

EVALUATION OF NOVEL 6-SUBSTITUTED BENZIMIDAZOLE-2-CARBAMATES FOR POTENTIAL ANTITUMOR ACTIVITY

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ABSTRACT

Purpose: The aim of the present work was to evaluate novel series of 6-substituted benzimidazole-2-carbamates for antitumor activity.

Method/approach: Evaluation of antitumor activity was carried out *in vitro* in human malignant cell lines A549 (lung), SK-MEL-2 (melanoma), COLO 205 (colon) and JURKAT (leukemia) by sulforhodamine (SRB) assay. The test compounds were compared with adriamycin (doxorubicin) as positive control for antitumor activity.

Findings: Significant activity against cell proliferation was demonstrated by the test compounds in *in vitro* cytotoxicity assays.

Application: The findings of the study can be used to develop novel 6-substituted benzimidazole-2-carbamates as potent antitumor agents.

Social value: As cancer is still a dreaded disease, the present work provides scope for introduction of a new molecule belonging to benzimidazole class of compounds for treatment of cancer having broad spectrum of activity and minimal side effects.

Research Value: The series of novel 6-substituted benzimidazole-2-carbamates can be structurally optimized further to yield a compound with remarkable *in vitro* and *in vivo* antitumor properties which can then be translated in to clinic if found to exhibit minimum toxicity.

Conclusion: It can be concluded that all the compounds tested showed potent *in vitro* antitumor activity in cell lines tested.

Keywords: Benzimidazole; antitumor; microtubule; antitubulin.

INTRODUCTION

The rate of mortality due to cancer still remains high throughout the world. Although the exact cause of cancer is not known, certain environmental factors may enhance the risk for tumors. Surgery and/or radiotherapy are used if the cancer is detected in the early stages. In the advanced stages, treatment comprises of combining options such as surgery and chemotherapy. A large number of chemotherapeutic agents are available to treat cancer. But these agents are not effective against all types of tumors. Therefore, the search for novel chemotherapeutic agents which will selectively target only tumor cells and those exhibiting broad spectrum of activity with minimal side effects continues.

Benzimidazoles are of considerable interest as they are reported in literature to have a wide range of therapeutic activities.¹⁻⁶ The 2-substituted benzimidazoles have general formula as shown in fig.1 where R₁=H, alkyl group, R₂=NHCOOR (where R is aliphatic hydrocarbon of less than seven C-atoms), R₃=H, X, alkyl, alkoxy and R₄=H, X, oxychloro, nitro, methyl or ethyl. They have been reported to possess antifungal, anthelmintic, antitumor and vascular damaging properties.⁷ Oncodazole, a benzimidazole-2-carbamate derivative is

reported to have antifungal, antitumor and anthelmintic properties. Due to its poor aqueous solubility, it exhibits poor oral bioavailability and reduced activity against solid tumors. Several analogs of Oncodazole have been prepared to improve the antitumor activity and water solubility.^{8,9}

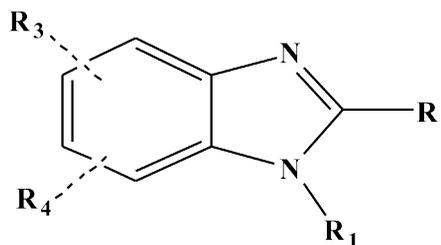


Fig.1: General Structure of 2-Carballoxyamino Benzimidazoles

The cytotoxicity and antitumor activity of substituted benzimidazole-2-carbamate derivatives have been attributed to their ability to bind to tubulin protein and arrest the mitotic phase of cell division. Microtubules are an attractive therapeutic target for cancer. Natural agents (vinca alkaloids, colchicine and podophyllotoxin), semi-

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synthetic (taxotere) and synthetic (oncodazole) bind to them and interfere with their assembly through distinct mechanisms.¹⁰

The entire series of known antifungal and anthelmintic benzimidazole-2-carbamic acids have been investigated for antitumor properties.¹¹⁻¹³ Therefore, using the structures of Oncodazole (fig.2) and methyl-N-[6-(3,4,5-trimethoxybenzyloxy)imidazo[1,2-b]pyridazin-2-yl] carbamate as lead molecules (fig. 3), novel 6-substituted benzimidazole-2-carbamic acid alkyl esters have been designed (fig. 4) and synthesized as reported by us earlier.¹⁴ The present work describes the evaluation of novel 6-substituted benzimidazole-2-carbamates for *in vitro* cytotoxicity in various cancer cell lines.

