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Research Article

SYNTHESIS AND CYTOTOXIC STUDIES OF 2, 3-DIMETHYLINDOLES AND TETRAHYDROCARBAZOLES

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ABSTRACT

Objective: The objective of this study was to report the synthesis of 2,3-dimethylindoles and tetrahydrocarbazoles *via Fisher* indole synthesis and evaluation of their the anticancer properties.

Methods: A simple and more efficient method for the synthesis of 2,3-dimethylindoles and tetrahydrocarbazoles has been described using Phenylhydrazine hydrochlorides and different cyclic and acyclic ketones in presence of antimony phosphate as catalyst in methanol solvent at reflux temperature in one pot reaction.

Results: The synthesized compounds were characterized by spectroscopic techniques (^{1}H NMR, ^{13}C NMR, LC-MS and elemental analysis) and tested for anticancer activity against five different cell lines such as kidney adenocarcinoma (ACHN), pancreas carcinoma (Panc1), lung carcinoma (GIII) (Calu1), non-small cell lung carcinoma (H460), colon cancer cell (HCT116) and normal breast epithelium (MCF10A) cell lines. The results indicated that the compounds 3a and 3b exhibit promising activity against both lung carcinoma and pancreas carcinoma cell line with IC_{50} value 2.7, 3.1, 2.8 and 3.2nM. Whereas compound 5d shows high activity against lung carcinoma cell line alone with IC_{50} 2.5nM and remaining derivatives exhibited good to moderate activity.

Conclusion: The differently substituted 2,3-dimethylindoles and tetrahydrocarbazoles have reported to posses significant activity. Since their synthesis made very simple in this report, the method of synthesis and promising results of 2,3-dimethylindoles and tetrahydrocarbazoles on different cancer cell lines opens an opportunity among researcher for their further study of such moieties.

Keywords: Cytotoxic activity, 2,3-dimethylindoles, Tetrahydrocarbazoles, Antimony phosphate, Fisher indole synthesis.

INTRODUCTION

Indole has been an important structural constituent in many natural and synthetic alkaloids [1]. Besides, a number of significant synthetic drugs also contain an indole ring [2]. Hence, indole derivatives reported to possess wide variety of biological and pharmacological properties [3-18]. In particular 2,3-substituted indoles and tetrahydrocarbazoles have been reported anticancer activity [19,20] against different cancer cell lines like human kidney cancer cell line and human lung cancer cell line. Further, some indole sulfonamide derivatives [21] displayed cytotoxicity against HuCCA-1(cholangio carcinoma), HepG2 (hepatocellular carcinoma), A-549 (lung carcinoma) including MOLT-3(lymphoblastic leukemia) cell line. Recently Zhuang *et al.,* revealed encouraging result wherein the 2, 4-disubstituted furo [3,2-b]indoles [22] exhibited high *in-vitro* selectivity against NCI-60 human cancer cell lines. Apart, from these the indole retinoid [23],Isoxazolo[5',4':5,6]pyrido[2,3-b]indoles [24] were possess significant anticancer activity both *in-vitro* and *in-vivo.* Interestingly, the mannich bases of tetrahydrocarbazoles [25] were also known to possess potent cytotoxic activity against human cancer cell lines including human non-small lung cancer cells (A549), human gastric adenocarcinoma (SGC), human colon cancer cell (HCT116), human myeloid leukemia cells (K562) with one multi-drug resistant subline (KBVCR). On the basis of these results we speculated that indole and tetrahydrocarbazole could be an excellent antiprolifirating agent against the kidney adenocarcinoma, pancreas carcinoma, lung carcinoma (GIII) and colon cancer cell lines. These reports encouraged us to synthesize the 2,3-dimethyl indoles and tetrahydrocarbazoles using antimony phosphate catalyst. Although many methods available to prepare indoles and tetrahydrocarbazoles [26] the *Fisher* indole synthesis using ketones and arylhydrazines remain the most widely employed synthetic procedure [27,28]. Thus many catalysts have also been reported to catalyse the *Fisher* indolization [29-34]. But they lead to low yield, toxic, corrosive and difficult to isolate the product from the reaction mixture.

