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Synthesis and Structural Elucidation of Benzimidazole Derivatives Incorporating 4-Chloropyridine and their Antiproliferative Potential

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Abstract: *New benzimidazole derivatives (2-((benzylthio)methyl)-1H-benzo[d]imidazol-1-yl)(4-chloropyridin-2-yl)methanones(3)) have been created, as shown in scheme 1. These derivatives comprise 4-chloropyridine-2-carbonyl and N-methylpicolinamide moieties in one side chain at the 1H position of benzimidazoles. The intermediates and final compounds were purified, and IR, 1H NMR, and their bioactivity antiproliferative activity of the produced compounds, which displays the better activity, have corroborated their chemical structures.*

Keywords: Benzimidazoles, antiproliferative activity, Sonication

I. INTRODUCTION

Due to its widespread prevalence and high fatality rate, cancer is today the disease for which a cure is most urgently needed. It is crucial to find new, powerful anticancer medications with fewer adverse effects. Although there are numerous potential causes of cancer, normal cell mutations are the main culprits [1, 2]. There have been reports of the anticancer potential of glutamic acid derivatives, curcumin-I Knoevenagel's condensates and their Schiff bases, and platinum compounds [3-5]. Cancers such as carcinoma, sarcoma, lymphoma, or leukaemia can affect many organs. Thalidomide, which had previously been taken off the market because of its teratogenic effects, has now been authorised for the treatment of Kaposi's sarcoma, multiple myeloma, and metastatic prostate cancer [6]. Due to subpar living conditions and medical infrastructure, the number of cancer cases is rising alarmingly quickly on the Indian subcontinent, with lung cancer leading the list, while globally it has been viewed as a major blow to mankind. In recent years, tailored micro anticancer medicines have been developed using nanotechnology [7–11]. According to several earlier studies, the most prevalent heterocycle with effective cytotoxic characteristics against various cancer cell lines is benzimidazole.

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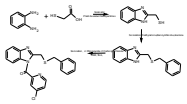


II. EXPERIMENTAL

The experimental study required the acquisition of chemicals and solvents from Qualigens and S.D.Fine Limited India. The analytical chromatography (TLC) silica gel G was purchased from S.D. Finechem in India. Uncorrected melting points were established in open glass capillaries using Kjeldahl flasks filled with liquid paraffin. Using TMS as an internal standard, proton magnetic resonance spectra (1 H NMR) were captured using a Bruker 300 MHz apparatus (Bruker, Germany). In ppm, chemical changes are indicated. Using an infrared spectrophotometer (FTIR-8400S, Fourier transform), the compound's infrared spectra was captured in KBr (Shimadzu).

Synthesis of (1H-benzo[d]imidazol-2-yl)methanethiol (1):

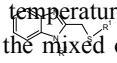
The reaction was carried out in a round-bottomed flask using a combination of 10 orthophenylenediamine, 0.2 gm plant-assisted nanoparticles, and 12 mmole mL thioglycolic acid. The reaction was then completed and verified by TLC analysis [3]. The mixture was also chilled on ice before being turned alkaline by adding a 30% ammonia solution. Filtered, dried, and recrystallized from a suitable solvent, the precipitate that had formed. MP; 155-158^{o}C Yield; 88%

Synthesis of 2-((benzylthio)methyl)-1H-benzo[d]imidazole (2):

A suspension of benzyl chloride (10 mmol), triethylamine (10 mmol), and (1H-Benzo[d]imidazol-2-yl)methanethiol (10 mmol) was added to dry acetone (10 ml). The acetone was then evaporated after the reaction mixture was sonicated for two to three hours at room temperature. The residue was mixed with distilled water, and the precipitate that resulted was filtered, water-washed, dried, and recrystallized using an appropriate solvent. TLC and spectrum data were used to establish the compound's purity. MP; 168-172oC Yield; 84% IR (KBr) (cm-1): 3080 (N-HStr.), 3180 (Ar-HStr.), 660 (C-S Str.), 1620-1530 (C=C & C=N Str.), and 3.9 (H1 NMR, DMSO-d6) (s, 4H, -CH2-S-CH2-).