Methyl-N-[6-(3,4,5-trimethoxybenzyloxy)imidazo[1,2-b]pyridazin-2-yl] carbamate has 3,4,5-trimethoxy benzyl oxy group at 6-position and methyl carbamate group at 2-position This compound has been reported to be one of the most potent cytotoxic agent with broad spectrum of anticancer activity and also effective against multi-drug resistant tumors.¹⁵⁻¹⁷

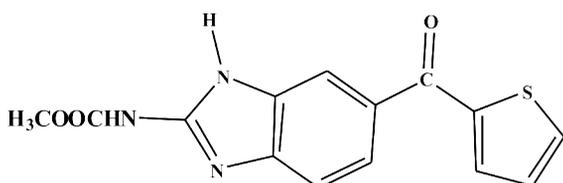


Fig. 2: Structure of Oncodazole

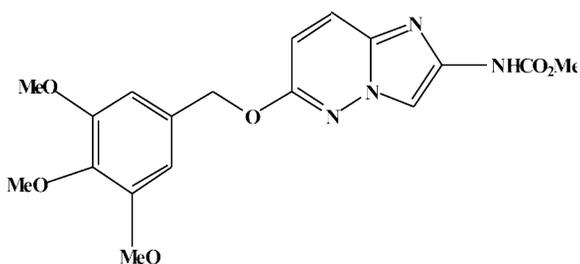
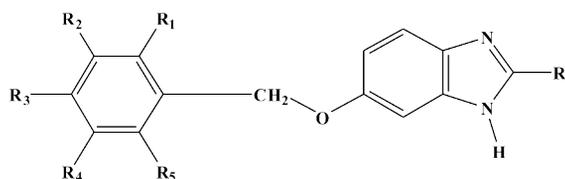


Fig. 3: Structure of Methyl-N-[6-(3,4,5-trimethoxybenzyloxy)imidazo [1,2-b]pyridazin-2-yl] carbamate



Compounds	R	R1	R2	R3	R4	R5
1a	-NHCO ₂ Me	H	-OCH ₃	-OCH ₃	-OCH ₃	H
1b	-NHCO ₂ Et	H	-OCH ₃	-OCH ₃	-OCH ₃	H
2a	-NHCO ₂ Me	-OCH ₃	-OCH ₃	H	H	H
2b	-NHCO ₂ Et	-OCH ₃	-OCH ₃	H	H	H
3a	-NHCO ₂ Me	-OCH ₃	H	H	-OCH ₃	-H
3b	-NHCO ₂ Et	-OCH ₃	H	H	-OCH ₃	-H

Fig. 4: Structures of novel 6-substituted benzimidazole-2-carbamates

MATERIALS AND METHODS

Pharmacological evaluation

A series of six test compounds (1a-b, 2a-b and 3a-b) were evaluated for *in vitro* cytotoxicity in various human cancer cell lines like A549 (lung), SK-MEL-2 (Melanoma), COLO-205 (Colon) and JURKAT (leukemia). Adriamycin was used as standard (positive control) and sulforhodamine B (SRB) assay procedure was used for the determination.¹⁸ Solutions of test compounds and standard were prepared in DMSO and suitably diluted to give final concentration of 10⁻⁷, 10⁻⁶, 10⁻⁵ and 10⁻⁴ M. The ability of test compounds to inhibit proliferation was determined. Cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2mM L-glutamine. Cells were inoculated in to 96 well micro-titer plates in 90 µl and plating densities were adjusted depending on doubling time of individual cell lines. The plates were incubated at 37 °C for 24 h prior to addition of the test compounds. One plate of each cell line was fixed *in situ* with trichloroacetic acid (TCA). A 10 µl of varying dilutions of both test and standard drugs were added to the appropriate micro-titer wells containing 90 µl of medium. After addition, plates were incubated for 48 h and assay was terminated by the addition of cold TCA and incubated for 60 min at 4°C. SRB reagent was added to each of the wells and plates were incubated for 20 min at R.T. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried, bound dye was eluted with 10 mM Trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm.

RESULTS

Table 1 and Table 2 show *in vitro* cytotoxicity data of test compounds in various cancer cell lines. Percent growth is expressed as the ratio of average absorbance of the test well/average absorbance of the control wells multiplied by 100 and values reported are average of three experiments. The GI50 value which is the concentration of the drug that produces 50% inhibition of cells is given in Table 3. Compounds with GI50 value of ≤ 10⁻⁶ molar drug concentration are considered to demonstrate good inhibitory activity.