Hence, in continuation of simple, convenient and more efficient synthesis of indoles *via Fisher* indolization [35-38] herein, we report an antimony phosphate as a novel catalyst which serves as cheaper, less toxic, easy to handle reagent. Therefore, we optimized the reaction condition for indolization by heating equimolar mixture of phenylhydrazine hydrochloride (**1a**), ethylmethylketone (**2a**) in MeOH solvent using antimony phosphate as catalyst. To our delight, the reaction was completed in less than 7h and afforded excellent yield of 2,3-dimethyl indole(Scheme1&2). Thus similar approach was attempted to synthesis remaining 2,3-dimethyl indole and tetrahydrocarbazole derivatives (Table 1).The synthesized compounds were tested against various *in-vitro* cancer cell lines which comprise ACHN, Panc1, Calu1, H460, HCT116, and MCF10A by using propidium iodide(PI)staining assay [39].

MATERIALS AND METHODS

Chemistry

The TLC was performed on alumina silica gel 60 F254 (Merck). The mobile phase was hexane and ethyl acetate (8:2 v/v) and detection was made using UV light (254 nm). Melting points of the synthesized compounds were determined by electrothermal apparatus in open capillaries and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra recorded on Brucker (Bangalore, India) AM 400 (at 400 and 100 MHz, respectively) model spectrophotometer in $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts are expressed as δ values relative to TMS as internal standard. Mass spectra were recorded on a Jeol SX 102=DA-6000(10 kV) FAB mass spectrometer and elemental analysis were carried out using Heraus CHN rapid analyzer. All compounds gave C, H, and N analysis within +/- 0.5% of the theoretical values.

General procedure for the synthesis of 2,3–dimethylindoles and tetrahydrocarbazoles **3a-b and 5a-i**

A mixture of mole equivalent of phenylhydrazine hydrochloride 2.0g (0.013mol) and cyclohexanone 1.36g (0.013mol) or ethylmethylketone 0.99g (0.013 mol) with 0.28g antimony phosphate ($SbPO_4$)(10 mol%) as catalyst in and 10 ml MeOH solvent

was taken in a round bottom flask. The whole reaction mixture was refluxed on water bath for the appropriate time. The completion of the reaction was monitored through TLC[hexane and ethyl acetate (8:2 v/v)]. The reaction mixture was cooled to room temperature and poured into water (10 mL) quenched with sodium bicarbonate and extracted with EtOAc (3X10 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get crude solid. The crude product was purified by column chromatography with silica gel (60–120 mesh, petroleum ether: ethyl acetate, 8:2 v/v) furnished the analytically pure products. All the products were characterized by 1*H* NMR, ^{13}C NMR, LC-MS and Elemental analysis.

Spectral Data

2, 3-dimethyl-1*H*-indole(3a, $C_{10}H_{11}N$)

Brown solid; m.p. 103-105^{0}C(lit [40]106-107^o C). ^{1}H NMR (400 MHz, DMSO-d_6) :(δ /ppm,):10.61(s,1H), 7.35(d, 1H, *J* = 7.60 Hz), 7.22(d, 1H, *J* = 1.20 Hz), 6.99-7.00(m,2H), 2.31(s,3H), 2.16(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) :(δ /ppm,):159.5, 146.4, 135.1, 132.8, 130.4, 129.5, 127.6, 122.8, 119.3, 25.3; MS. m/z =144.2 (M^+ +1). Anal. Calcd.for $C_{10}H_{11}N$(%): C: 82.72, H: 7.64, N: 9.65. Found: C: 82.80, H: 7.50, N: 9.50.

5-fluoro-2, 3-dimethyl-1*H*-indole(3b, $C_{10}H_{10}FN$)

Crystalline solid, m.p. 61-62^{0}C(lit [41]60-61^{o}C). ^{1}H NMR (400 MHz, DMSO-d_6) :(δ /ppm,):10.70(s,1H), 7.17(q, 1H, *J*=4.80 Hz,), 7.07(dd, 1*H*, *J*=2.40,10.00 Hz,),6.77 (m, 1H), 2.82(s, 3H), 2.10(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) :(δ /ppm,): 159.3, 132.6, 131.5, 129.9, 110.4, 108.9, 107.4, 103.1, 11.6, 8.1; MS. m/z =162.4 (M^+ +1). Anal. Calcd.for $C_{10}H_{10}FN$(%): C: 73.60, H: 6.18, N: 8.58. Found: C: 74.05, H: 6.51, N: 9.04.

