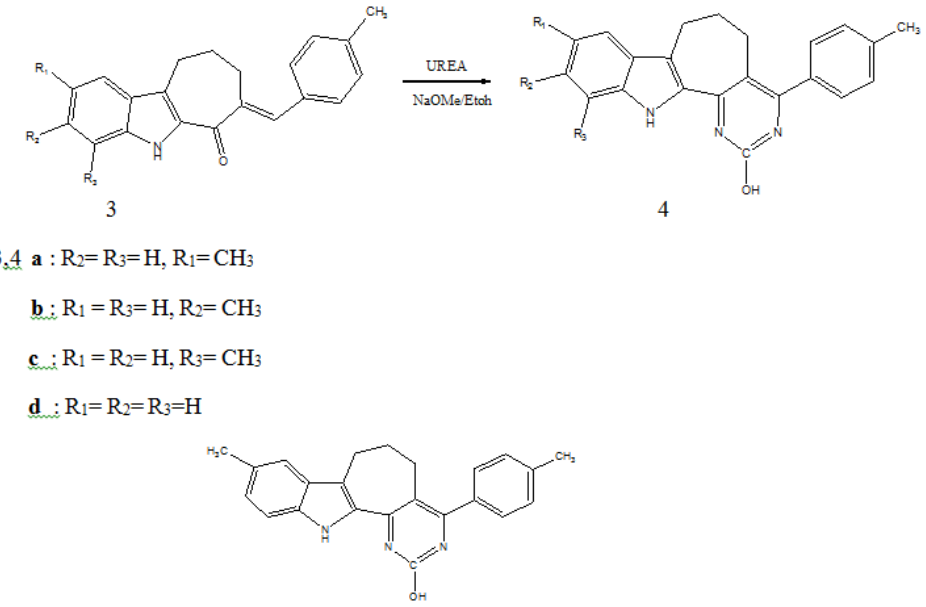


Scheme 1: Formation of 2-(4′-methyl)benzylidine-1-oxo-3,4,5,10 tetrahydrocyclohept[*b*]indole

Preparation of 2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)phenylpyrimido-[3′,4′:5,4] cyclohept[*b*]indole (4a)

2-(4′-methyl)benzylidine-7-methyl-1-oxo-3,4,5,10tetrahydrocyclohept[*b*]indole(3a) was treatedwith urea in sodium methoxide at 100℃ for 18 hours[22]. The product obtained was purified by column chromatography to yield a 2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)phenyl-pyrimido-[3′,4′:5,4]cyclohept[*b*]-indole(4a). IR] Spectrum of 2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)-phenyl-pyrimido-[3′,4′:5,4]cyclohept-[*b*]indole(4a) showed strong absorption bands at 3325 cm^{-1} and 3757 cm^{-1} ascribed to -NH and-OH groupsrespectively. The ^{1}H-NMR spectrum of (4a)2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)phenylpyrimido[3,′4′:5,4]cyclohept[*b*]indolein $CDCl_3$ showedthat a multiplet corresponding to six proton in the region δ 2.07-3.13 for C_5-H_2, C_6-H_2 and C_7-H_2. A two three proton singlet at δ 2.50 and δ 2.38 corresponding to C4'-CH_3 and C_9-CH_3 respectively. An aromatic cluster with seven protons integration appeared as a multiplet at δ7.09-7.59 due to C_8-H, C_{10}-H, C_{11}-H, C_2'-H, C_3'-H, C_5'-H, C_6'-H and a singlet appeared at δ 7.37 for -OH proton. A singlet at δ 11.30 for N_{12}-H. Elemental analysis and mass spectral values were in good agreement with the molecular formula. The other carbazole derivatives 3b-3dreacted similarly with urea to yield the corresponding pyrimido carbazole (3b-d). The reaction for

