FACILE APPROACH FOR THE SYNTHESIS OF PYRIDAZINO[4,5-B]INDOL-4-ONE EXHIBITING ANTIPROLIFERATIVE ACTIVITY

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Abstract:

Efficient and eco-friendly approach was made for the synthesis of a series of pyridazinone indoles from commercially available indole-3-carboxaldehyde and carbonyl diimidazole. The strategy was found to be efficient for an easy access of varieties of pyridazino[4,5-b]indol-4-ones under mild conditions. One pot carbonylation of indole nucleus and substituted hydrazine have been achieved with carbonyldiimidazole. A library of synthesized compounds 3a-p was evaluated for their potential towards antiproliferative behavior against HeLa cell lines. Among the series pyridazino[4,5-b]indol-4-ones, 3h, 3i, 3j and 3m come out to be promising candidates exhibiting moderate to high antiproliferative activities.

Keywords: pyridazino[4,5-b]indol-4-one, carbonyldiimidazole, antiproliferative, HeLa cells

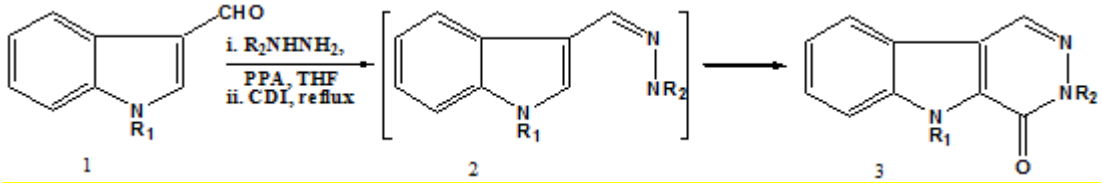
Introduction

Heterocycles dominated medicinal chemistry over carbocycles posessing wide range of pharmacological activities. Among the heterocycles, structurally decorated indoles have been a topic of substantial research interest and continue to be one of the most active areas of heterocyclic chemistry. Wide range of indole derivatives are biologically active, lead compounds for drug development, and involved in broad spectrum of pharmacological activity. The aza *β*-carboline indoles especially, 5H-pyridazino[4,5-b]indoles have been covered in literature in terms of synthesis and biological activities associated with large number of its derivatives. These include antihypertensive,[1] antiarrhythmic,[2] positive inotropic,[3] thromboxane A synthetase inhibitory,[4] MAO inhibitory,[5] serotonine antagonistic,[6] antihistaminic,[7] and anxiolytic,[8] activities. anthelmintic and antiprotozoal activity,[1] antitumor activity,[2] cytotoxicity to tumor cell lines,[3] antihypertensive,[4] antimicrobial[5] serotonine antagonistic, antitumor agents,[6] antihistaminic, anxiolytic, and HIV-1 reverse transcriptase inhibitory activities.[7a] Synthesis of related indole derivatives[7b,7c] such as carbazoles, *γ*-carbolines, *β*-carbolines, *γ*-carboline, azaindoles has been developed from our group. Mohamed Nafie[8] et al recently reported synthesis of a library of some pyridazino[4,5-b] indole and chemo-selective mono/dialkylation at 3 and 5-nitrogen for the breast cancer therapy.

Taking into consideration the importance of indole alkaloids possessing various pharmacological activities, new synthetic method for 5,3-dialkyl-3,5-dihydro-4*H*-pyridazino[4,5-b]indol-4-one was chosen as targets of interest. Literature survey revealed several synthetic methods for simple and complex indole derivatives, of which these systems are less explored than *β*-carbolines and *γ*-carbolines with respect to their biological activities as well as synthetic studies. Most of these systems are having indole skeleton with fused benzene ring with or without one heteroatom. Especially all the methods involved in the synthesis of pyridazines make the use of indole-2,3-dicarbonyls or indole-2,3-bisfunctionality.

Result and discussion

Here we report one pot two stage preparation of 5,3-dialkyl-3,5-dihydro-4*H*-pyridazino[4,5-b]indol-4-one from commercially available alkyl hydrazines, CDI and N-alkyl indole-3-carboxaldehyde. Treatment of N-alkyl indole-3-carboxaldehyde **1** with alkyl hydrazine, results corresponding hydrazone **2**, which upon addition of CDI in presence of catalytic PPA in dry THF furnished desired aza *β*-carbolines **3** (**Scheme 1**).



Scheme 1. Synthesis of substituted 3,5-dialkyl-3,5-dihydro-4*H*-pyridazino[4,5-b]indol-4-one

Attempt to isolate intermediate hydrazone **2** and subsequent cyclization with CDI in presence of catalytic PPA as a separate step did not significantly affect the yield of indole *β*-carbolines. Certainly employing this sequence in a single pot saves reaction duration, efforts and manpower. It is worth to note that when indole nitrogen sets free (entry **3a** to **3d**) and/or N-tosyl derivative, yield of pyridazino indoles **3a-3p** was albeit low and forming complex tlc picture. Based on these observations, it was concluded that N-alkyl indole accelerates cyclization step due to increased nucleophilicity of heterocyclic nucleus and avoids any side reactions. The versatility of the methodology was then tested with various alkyl hydrazines. Among the different acid catalysts tested, (Table 1), PPA found to be efficient for this conversion without leading any side products. Alkyl and aryl carboxylic acids are excluded due to possible amide, carbamate and any other side products formation with CDI.