Table 1: *in vitro* anticancer activity of synthesized compounds in A549 (Lung) and COLO-205 (Colon) cancer cell lines.

Compound	% control Growth in Molar Drug Concentration*							
	A549				COLO-205			
	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
1a	24.8	23.7	20.3	14.4	12.6	10.1	7.4	5.8
1b	22.8	21.2	16.2	10.3	17.2	11.7	10.5	9.7
2a	81.7	76.8	71.9	62.1	98.9	80.6	59.7	42.8
2b	93.2	88.1	77.3	59.3	78.8	66.1	46.2	37.2
3a	75.6	64.3	56.5	48.6	87.2	70.6	55.2	44.9
3b	78.8	68.3	58.4	52.9	77.4	65.4	52.8	35.6
Adriamycin	42.7	21.5	17.0	2.9	58.7	42.1	19.2	11.3

*values are given as average of three experiments.

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Table 2: *in vitro* anticancer activity of synthesized compounds in SK-MEL-2 (Melanoma) and Jurkat (Leukemia) cancer cell lines.

Compound	% control Growth in Molar Drug Concentration*							
	SK-MEL-2				JURKAT			
	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
1a	24.7	22.0	19.1	13.9	73.5	61.6	55.7	52.3
1b	31.4	26.5	17.1	13.5	67.7	58.5	55.7	51.2
2a	22.0	11.6	0.8	-17.3	62.8	-2.0	-12.8	-29.8
2b	-6.4	-13.0	-19.8	-30.4	-3.0	-13.5	-19.7	-32.6
3a	19.0	15.2	5.5	-4.3	57.7	13.2	-2.6	-11.9
3b	-3.2	-7.4	-11.2	-16.8	-1.8	-10.5	-18.3	-25.6
Adriamycin	45.7	-30.3	-55.7	-59.0	8.2	-19.6	-26.8	-48.9

*values are given as average of three experiments.

TABLE 3: GI50 values of the compounds in various cell lines for *in vitro* cytotoxicity testing.

Compound	GI50			
	A549	SK-MEL-2	COLO-205	JURKAT
1a	<0.1	<0.1	<0.1	>100
1b	<0.1	<0.1	<0.1	>100
2a	>100	<0.1	79.0	<0.1
2b	>100	<0.1	53.9	<0.1
3a	>100	<0.1	72.6	<0.1
3b	>100	<0.1	51.6	<0.1
Adriamycin	<0.1	<0.1	<0.1	<0.1

DISCUSSION

Compounds 1a and 1b exhibited good activity against three human malignant tumor cell lines such as A549 (lung), SK-MEL-2 (melanoma) and COLO-205 (colon). These compounds were inactive against JURKAT leukemia cell line. The activity was found to be better than standard adriamycin for compounds 1a and 1b in cell lines A549 and COLO-205. The GI50 values were less than 0.1 micro molar drug concentrations for cell lines A549, SK-MEL-2 and COLO-205. Compounds 2a-b and 3a-b showed excellent activity against cell lines SK-MEL-2 and JURKAT leukemia. These compounds were inactive against A549 (lung) and COLO-205 (colon) cell line. Compounds 2a-b and 3a-b were effective even at concentrations of 10⁻⁶ M as is evident from the percent control growth values obtained in these cell lines. The percent control growth was found to decrease with increase in concentration for all the test compounds. The percent control growth for compounds 1a-b was found to be low (5-15%) in cancer cell lines such as A549, COLO-205 and SK-MEL-2 at 10⁻⁴ μM drug concentration.

Substitution of groups in novel 6-substituted benzimidazole-2-carbamates were investigated with respect to the position of methoxy groups in side chain aromatic ring at 6-position. Compounds 1a-b containing 3, 4, 5-trimethoxy benzyl group exhibited high *in vitro* cytotoxic potency in three out of four cell lines tested. However, even the dimethoxy benzyl group containing compounds 2a-b and 3a-b exhibited good antitumor

activity against SK-MEL-2 and JURKAT leukemia cell proliferation. Thus removal of any one of the methoxy groups does not abolish antitumor activity. It is possible to alter the lipophilic and steric effects of the linear alkyl groups like methyl, ethyl in the carbamate group at 2-position. The cytotoxic potency of ethyl esters 1b, 2b and 3b were found to be generally better than the corresponding methyl esters 1a, 2a and 3a.