2,3,4,9-tetrahydro-1*H*-carbazole(5a, $C_{12}H_{13}N$)

Crystalline brown solid; m.p. 118-117 ^{o}C (lit [40]116-118^o C). MS. m/z = 172.2 (M^++1). Anal. Calcd.for $C_{12}H_{13}N$(%): C: 84.17, H: 7.65, N: 8.18.Found: C: 84.05, H: 7.85, N: 7.96

3-methyl-2,3,4,9-tetrahydro-1*H*-carbazole(5b, $C_{13}H_{15}N$).

Crystalline brown solid; m.p. 108-110^{o}C(lit [40]109-110^o C). ^{1}H NMR (400 MHz, DMSO-d_6):(δ /ppm,):10.58 (s,1H), 7.30(d, 1H, *J*=7.6 Hz,), 7.21(d, 1H,*J*=8.0 Hz,), 6.91(m, 2H), 2.70-2.71(m, 3H), 2.18(t, 1H, *J*=9.60 Hz,), 1.84-1.85(m, 2H), 1.45-1.46 (m, 1H), 1.09(d,3H, *J* = 6.40 Hz,). ^{13}C NMR (100, MHz, DMSO-d_6):(δ /ppm,):135.8, 134.0, 127.1, 119.8, 117.8, 116.9, 110.4, 107.9, 31.0, 29.1, 29.1, 22.3, 21.6 ; MS.m/z =186.4 (M^+ +1). Anal. Calcd.for $C_{13}H_{15}N$(%): C: 84.28, H: 8.16, N: 7.56. Found: C: 84.01, H: 8.34, N: 7.38.

6-methoxy-2,3,4,9-tetrahydro-1H-carbazole(5c, $C_{13}H_{15}NO$)

Crystalline brown solid; m.p. 87-89^{o}C(lit [40]88-90^o C). ^{1}H NMR (400 MHz, DMSO-d_6) :(δ /ppm,): 1.74-1.94 (m, 4H), 2.56 -2.74 (m, 4H), 3.70 (s, 3H), 6.60 (dd, 1H, *J* = 8.4 Hz,*J* = 2.08 Hz), 6.80(s, 1H), 7.10(d, 1H, *J* = 8.4 Hz), 10.40(s, 1H) ppm. MS. m/z = 202.1 (M^++1). Anal. Calcd.for $C_{13}H_{15}NO$(%): C: 77.58, H: 7.51, N: 6.96. Found: C: 77.39, H: 7.89, N: 7.37.

6-methyl-2,3,4,9-tetrahydro-1*H*-carbazole(5d, $C_{13}H_{15}N$)

Crystalline brown solid; m.p. 87-89^{o}C(lit [40]88-90^o C). ^{1}H NMR (400 MHz, DMSO-*d6*):(δ /ppm,): 10.40(s, 1H), 7.10(d, 1H, *J* = 8.4 Hz), 6.80(s, 1H), 6.60 (dd, 1H, *J* = 8.4 Hz,*J* = 2.08 Hz), 3.70 (s, 3H), 2.56 - 2.74 (m, 4H), 1.74-1.94 (m, 4H), ppm. MS. m/z = 202.1 (M^++1). Anal. Calcd.for $C_{13}H_{15}NO$(%): C: 77.58, H: 7.51, N: 6.96. Found: C: 77.39, H: 7.89, N: 7.37.