Entry	R_1	R_2	% Yield[a](t)	%Yield[b](t)	% Yield[c](t)	% Yield[d](t)
3a	H	H	NR	12(10)	20(9)	28(10)
3b	H	Me	05(12)	NR	21(9)	36(10)
3c	H	Et	17(10)	NR	16(8)	27(10)
3d	H	n-Pr	12(12)	22(10)	28(9)	21(10)
3e	Me	H	19(10)	15(8)	30(8)	43(8)
3f	Et	H	27(10)	13(10)	28(7)	47(9)
3g	Me	Me	31(10)	28(9)	46(7)	90(6)
3h	Me	Et	28(10)	31(7)	42(6)	82(8)

3i	Me	n-Pr	29(8)	29(8)	52(7)	91(5)
3j	Me	n-Bu	32(10)	14(10)	55(9)	83(7)
3k	Et	Me	30(8)	35(7)	49(8)	87(7)
3l	Et	Et	25(7)	29(8)	53(10)	84(8)
3m	Et	n-Pr	22(8)	33(6)	44(10)	81(8)
3n	Et	n-Bu	14(10)	27(8)	40(9)	86(6)
3o	n-Pr	n-Pr	11(10)	30(8)	39(8)	83(7)
3p	n-Bu	n-Bu	NR	NR	42(9)	80(8)

[a] $AlCl_3$. [b]camphor sulphonic acid. [c]oxalic acid. [d]polyphosphoric acid. [t]time in hrs

Table 1. 3,5-dialkyl-3,5-dihydro-4*H*-pyridazino[4,5-b]indol-4-one compounds

Evaluation of antiproliferative activity

A library of synthesized pyridazino indole-4-one's **3a** to **3p** were tested for their antiproliferative activity studies against HeLa and normal cell lines. The compounds **3j** showed higher anti-proliferative activity against HeLa cell line with IC_{50} value of 3.84 μM. Moreover, the compounds **3h**, **3i** and **3m** also showed higher anti-proliferative activity against HeLa cell line with IC_{50} values as 5.36, 5.26 and 5.23 μM respectively. Rest of the compounds showed moderate to low IC_{50} values ranging from 30.4 to 14.2 μM.

Entry	**% Cell of Viability HeLa cells**				**% of Cell Viability of normal cells**
	48 h	**72 h**	**92 h**	**IC_{50} in μM**	**72 h**
3a	31.2	25.3	19.4	14.2	93.3
3b	29.6	21.0	16.7	11.3	91.8
3c	17.3	16.7	12.24	7.84	96.6
3d	36.6	18.16	16.71	17.2	95.2
3e	26.2	19.2	12.3	10.4	92.4
3f	24.7	17.9	13.1	11.1	93.2
3g	26.2	15.9	12.1	6.86	94.2
3h	27.2	18.32	16.32	5.36	96.2
3i	27.2	18.32	16.1	5.26	96.2
3j	35.6	18.9	14.22	3.84	98.4
3k	23.7	16.11	10.16	9.22	95.2
3l	28.7	18.11	13.18	6.34	89.9
3m	25.7	19.4	14.13	5.23	96.3
3n	23.7	16.4	13.13	30.4	97.3
3o	36.1	18.2	15.14	17.3	96.4
3p	36.1	18.2	15.14	27.4	96.4

Table 2. Antiproliferative activity against HeLa and normal cells.

Experimental

General Remarks

Chemicals and solvents received from commercial sources were used without further purification. ^1H NMR spectra and ^{13}C NMR spectra were recorded on Bruker (300MHz, 400 MHz and 500 MHz) spectrometer. Coupling constants (*J*) are reported in hertz (Hz) and chemical shifts are reported in parts per million (δ). Melting points were determined using a Thomas Hoover capillary melting point apparatus and uncorrected. Column chromatography was performed using silica gel (100-200 mesh). Exact mass measurements were performed on Bruker impact HD Q-TOF analyzer in the ESI mode. IR spectra were recorded by a Shimadzu FT-IR 8400 Spectrometer. The routine monitoring of reactions were performed using TLC (Merck kieselgel 60 0.20 mm layer, UV254).

General procedure for 3a-3p

Alkyl hydrazine (0.025mol) was added to a stirred solution of Indole-3-carboxaldehyde (0.025mol) and PPA (catalytic) in dry THF (15mL) at rt under N_2 atmosphere. The resulting mixture was refluxed for 30 min to ensure the complete consumption of indole-3-carboxaldehyde (tlc check). Reaction mixture was brought to rt and CDI (0.03 mol) was added portion wise in dry THF by means pressure equalizing funnel over the period of 10 min. The reaction mass was again refluxed for 8-10 h (tlc check). It was colled to rt and quenched with addition of little cold water. THF was stripped out under reduce pressure. Crude pyridazino[4,5-b]indol-4-one is obtained after normal extractive aqueous work up using ethyl acetate. After silica gel (100-200 mesh size) column chromatography desired **3a-3p** are obtained pure enough for spectral analysis.

Conclusion

CDI and catalytic PPA found to be efficient combination for the single pot carbonylation of indole and hydrazone offering cyclization at C2 and C3-hydrazone to afford new derivatives of 5,3-dialkyl-3,5-dihydro-4*H*-pyridazino[4,5-b]indol-4-ones. All synthesized molecules are well characterized with spectral analysis and evaluated for their antiproliferative activity against HeLa and normal cell lines.

Acknowledgements
DGH thanks CIF, SPPU for spectral characterisation. Author DDG is grateful to the BPHE Society's Ahmednagar College, Ahilyanagar for supporting this research work.

Conflict of interest
The authors declare no conflict of interest.

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