CONCLUSION

A structure based drug design approach was utilized to construct novel 6-substituted benzimidazole-2-carbamates containing essential pharmacophore features necessary to exhibit antitumor activity. These molecules were evaluated for cytotoxic effects on tumor cells. It can be concluded that the compounds exhibit potent antitumor activity in cell lines tested.

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REFERENCES

- Spasov AA, Yozhitsa IN, Bugaeva LI, Anisimova VA. Benzimidazole Derivatives: Spectrum of pharmacological activity and toxicological properties (A Review). Pharm Chem J 1999; 33(5):6-17.
- Walia R, Hedaitullah Md, Naaz SF, Iqbal K, Lamba HS. Benzimidazole derivatives. An overview. Int J Res Pharm Chem 2011;1(3) :565-74.
- Srikanth L, Varun Raj V, Raghunandan N, Venkateshwerlu L. Recent Advances and Potential Pharmacological activities of benzimidazole derivatives. Der Pharm Chem 2011;3(2):172-93.
- Bansal Y, Silakari O. The therapeutic journey of benzimidazoles: A Review. Bioorg Med Chem 2012;20:6208-36.
- Desai JV, Chavan RS, Chaudhari NS, Patil ST. A short review on chemistry and potential activities of benzimidazole molecule. Int J Pharm Tech. 2013;5(1):2477-94.
- Kapuriya K, Ganure A, Davda A, Kitawala M, Topiya H. Benzimidazole: A promising lead for anticancer drug design. Univ J Pharm. 2012;2(3):57-62.
- Malathi Raghunath, Viswanathan CL. Benzimidazole-2-carbamic acid as a privileged scaffold for antifungal, anthelmintic and antitumor activity: A Review. Int J Pharm Pharm Sci. 2014;6(5):17-25.
- De Brabander MJ, Van de Velre RML, Aerts FEM, Borgers M, Janssen PAJ. The effects of Methyl [5-(2-thienyl carbonyl)-1H-benzimidazol-2-yl]

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- carbamate, (R 17934, NSC 238159), a new synthetic antitumoral drug interfering with microtubules, on mammalian cells cultured *in vitro*. *Cancer Res.* 1976;36:905-16.
9. Kruse LI, Ladd DL, Harrsch PB, McCabe FL, Ming Mong S, Faucette L et al. Synthesis, Tubulin binding, antineoplastic evaluation, and structure-activity relationship of Oncodazole analogues. *J. Med. Chem.* 1989; 32, 409-17.
 10. Nguyen TL, McGrath C, Hermone AR, Burnett JC, Zaharevitz DW, Day BW et al. A common pharmacophore for a diverse set of colchicine site inhibitors using a structure-based approach. *J Med Chem.* 2005;48: 6107-16.
 11. Ireland CM, Gull K, Gutteridge WE, Pogson CI. The interaction of benzimidazole carbamates with mammalian microtubule protein. *Biochem Pharmacol.* 1979;28:2680-82.
 12. Lacey E, Watson TR. Structure-activity relationships of benzimidazole carbamates as inhibitors of mammalian tubulin, *in vitro*. *Biochem Pharmacol.* 1985;34(7):1073-77.
 13. Zhao Y, Pourgholami MH, Morris DL, Collins GJ, Day AI. Enhanced cytotoxicity of benzimidazole carbamate derivatives and solubilisation by encapsulation in cucurbit[n]uril. *Org Biomol Chem.* 2010;8:3328-37.
 14. Malathi Raghunath, Viswanathan CL. Synthesis, characterization and evaluation of novel 6-substituted benzimidazole-2-carbamates as potential antimicrobial agents. *Int J Pharm Pharm Sci.* 2014;6(9):372-5.
 15. Hodgson ST, Con Jenkins D, Knick V, Rapson E, Watts SDM. Synthesis and Biological Properties of 1069C: A new synthetic antitumor agent with very low cross-resistance potential. *Bioorg Med Chem Lett.* 1992; 2(10):1257-62.
 16. Denyer CV, Reddy MT, Con Jenkins D, Rapson E, Watts SDM. Synthesis of Antitumor Carbon-Bridged Imidazopyridazine carbamates. *Arch Pharm. (Weinheim)* 1994; 327: 95-8.
 17. Judson I, Briasoulis E, Raynaud F, Hanwell J, Berry C, Lacey H. Phase I trial and pharmacokinetics of the tubulin inhibitor 1069C85- a synthetic agent binding at the colchicine site designed to overcome multidrug resistance. *Brit J Can.* 1997;75(4):608-13.
 18. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Can Inst.* 1190;82(13):1107-12.