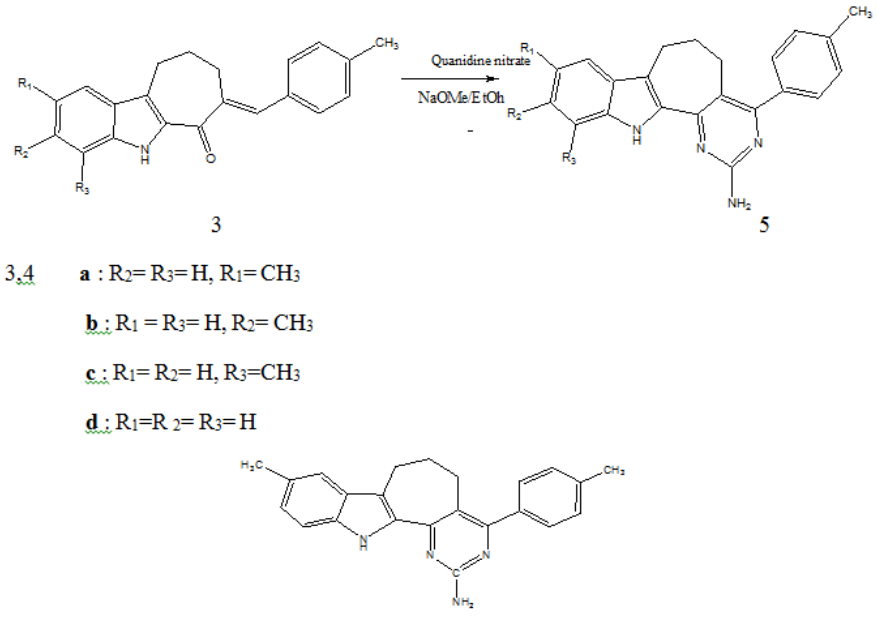
the formation of 2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)phenylpyrimido-[3′,4′:5,4] cyclohept-[*b*]indole (4b-4d) has been showed in Scheme 2 ($C_{23}H_{21}N_3O$).



Scheme 2: Synthesis of 2-hydroxy-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)phenylpyrimido-[3′,4′:5,4] cyclohept[*b*]indole

Preparation of 2-amino-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)phenylpyrimido-[3′,4′:5,4] cyclo-hept[*b*]indole (5a)

2-(4′methyl)benzylidene-1-oxo-3,4,5,10-tetrahydro cyclohept[*b*]indole (3a) was treatedwith guanidine nitrate insodium methoxide at 100ºCfor 18 h to give a single product [22]. It is purified by column chromatography. Then the product was found to be 2-amino-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)phenylpyrimido-[3′,4′:5,4] cyclohept[*b*]indole (5a). It was characterized by IR, ^{1}H-NMR spectra and elemental analysis. Its IR spectrum of (5a)showed that a strong absorption at 3325 cm^{-1} and 1620 cm^{-1} corresponding to presence of -NH and >C=N stretching respectively. The ^{1}H-NMR spectrum of (5a) showed singlet at δ 2.38 for the presence of C_9-CH_3 protons. A two proton singlet appeared at δ 7.26 was assigned to C_2 -NH_2. The complex multiplet for seven aromatic protons range from δ7.08 to δ7.59 for C_8-H, C_{10}-H, C_{11}-H, C_2'-H, C_3'-H, C_5'-H, C_6'-H. The C_5, C_6 and C_7 methylene protons appeared as multipletranges δ 2.08-3.13. The C_4-CH_3 appeared as a three proton singlet at δ 2.38. The indole -NHappeared as asinglet at δ 11.31. The elemental analysis of similar compounds 5b-5d was derived from 3b-3dwas compatible well with the molecular formula ($C_{23}H_{22}N_4$) [23].



Scheme 3: Synthesis of 2-amino-9-methyl-5,6,7,12-tetrahydro-4-(4′-methyl)phenylpyrimido-[3′,4′:5,4] cyclo-hept[*b*]indole

Antimicrobial activity of some newly prepared annelated cyclohept[*b*]indole derivative

The synthesized compounds were screened for their antibacterial activity against *Pseudomonas aeruginosa, Aeromonas hydlophila, Thiobacillus thidurance, Serratia marcescens, Acinetobacter Baumannii and antifungal activity against Aspergillus niger, Candida topicali* by disc diffusion method using Mueller Hinton agar medium for bacteria and sabrose Dextrose Agar medium for fungus. In this disc diffusion technique, a concentration gradient of the drug in a nutrient medium after an inoculation period is observed and the clear zone of inhibition is noted around the disc due to diffusion of drug and growth of the bacteria and fungi.

The diameter of the inhibition zone denotes the relative stability of the microorganism to a particular microbe. Table 1 include zone of inhibition of compounds 2-hydroxy-4-(4′-methyl)phenyl-5,6,7,12-tetrahydropyrimidocyclohept[*b*]indole (4d) and 2-amino-4-(4′-methyl)phenyl-5,6,7,12-tetra-hydro-pyrimido-cyclohept[*b*]indole (5d) against bacteria and fungus.

