Synthesis and Anticancer Activity of Some Novel-1H-BENZO[D]IMIDAZOL-1YL) PYRIDIN-2-YL) (1H-IMIDAZOL-1YL) METHANONES

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Abstract **- The chemistry of benzimidazoles and its derivatives has been studied for over a century due to their diverse biological activities. They possess antibacterial, antiviral, antihypertensive, anthelmintic and antiinflammatory pharmacological activities. Benzimidazoles formed by the fusion of Picolinic acid moiety, have been reported to be chemotherapeutically active. In view of various biological activities and its enormous importance of benzimidazoles, we have made an attempt to synthesize and characterize some new (4-(2-substituted-1H-benzo[d]imidazol-1-yl) pyridin-2-yl) (1H-imidazol-1-yl) methanone derivatives and evaluate them for anticancer activity. 2-(substituted)-1H-benzo[d]imidazoles were treated with (4-chloropyridin-2-yl) (1H-imidazol-1-yl) methanone in presence of potassium tert-butoxide and dry N, N-dimethylformamide to give (4-(2-substituted-1H-benzo[d]imidazol-1-yl) pyridin-2-yl) (1H-imidazol-1-yl) methanones. All the intermediates and final compounds were purified and their chemical structures have been confirmed by IR, 1H NMR and Mass spectral data. All the newly synthesized compounds were screened for their anticancer activity by MTT assay and analyzed statistically. Compounds showed considerable anticancer activity when compared with Cisplatin.**

Index Terms **- Benzimidazoles, Picolinic acid, anticancer activity, Antimicrobial activity, Cisplatin.**

INTRODUCTION

The research of anticancer drugs in the past several decades has shown significant progress and has cured substantial number of patients. Still, it is the intense area of investigation due to the complex physiological changes in the cell functionality, metastasis and apoptotic mechanisms. Hence it has multiple ways of therapeutic strategies ranging from chemotherapy (nitrogen mustard), anti-metabolites to irradiation of cancerous tissues, recently developed targeted therapy[1].

The overall cancer incidence rates were stable from 1995 through 1999, while cancer death rates decreased steadily from 1993 through 1999, which reflects the combined impact of improved screening, prevention and treatment[2,3]. In the past few years lots of compounds were screened for anticancer activity due to the availability of various cell lines and screening methods[4].

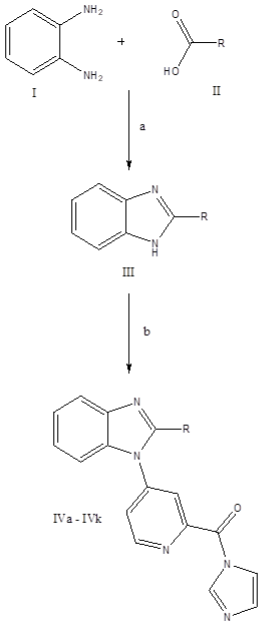
In this process of investigation, many benzimidazole derivatives proved their therapeutic ability against cancer in the previous literature[5]. Benzimidazoles are reported for their antibacterial[6], antimicrebial[7], antitubercular activity[8], analgesic and anthelmintic activity[9]. Many benzimidazoles are also reported as anticancer agents and this has laid base for our intention to synthesize some novel (4-(2-substituted-1H-benzo[d]imidazol-1yl) pyridin-2-yl) (1H-imidazol-1-yl) methanones and to test their ability as anticancer agents.

MATERIALS AND METHODS

The chemicals and solvents used for the experimental work were commercially procured from E.Merck, India, S.D. Fine Chem, India and Qualigens, India. Silica gel G used for analytical chromatography (TLC) was obtained from S.D. Fine Chem, India. Melting points were determined in an open glass capillary using Kjeldahl flask containing liquid paraffin and are uncorrected. The proton magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker 300 MHz instrument (Bruker, Germany) in DMSO/$CDCl_3$ using TMS as internal standard. Chemical shift (δ) are expressed in ppm. The infrared spectra of compounds

were recorded in KBr on a FTIR- 8400S, Fourier Transform (Shimadzu), Japan infrared spectrophotometer. Mass spectra were recorded on LC-MS/MS (API-400 TM), Applied Biosystems, MDS SCIEX (CANADA).