3-phenyl-2,3,4,9-tetrahydro-1*H*-carbazole(5e, $C_{18}H_{17}N$)

Brown solid; m.p.118-120 ^{o}C(lit [40]121-123^o C). ^{1}H NMR (400 MHz, $CDCl_3$) :(δ /ppm,): 7.80 (s, 1H), 7.40 (d, 1H *J*=7.6 Hz,), 7.34-7.28 (m, 5H), 7.25-7.11 (m, 1H), 7.09-7.05(m, 2H), 3.09-3.05(m, 2H), 2.85-2.80 (m, 3H), 2.21-2.13 (m, 2H). MS. m/z = 248.2 (M^+ +1). Anal. Calcd.for $C_{18}H_{17}N$(%): C: 87.41, H: 6.93, N: 5.66. Found: C: 87.24, H: 7.16, N: 5.42.

4-(2,3,4,9-tetrahydro-^{1}H-carbazol-3-yl)benzonitrile(5f, $C_{19}H_{16}N_2$)

Solid; m.p.165-170^{o}C (lit [36]170^{o}C). MS: m/z=273.0 (M^++1). Anal. Calcd.for $C_{13}H_{15}N$(%): C: 83.79, H: 5.92, N: 10.29. Found: C: 83.55, H: 6.22, N: 10.0.

6-fluoro-3-methyl-2,3,4,9-tetrahydro-1*H*-carbazole(5g, $C_{13}H_{14}FN$)

Brown solid, m.p.104-106^{0}C(lit [40]105-106^{o}C). ^{1}H NMR (400 MHz, DMSO-d_6) :(δ /ppm,):10.66 (s,1H), 7.15 (dd, 1H *J*=8.8 Hz,*J*=4.40 Hz), 7.00 (dd, 1H, *J*=2.80 Hz, *J*= 10.0Hz), 6.74 (m, 1H), 2.68 (d, 3H, *J*=2.40 Hz), 2.10 (t, 1H, *J*=9.60 Hz), 1.79-1.81 (m, 2H), 1.40-1.41 (m,1H), 1.04 (d, 3H, *J*=6.40 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) :(δ /ppm,):158.9, 156.6, 135.9, 132.3, 128.1, 110.5, 108.7, 102.8, 31.2, 29.5, 29.2, 22.9, 21.6; MS. m/z = 204.2 (M^++1). Anal. Calcd.for $C_{13}H_{14}FN$(%): C: 76.82, H: 6.94,N: 6.89. Found: C: 77.19, H: 7.27,N: 7.05.

5, 7-difluoro-2,3,4,9-tetrahydro-1*H*-carbazole(5h, $C_{12}H_{12}FN$)

Brown solid, m.p.110-115 ^{0}C(lit [40]110-115^o C). ^{1}H-NMR (300 MHz, $CDCl_3$) :(δ /ppm,): 7.73 (s, 1H), 6.76 (dd, 1H, *J*=12.1 Hz,*J*= 2.0 Hz), 6.53 (m, 1H), 2.86 (s, 2H), 2.67 (s,2H), 1.86 (s,4H). ^{13}C NMR (100, MHz, $CDCl_3$):(δ /ppm,):147.1, 128.9, 127.5, 126.7, 121.7, 119.7, 118.2, 110.9, 41.6, 30.8, 29.7, 23.9. MS. m/z = 208.2 (M^++1). Anal. Calcd.for $C_{12}H_{12}FN$(%): C: 69.55, H: 5.35, N: 6.76. Found: C: 69.26, H: 5.67, N: 6.66.

6-fluoro-3-phenyl-2,3,4,9-tetrahydro-1*H*-carbazole(5i, $C_{18}H_{16}FN$)

Brown solid; m.p.115-120 ^{0}C(lit [40]115-120^o C). ^{1}H NMR (400 MHz, DMSO-d_6) :(δ /ppm,): 10.81 (s, 1H), 7.33-6.38 (m, 4H), 7.31-7.24 (m, 2H), 7.11-7.08 (m, 1H), 6.84-6.79 (m, 1H), 3.02-3.00 (m, 1H), 2.99-2.85 (m, 3H), 2.70-2.63 (m, 1H) 2.51-2.50 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) :(δ /ppm,): 160.8, 158.7, 153.1, 147.8, 138.6, 135.6, 131.8, 130.0, 128.1, 127.3, 109.8, 96.6, 45.1, 30.5, 12.4 ; MS. m/z = 266.2 (M^+ +1). Anal. Calcd.for $C_{18}H_{16}FN$(%): C: 81.48, H: 6.08, N: 5.28. Found: C: 81.21, H: 6.40, N: 5.63.