Synthesis of (2-((benzylthio)methyl)-1H-benzo[d]imidazol-1-yl)(4-chloropyridin-2-yl)methanone(3):

Triethylamine was added to a solution of 2 (10 mmol) in dry N,N-dimethylformamide before the reddish-brown liquid was sonicated at room temperature for two hours. After treating the contents with 4-chloropyridine-2-carbonyl chloride (10 mmole), the contents were sonicated at 40 oC for four hours. The liquid was placed into ethylacetate after cooling to ambient temperature. To produce (2-(((benzylthio)methyl)-1H-benzo[d]imidazol-1-yl)(4-chloropyridin-2-yl)methanone, the mixed organics were washed with brine, dried over sodium sulphate, and concentrated. M.P; 226-228oC Yield; 87%. IR (KBr) (cm-1) values include 3175 (Ar-H str.), 1630–1560 (C=C & C=N str.), 670 (C-S str.), 1270 (C-N), 1680 (C=O), and 740 (C-S str). (C-Cl). 9.1 – 7.4 (m, 12H, Ar-H), 3.6 in the H1NMR (DMSO-d6) (s, 4H, -CH2-S-CH2-).



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Sr.No	R1	R2	Mol.Formula	Mol.Wt	MP	Yield
			$C_{21}H_{16}ClN_3OS$	393.89	226	60
			$C_{16}H_{12}ClN_3O_2S$	345.8	260	75
			$C_{20}H_{13}ClN_4O_2S$	408.86	225	72
			$C_{21}H_{16}ClN_3O_3S_2$	457.95	250	68
			$C_{21}H_{14}ClN_3O_2S$	407.87	230	72
			$C_{22}H_{18}ClN_3O_2S$	423.92	215	62

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			$C_{21}H_{13}ClN_4O_4S$	452.87	255	75
			$C_{21}H_{13}ClN_4O_4S$	452.87	263	70
			$C_{21}H_{13}Cl_2N_3O_2S$	406.87	254	78

Biological Evaluation

III. ANTIPROLIFERATIVE ACTIVITY

Using Mosmann's MTT assay method, a panel of tumour cell lines, including A549 (a cell line for human lung cancer), A498 (a cell line for human chronic myeloid leukaemia), HeLa (a cell line for human acute monocytic myeloid leukae mia), HepG2 (a cell line for human liver cancer), A375 (a cell line for human malignant melanoma), and HEK293 (a ce ll line for human embryonic

The reduction of soluble MTT (0.5 mg/ml, 100 l) to a blue-violet formazan product, mostly by mitochondrial reductase activity happening inside live cells, provides the basis for the measurement of MTT. 23 The cytotoxicity test cells were cultivated in RPMI 1640 media, 10% foetal calf serum, penicillin, and streptomycin supplements, and were maintained at 37°C and 5% CO2 humidification. Following a quick plating procedure in 96-well plates with a total capacity of 100 ml and a cell density of 1-2.5 x 10 4 cells per ml, the cells were given 24 hours to adhere before being treated with test medicines in DMSO solution (final concentration 10-5, 10-6, 10-7 mol L-1). Triplicate wells were treated with medium and reagents, and cell viability was assayed after 96 hours of continuous drug exposure to the tetrazolium compound. After removing the supernatant medium, 150 mL of DMSO solution was applied to each well. The OD570 was calculat ed using a microplate reader after the plates were gently stirred with a mechanical mixer until the colour response was u niform. The absorbance of untreated wells in the MTT experiment was decreased by 50% of the vehicle at the 50% inhi bitory concentration (IC50). Three separate experiments were conducted, and three assays were run in triplicate. The st andard errors were less than 10%, and the results were highly reproducible between wells.By contrasting the results with those of the standard antiproliferative medication methotrexate, all synthesised compounds (1–9) were assessed for their in vitro antiproliferative efficacy against five human cancer cell lines. The MTT test was used to conduct an in vitro antiproliferative screening of the title compounds against the human malignant melanoma (A375), lung cancer

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cell line (A549), kidney cancer cell line (A498), cervical cancer cell line (HeLa), and liver cancer cell line (HepG2). 23 Table 1 provides a summary of the in vitro antiproliferative actions that were achieved.

Table1. Invitroantiproliferativeactivityofsynthesizedsubstitutedbenzimidazole derivatives studied using MTT assay (96 hrs)

	HEK293	A549	A498	HeLa	A375	HepG2		Specificity				
Sr.No	IC50(μM)				A549			A498	HeLa	A375	HepG2	cLogP#
1	0.85	0.425	0.312	0.425	0.625	0.058	0.80	3.00	1.00	1.00	15.00	3.837
2	1.5	0.85	0.625	4	5	0.112	0.80	3.00	0.40	0.6	7.00	3.806
3	0.425	0.212	0.312	0.062	0.078	0.056	0.80	1.00	3.8	7.00	3.00	3.837
4	0.25	0.525	0.312	4	4	0.425	0.80	3.00	0.25	0.35	1.00	3.576
5	1.5	0.85	0.625	4	4	0.425	0.80	3.00	0.56	0.4	3.00	3.060
6	0.25	0.425	0.312	0.425	0.825	0.078	0.80	3.00	1.00	1.80	13.00	3.060
7	4	1.5	1.25	1.5	1.6	0.625	0.80	3.00	1.00	1.00	7.00	3.559
8	1.5	0.85	0.625	1.5	09	0.078	0.80	3.00	2.00	0.35	3.00	3.851
9	0.425	0.212	0.312	0.85	1.5	0.625	0.80	1.00	0.5	0.35	1.00	4.471
MTX	0.022	0.022	0.022	0.022	0.022	0.022	1.00	1.00	1.00	1.00	1.00	0.94