SCHEME



Reagents : (a) 4N HCl;
(b) t-BuOK, DMF, anhydrous K_2CO_3, (4-chloropyridin-2-yl)(1H-imidazol-1-yl)methanone.

EXPERIMENTAL

Synthesis of 2-(substituted)-1H-benzo[d] imidazole (III):

A mixture of *o*-phenylenediamine (0.03 mol), 36 ml of 4 N HCl and different carboxylic acids (0.03 mol) were taken in a round bottom flask and the solution was refluxed for 3 hours. Further the solution was cooled on ice bath and made alkaline by the addition of dilute ammonia solution[10]. The product formed was filtered, dried and recrystallized from suitable solvents. M.P;170^{o}C, Yield; 79 %.

Synthesis of (4-(2-substituted-1H-benzo[d]imidazol-1-yl) pyridin-2-yl) (1H-imidazol-1-yl) methanones (IVa - IVk):

A solution of various 2-(substituted)-1H-benzo[d]imidazoles (0.005 mol) in dry N, N-dimethylformamide were treated with potassium *tert*-butoxide and the reddish-brown mixture was stirred at room temperature for 2 hr. The contents were treated with (4-chloropyridin-2-yl) (1H-imidazol-1-yl) methanone (0.005 mol) and potassium carbonate and then heated to 80^{o}C for 6 hr. The mixture was cooled to room temperature and poured into ethyl acetate[11, 12]. The combined organics were washed with brine, dried over sodium sulphate and concentrated to give (4-(2-substituted-1H-benzo[d]imidazol-1-yl) pyridin-2-yl) (1H-imidazol-1-yl) methanones. M.P;247^{o}C, Yield; 60 %. IR spectrum (KBr) (in cm^{-1}) : 3011 (Ar-H str.), 1615-1513 (C = C and C = N str.), 1259 (C - N), 1641 (C = O), 777 (C - Cl). H^1 NMR (DMSO-*d6*) (IVa) : (δ, ppm) 8.8 -7.1 (m, 11H, Ar-H). (M^+) *m/z :* 289.

Eleven derivatives of (4-(2-substituted-1H-benzo[d]imidazol-1-yl) pyridin-2-yl) (1H-imidazol-1-yl) methanones (IVa - IVk) were prepared by following the above procedure. The physical data of all the eleven compounds prepared are given in the Table – 1.

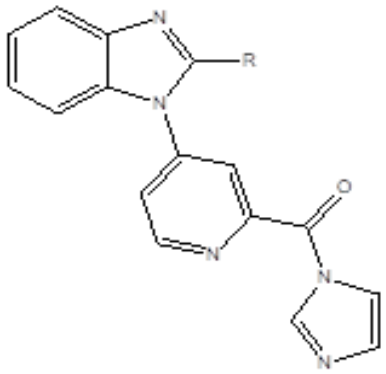
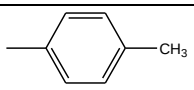
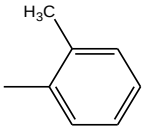
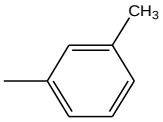
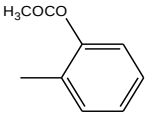
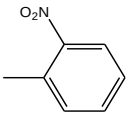
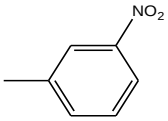
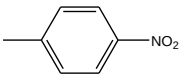
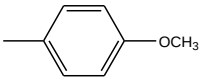
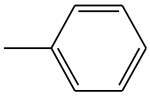


Table 1: Physical data of synthesized compounds (IVa – IVk)

S.NO.	Comp.	R	Mol. Formula	m.p. (^{o}C)	Yield%
1	IVa	—H	$C_{16}H_{11}N_5O$	247	60
2	IVb		$C_{23}H_{17}N_5O$	234	74

3	IVc		$C_{23}H_{17}N_5O$	195	62
4	IVd		$C_{23}H_{17}N_5O$	257	60
5	IVe		$C_{24}H_{17}N_5O_3$	240	65
6	IVf		$C_{22}H_{14}N_6O_3$	225	75
7	IVg		$C_{22}H_{14}N_6O_3$	238	62
8	IVh		$C_{22}H_{14}N_6O_3$	266	78
9	IVi		$C_{23}H_{17}N_5O_2$	212	75
10	IVj		$C_{22}H_{15}N_5O$	218	68
11	IVk		$C_{17}H_{13}N_5O$	215	65