Cytotoxicity assay

Propidium iodide (PI)staining assay[39]

PI was used to stain the nuclear changes of living and apoptotic cells. Briefly, ACHN, Panc1, Calu1, H460 and HCT116 cancer cell lines along with normal MCF10A cells (2X10^{6}cells/well) were incubated for 24 hour in 5% CO_2 at 37^{o}C with different concentrations of the synthesized compounds **3a-b** and **5a-i**. Gemcitabine and Flavopiridol were used as positive controls. The cells were further incubated for another 48 hours, harvested, homogenized in 200µl of 1% formaldehyde and again incubated for 15 minutes. The cells were washed twice with cold PBS (Phosphate Buffered Saline), and then 1ml of 10µg/ml PI was added into each well and incubated at 37^{o}C for 5 minutes in dark to allow nuclear penetration. After, being washed with cold PBS, the cells were detected by blue filter (515nm) fluorescent microscope (Olympus Corp., Shibuya-ku, Tokyo, Japan) at 400x magnification. Cytotoxicity of the synthesized compounds was determined by PI staining assay. According to the PI staining assay, IC_{50} (the concentration of compound required to inhibit 50% of cell growth) was determined. As showed in Table 1.The tested compounds shows good to moderate cytotoxic activity against ACHN, Panc1, Calu1, H460 and HCT116 cancer cell lines.

RESULT AND DISCUSSION

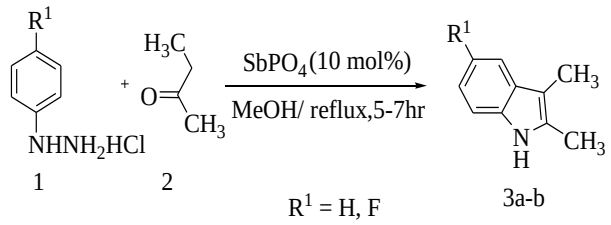
Chemistry

The synthesis of compounds **3a-b and 5a-f** was accomplished is as shown in Scheme 1&2 by using commercially available substituted phenylhydrazine hydrochlorides **1** and ethylmethylketone **2** /cyclohexanones **4** (Sigma – Aldrich, India) *via Fisher* indole synthesis [27,28]in presence of 10 mol % of antimony phosphate ($SbPO_4$) in MeOH solvent, refluxing the contents on water bath for 5-7h. All the purified products were characterized by spectroscopic techniques (^{1}H NMR, ^{13}C NMR, LC-MS and elemental analysis).The detailed physical and analytical data are listed in the experimental section.

Biological studies

Cytotoxic activity

The compounds **3a-b** and **5a-i** were evaluated for cytotoxic activity *in-vitro* against six cancer cell lines such as ACHN, Panc1, Calu1, H460, HCT116 and MCF10A by using propidium iodide (PI) staining assay [39]. IC_{50} values (in μM), which is the concentration required to inhibit 50% of cell viability by the test compounds after exposure to cells, have been determined.

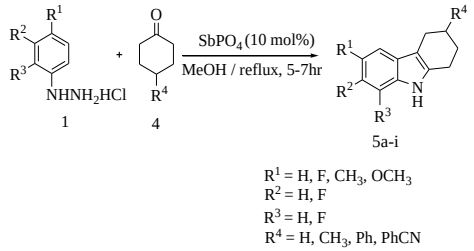


Scheme 1: Synthesis of 2,3-dimethylindoles 3a-b

The results are summarized in **Table 1.**The results indicated that the compounds **3a,** exhibited good to excellent anticancer potency against all cell line with IC_{50} **3.7, 2.8, 2.7, 3.2, 4.5**nM excluding HCT116 wherein it shows moderate activity with IC_{50} **4.5**nM**.** The compound **3b** shows good to high potency against three cell line (ACHN, Panc1&Calu1) with IC_{50} **3.8, 3.2, 3.1**nM but it showed moderate against H460 and HCT116 cell line.