Zone of inhibition observed against bacteria and fungi by the test compounds

The synthesized compounds were screened for their antibacterial and antifungal activity. The bacteria *Pseudomonas aeruginosa, Aeromonas hydrophila, Thiobacillus thidurance, Serratia marcescens* and *Acinetobater baumanii* and the fungi *Aspergillus niger, Candida tropicali* were collected from Kongunadu microbial culture center, Kongunadu Arts and science College, Coimbatore.

All the microbial cultures were subjected to analyse their susceptibility/resistance pattern to test samples by well diffusion method using Mueller Hinton agar medium (Cat. No. M1084, HiMedia, India) for bacteria and Sabrose Dextrose Agar medium for fungus. Sterile medium was dispensed into sterile petri dishes aseptically. Enriched broth cultures (24 h for bacteria and 48 h for fungus, incubated) were used as inoculums. Using sterile cotton swab, the test organisms were swabbed over the surface e of the agar plate aseptically. In each of these plates, wells (10 mm) were cut out using sterile cork borer.

The samples were dissolved in the DMSO (Dimethyl Sulfoxide) and different concentration (20-80 µg/ml) of the sample was loaded onto the wells. Incubate the plates at 37^{o}C (for 24 h for bacteria and 96 h for fungus) upright position of the plates. Solvent was used as control. After the incubation, the diameters of inhibition zones were observed. If the zone is observed, the inhibition zone was compared with the Performance Standards for Antimicrobial disk Susceptibility Tests CLSI vol. 25 No 1 January 2005 (Chart of Kirby-Bauer sensitivity method modified in July 1966 (Scherring Corporation, U.S.A., Bloomfield, New Jersey) and classified as resistant, intermediate and sensitive. The intermediate strains were also scored as resistant. The values are shown in Table 1.

Table 1: Assay of antimicrobial activity

S. No.	Name of organism	Zone of inhibition (mm)									
		Compound 1 (µg/ ml)					Compound 2 (µg/ ml)				
		R	20	40	60	80	R	20	40	60	80
	Antibacterial activity										
1	*Pseudomonas aeruginosa*	21	2	3	6	7	22	2	4	5	6
2	*Aeromonas hydrophila*	19	3	4	5	9	21	7	9	11	13
3	*Thiobacillus thidurance*	18	3	2	3	5	19	5	6	8	9
4	*Serratiamarcescens*	19	-	2	4	7	20	6	7	8	8
5	*Acinetobater baumanii*	22	2	5	8	9	22	3	5	8	9
	Antifungal activity										
6	*Aspergillus niger*	21	-	-	2	3	20	2	-	-	-
7	*Candida tropicali*	21	1	2	4	9	20	2	2	4	5

*R-inhibition zone of the streptomycin and nystatin used as a standard against bacterial and fungal strains respectively; Compound 1: 2-hydroxy-4-(4′-methyl)phenyl-5,6,7,12-tetrahydrocyclohept[b]indole (4d); Compound 2: 2-amino-4-(4′-methyl)phenyl-5,6,7,12-tetrahydrocyclohept[b]indole (5d)

CONCLUSION

In conclusion 2-hydroxy-4-(4′-methyl)phenyl-5,6,7,12-tetrahydrocyclohept[*b*]indole(4a-d)and 2-amino-4-(4′-methyl)phenyl-5,6,7,12-tetrahydrocyclohept[*b*]indole(5a-d)have beensynthesized from the newly developed synthon 2-(4′-methyl)benzylidene-7-methyl-1-oxo-3,4,5,10-tetrahydrocyclohept[*b*]indole(3a -d) by reacting with the reagent urea and guanidine nitrate.

From our results on the biological activity of the cyclohept[*b*]indole derivatives, it was found that the compound (I) 2-hydroxy-4-(4′-methyl)phenyl-5,6,7,12-tetrahydropyrimidocyclohept[*b*]indole(4d) found better activity against the organism.

A. baumanii and compound(2) 2-amino-4-(4′-methyl)-phenyl-5,6,7,12-tetrahydrocyclohept[*b*]indole(5d) showed better activity against the organism *Aeromonas hydrophilla and A. baumanii*,moderate activity against *Thiobacillus thidurane.*

2-amino-4(4′-methyl)phenyl-5,6,7,12-tetrahydropyrimidocyclohept[*b*]indole(5d) were found better activity against the organism *A. hydrophilla.*

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