INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS

VOL.6 ISSUE 3, 2018; 400 – 405 ; http://ijpda.com; ISSN: 2348-8948

Research Article

In vivo Evaluation of Antiproliferative Activity of a Novel Benzimidazole Derivative Against Daltons Lymphoma Ascitic in Swiss Albino Mice

P. T. Manju[1*], A. Anton Smith[2], V. Padmaja[1]

[1]College of Pharmaceutical sciences, Govt. Medical College, Trivandrum, Kerala, India.

[2]Department of Pharmacy, Annamalai University, Annamalai nagar, Chidambaram, Tamilnadu, India.

Date Received: 9rd March 2018; Date accepted: 17th March 2018; Date Published: 18th March 2018

Abstract

The present work was to evaluate the antiproliferative activity of a novel benzimidazole derivative, [N-[3-chloro-2-oxo-4-(2-hydroxyphenyl)-4-oxoazetidin-1yl]-2-(2-methyl-1H-benzimidazol-1-yl)acetamide] against tumour induced in female Swiss albino mice. The effective dose of the test compound was determined as 10 mg/kg body weight. Two doses of the test compound were used for the evaluation. 5- Fluorouracil at a dose of 20 mg/kg body weight was used as the standard. Derived, haemotological and biochemical parameters were evaluated. RBC, Hb level, platelet count and packed cell volume that reduced significantly in cancer control group became normal after treatment with test drug. WBC count that increased significantly in DAL control group became normal by treatment with test drug. The results strongly support the antiproliferative activity of novel benzimidazole derivative [N-[3-chloro-2-oxo-4-(2-hydroxyphenyl)-4-oxoazetidin-1yl]-2-(2-methyl-1H-benzimidazol-1-yl)acetamide] against cancer induced female Swiss albino mice.

Keywords: Antiproliferative activity, Benzimidazole, DAL, Haematological parameters.

Introduction

Benzimidazole is a heterocyclic aromatic compound. It is bicyclic in nature and consists of the fusion of benzene and imidazole [(1)]. Compounds that contain Benzimidazole nucleus possess a lot of medical and biological activities, such as antitumour [(2,3)], antibacterial [(4-7)], antiviral [(8-12)], antifungal [(13)], anti- inflammatory [(14)], analgesic [(15)] and anticonvulsant properties [(16)].

Cancer, the deadliest and complex genetic disease that is characterized by uncontrolled proliferation and spread of abnormal cells [(17)]. Cancer causes myelosuppression and anaemia, because of the reduction of RBC content and haemoglobin level [(18)]. Cancer also affects liver functions. Elevated level of total cholesterol, triglycerides, AST, ALT and ALP in serum indicates liver damage. Based on the above reports various Benzimidazole derivatives were designed and screened for the physicochemical parameters with the aid of bioinformatics tools like ACD Lab Chemsketch, Molinspiration, PASS, Schrodinger Glide XP etc. With the help of these parameters, compound (N-[3-chloro-2-oxo-4-(2-hydroxyphenyl)-4-oxoazetidin-1yl]-2-(2-methyl-1H-benzimidazole-1-yl)acetamide) was selected. This compound proved to possess significant antiproliferative property against MCF 7 and Hep2 cell lines. In the present work, compound (N-[3-chloro-2-oxo-4-(2-hydroxyphenyl) -4-oxoazetidin-1yl]-2-(2-methyl-1H-benzimidazol-1-yl)acetamide) was selected to study the *in vivo* anticancer activity in cancer induced female Swiss albino mice. Derived, biochemical and haematological parameters were evaluated to ascertain the antiproliferative activity of the test drug.

Materials and Methods

Acute Toxicity Study

Animals

Wistar Albino rats of either sex, having 150-200g were acquired from the animal house. They were maintained by feeding with standard pellet diet and were given water *ad libitum.* The animals were maintained in polycarbonate cages, within a properly ventilated animal house in a 12 h light-12 h dark cycle. The animals were kept overnight fasting for experimental purpose. But water was given *ad libitum.* The whole experiments were done according to the guidelines of Animals (CPCSEA) New Delhi, India. The approval for the entire study was issued by the Institutional Animal Ethics Committee. (IAEC) (IAEC/KMCP/210/2015-2016)

Acute toxicity class method [19]

As per the Organization of Economics Co-operation and Development (OECD) the acute toxicity study was performed. OECD 423 is a stepwise procedure in which three animals of single sex were used in each step. On an overage, 2-4 steps may be necessary for the proper judgement of test substance and it depends on the mortality and morbidity. In this method, different defined doses (5, 50, 300 and 2000 mg/kg body weight) were used and from the results, the substance can be ranked and classified according to the "Globally Harmonized System'' (GHS).