It is noteworthy to mention that the compound **5d** showed excellent binding selectively against calu1 cell line with IC_{50} **2.5**nM compare to other cell lines where it showed moderate activity.

Apparently, cytotoxic effect was evaluated against all the tested cell lines along with positive controls Gemcitabine and Flavopiridol. The tested compounds exhibited good to excellent activity against cell lines when compare to positive control.



Scheme 2: Synthesis of tetrahydrocarbazoles 5a-i

Similarly same way the compound **5c** has good binding selectivity for ACHN cell line with IC_{50} **3.8**nM compare to other cell lines. When we compare the overall activity, more number of synthesized compounds shows good activity against ACHN cell line than other cell lines. But all the synthesized compounds showed poor activity towards HCT116 cell line.

Another feature of these synthesized compounds is they have no affinity towards the normal **MCF10A** cell line. Further, SAR study reveals that the introduction of fluoro group in the compound **3a** does not alter the anticancer activity.

Table 1: Results of anticancer potency of compounds 3a-b and 5a-i in selected human cancer cell lines (IC_{50} (μM))[a]

Entry	ACHN [b]	Panc1[c]	Calu1[d]	H460[e]	HCT116[f]	MCF10A[g]
3a	**3.7**±0.23	**2.8**±0.51	**2.7**±0.63	**3.2**±0.07	4.5±0.85	>10
3b	**3.8**±0.33	**3.2**±0.01	**3.1**±0.23	4.2±1.43	5.1±1.53	>10
5a	**3.7**±0.28	4.8±1.19	4.7±1.27	4.9±1.77	4.9±1.13	>10
5b	6.6±3.33	5.7±2.03	4.9±1.63	5.8±2.27	5.2±2.43	>10
5c	**3.8**±0.32	4.4±1.18	4.5±1.16	4.3±1.43	4.6±1.23	>10
5d	6.2±2.82	5.0±1.37	**2.5**±0.06	4.6±1.64	6.5±3.03	>10
5e	6.1±1.98	5.2±2.53	4.6±1.23	5.0±2.01	5.1±1.73	>10
5f	6.3±3.28	5.3±2.93	4.9±1.73	5.1±2.13	5.2±2.01	>10
5g	6.1±2.03	6.6±3.78	6.4±3.25	5.9±2.93	6.9±3.43	>10
5h	7.2±4.32	7.4±4.23	6.9±3.93	7.1±4.13	7.2±4.25	>10
5i	6.5±3.83	5.8±2.63	4.9±1.23	5.7±2.83	5.4±2.93	>10
Flavopiridol	0.17±. 3.29	0.34± 2.85	0.45± 2.87	0.27± 2.73	0.24± 3.39	>10±3.2
Gemcitabine	0.45± 3.03	0.56± 2.65	0.64± 2.69	0.58± 2.44	0.72± 2.95	>10±3.2

[a]Mean values from three separate experiments. [b]Kidney adenocarcinoma. [c]Pancreas carcinoma. [d]Lung carcinoma(GIII). [e]Lung carcinoma. [f]Colon cancer. [g]Normal breast epithelium.

CONCLUSION

In conclusion, we have demonstrated a simple and high efficient method for the synthesis of 2,3-dimethylindoles (3a-b)and tetrahydrocarbazoles (5a-i),using antimony phosphate as catalyst. All the synthesized compounds were tested for anticancer activity against five different cell lines such as kidney adenocarcinoma (ACHN), pancreas carcinoma (Panc1),lung carcinoma (GIII)(Calu1),lung carcinoma (H460), colon cancer (HCT116), and normal breast epithelium (MCF10A) cell lines. The result shows that compounds 3a and 3b exhibit more significant activity against both lung carcinoma and pancreas carcinoma cell line with IC_{50} value 2.7, 3.1, 2.8 and 3.2nM. Whereas the compound 5d shows high potent activity against lung carcinoma cell line alone with IC_{50} 2.5nM while remaining derivatives were moderately active.

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