BIOLOGICAL EVALUATION

Cytotoxic Activity:

Microculture tetrazolium (MTT) assay

Materials:

RPMI-1640 (Himedia, Mumbai, India),

Trypsin 0.25% (Gibcous, USA)

FBS (fetal bovine serium) (Gibcous USA)

MTT 4 mg/ml (Himedia)

DMSO (Merck, India)

Lysis buffer (15% SLS in 1:1 DMF and water)

Composition of RPMI; 9.54 gm/lit, 10% FBS, 2000 mg sodium bicarbonate, 250 µl each of penicillin (60 mg/ml), streptomycin (100 mg/ml), amphotericin (200 mg/ml).

Microculture tetrazolium assay (MTT) is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2,5-diphenyl) tetrazolium bromide (MTT) to water insoluble formazan crystals with mitochondrial dehydrogenase enzyme, which gives direct correlation of viable cells[12, 13].

Method: 0.1 ml of the cell suspension (containing 5 x 10^5 cells / 100 µl) and 0.1 ml of the compound solution (10, 20, 50, 100, 150 and 200 µg in DMSO such that the final concentration of DMSO in media is less than 1%) was added to the 11 well plates and kept in carbon dioxide incubator with 5% CO_2 at 37°C for 72 hours. Blank contains only cell suspension and control wells contain 1% DMSO and cell suspension[14]. After 72 hours, 20 µl of MTT was added and kept in carbon dioxide incubator for 2 hours followed by 80 µl of lysis buffer (15% SLS in 1:1 DMF and water). The plate was covered with aluminium foil to protect from

light and then the 11 well plates were kept on rotary shaker for 8 hours[15,16].

After 8 hours the 11 well plates were read on ELISA reader for absorption at 562 nm. The readings were averaged, and viability of the test samples was compared with DMSO control as shown in Table -2.

Table – 2 Cytotoxic activities of synthesized benzimidazole derivatives. (IVa – IVk)

S. No	Comp.	HBL–100 cell lines IC_{50} values (µM)	HeLa cell lines IC_{50} values (µM)
1	IVa	480.00	430.06
2	IVb	375.12	280.66
3	IVc	252.00	241.06
4	IVd	401.11	385.00
5	IVe	263.00	268.11
6	IVf	82.07	136.03
7	IVg	118.65	126.13
8	IVh	137.63	149.08
9	IVi	310.00	260.00
10	IVj	--	--
11	IVk	408.04	--
12	Cisplatin	25.00	25.00

RESULTS AND DISCUSSION

The results on cytotoxic activity of the newly synthesized (4-(2-substituted-1H-benzo[d]imidazol-1-yl) pyridin-2-yl) (1H-imidazol-1-yl) methanones (IVa - IVk) by the given Scheme are presented in Table - 2. All the compounds synthesized in this series have exhibited moderate activity against both HBL-100 and HeLa cell lines except the compound IVj which has not shown any activity against both the cell lines employed. IVa (R = hydrogen) has shown least activity on both the cell lines used. Among the test compounds, compound IVf (R = 2-nitrophenyl) has shown more cytotoxic activity with IC50 values of 82.07 □m against HBL-100 cell lines whereas compound IVg (R = 3-nitrophenyl) has shown more activity with IC50 value of 126.13 □m against HeLa cell lines. The compound IVg and IVh have shown significant activity on both the cell lines. The compounds IVc and IVe have shown moderate activity against both HBL-100 and HeLa cell lines. The compound IVa has shown least activity on both the cell lines with IC50 values of 480.00 and 430.06 □m respectively. The compound IVk has shown activity against HBL-100 cell lines only.

CONCLUSION

The proposed benzimidazole derivatives were synthesized successfully. All the compounds were evaluated for anticancer activity. All the synthesized compounds were found to have good activity, among all the active compounds of benzimidazole derivatives, IVf, IVg and IVh showed good anticancer activity against HBL-100 cell lines and HeLa cell lines respectively. The compound IVk has shown activity against HBL-100 cell lines only while the compound IVj which has not shown any activity against both the cell lines employed. But, however, the cytotoxic activity of even the most active compounds is not at all comparable to that of the standard cisplatin (IC50 25.0 □m).

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