cLogPvalueofthesynthesizedcompounds(calculated fromChemB ioDrawUltra12.0.3).MTX:Methotrexate

The cytotoxicity of synthetic substances (19) against HEK293 was also examined.
We will better understand how these medications target cancer cells thanks to this work. A substan
e ought to be less hazardous to normal cells if it is more selective towards cancer cells. The series of synthesised compo
unds all shown strong antiproliferative activity against the tested human cancer cell lines, according to Table 1. In com
parison to normal methotrexate, all compounds generally demonstrated strong antiproliferative efficacy against HepG2
and A498 cancer cell lines. Unsubstituted naphthylbenzimidazole derivatives (1 and 3) from the produced compounds s
hown potential antiproliferative action against all examined cancer cell types. With IC50 values of 0.312 M and 0.078
M, respectively, compound 1 was found in Table 1 to be a strong antiproliferative agent against the cancer cell lines A4
98 and HepG2. With an IC50 value of 1.25 M against the HEK293 cell line, compound 1 was also demonstrated to be s
elective against cancer cell lines. Compared to the A498 and HepG2 cancer cell lines, respectively, this indicates a 4-
fold and 16
fold greater selectivity. Similar to compound 2, compound 3 shows 2, 4, and 8fold selectivity against the cancer cell lin
es A549, HeLa, and A375, respectively. 0 to show IC50 values of 162 M and 0.078 M against A549, HeLa, and A375 c
ancer cell lines and 0.625 M and 0.312 M against the HEK293 cell line, respectively (Table 1). The 2(benzimidazolyl)b
enzimidazole derivatives (1 and 6) are highly selective against HepG2 and have an IC50 value of 0.078 among the 1/2(
unsubstituted benzimidazolyl)benzimidazole derivatives (16). It demonstrates 16fold greater selectivity over the HepG2
cancer cell line due to its excellent antiproliferative action at M and 1.25 M. The cytotoxic effects of compounds (2 an
d 4) against the cancer cell types examined appeared to be lessened by the presence of a methyl linker between the naph
thalene and benzimidazole moieties. Similar to this, naphthalene (56)'s hydroxyl group was moved from the position to
lessen the cytotoxic impact. With an IC50 value of 0.078 M, rice field (8) was shown to be the most effective antiprolif
erative agent against the HepG2 cancer cell line among the 2-(5-
substituted benzimidazolyl)benzimidazole derivatives (7-9).
With an IC50 value of 2.5 M against the HEK293 cell line, compound 8 was also demonstrated to be specifically for ca
ncer cell lines. In comparison to the HepG2 cancer cell line, this indicates a 32-fold better selectivity.

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IV. CONCLUSION

The HepG2 cancer cell line was tested in this work using a series of benzimidazole derivatives (1–9) as possible antiproliferative agents. Given that benzimidazole derivatives are polycyclic aromatic compounds, the electronic ring o f benzimidazole plays a role in the antiproliferative action. Minor structural adjustments, such as lengthening the linker between substituents on the benzimidazole moiety, shifting the position of the bond, including additional functional mo ieties, or including substituents at various locations on the benzimidazole moiety, were assessed. The action against can cer cell lines was not considerably enhanced by the benzimidazole moiety. The reported logP values and the antiprolife rative effectiveness of synthesised drugs were not correlated. In contrast to nitro or chlorobenzimidazolylsubstituted na phthalene derivatives, however, exhibited greater antiproliferative activity and selectivity against the HepG2 cancer cell line. In comparison to the disubstituted benzimidazole derivatives (9), the monosubstituted benzimidazole derivatives (58) demonstrated superior selectivity for the HepG2 cancer cell line, particularly the chlorosubstituted molecule (8). In cancer cell lines, it demonstrated great selectivity with manyfold selectivity. Our findings suggest that this family of 2n aphthylbenzimidazole derivatives is a promising option for future study as a liver cancer therapeutic agent.

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