For the study, three healthy Wistar Albino rats having body weight of 150-200 g were chosen. They were subjected to overnight fasting but allowed free access to water. Then, the animals were subjected to treatment with test drug having dose of 50 mg/kg body weight, orally. For accessing the mortality and autonomic or behavioural responses, they were monitored for 24 h on an hourly basis. This was done regularly for 14 days to access the mortality or toxic symptoms. No death was observed. So the result was confirmed by repeating the same dose.

Antiproliferative activity

Animals

For this study, female Swiss Albino mice (20-25 g) were used. They were kept in micro nylon boxes at a temperature of 25±2°C and 12 h dark/light cycle. The animals were supplied with standard laboratory diet and water was given *ad libitum*. The approval for the study was obtained from Institutional Animal Ethics Committee (IAEC/KMCP/210/2015-2016). The segregation was based on the gender and a quarantine of 15 days was done before the experiment.

Cancer induction by DAL cells [20]

Dalton's Lymphoma ascites (DAL) cells was used for the evaluation of antiproliferative activity. The DAL cells were obtained from Amala Cancer Research Centre, Trissur, Kerala, India. Intraperitoneally, the cells were maintained *in vivo* in the experimental Swiss Albino mice. Thus the tumor cells were transformed. Then the transformed cells were isolated by aspiration from peritoneal cavity using saline. The cell count was done and further diluted to $1x10^6$cells/ml. Thus solution was given by i.p. allowed it to grow for 7 days before the treatments.

Experimental Design [21, 22]

30 Swiss Albino mice were divided into 5 groups each containing 6 animals:

G1:Normal control
G2:Tumor Control
} Receives normal diet and water

G3:Positive Control (5-Fluorouracil (5-FU) 20 mg/kg body weight i.p)

G4:Low dose treatment control (5 mg/kg, n=6)

G5:High dose treatment control (10mg/kg, n=6)

The treatment was started after 24 h of the last dose. It was given once daily for 14 days. After 24 h of the 14th day, all animals were sacrificed and the blood was withdrawn by retro orbital plexus method and various parameters were evaluated. The derived parameters include body weight, life span (1%) and cancer cell count. The body weight was assessed by weighing the animals on initial and 14 th, 15th day of the study. And then the average increase in body weight was determined. Percentage increase in life span [23] of treated group animals were calculated by the formula:

$$\% \text{ ILS} = \frac{\text{Lifespan of treated group}}{\text{Lifespan of control group}} - 1 \times 100$$

Cancer cell count was assessed by taking 0.1 ml of fluid from the peritoneal cavity of each mouse and then diluting with 0.8 ml of ice cold normal saline

or sterile phosphate buffer solution and 0.1 ml of tryphan blue (0.1 mg/ml). The total numbers of the living cells were counted using heamocytometer. Mary *et al*, (**1994**)

$$\text{Cell Count} = \frac{\text{Number of cells} \times \text{Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

The hematological parameters include WBC, RBC, Hb, Platelet count and PCV [24]. The serum enzyme and lipid profile [25] was evaluated by evaluating total cholesterol, triglycerides, AST, ALT [26] and ALP [27].

COBAS MIRA PLUS–S Auto analyzer from Roche Switzerland was used for all biochemical investigations. COBAS MICROS OT 18 from Roche was used for hematological tests. Biochemical investigations in blood sample were done by Hi-Tech instruments MAX MAT.

Results and Discussion

Acute toxicity study of the test compound was done as per OECD guidelines 423. After oral administration of 50 mg/kg body weight of test drug, the mortality and changes in autonomic or behavioural response were accessed by observing the rats every hour for 24 h. This was continued for 14 days. No death was observed. Thus the Maximum tolerated dose (MTD) was found to be up to 50 mg/kg body weight, as per OECD guidelines 423. From the 1/10th and 1/5th of MTD was selected and thus the effective doses were found to be 5 and 10 mg/kg.

Table. 1: Effect of test drug on Derived Parameters

Treatment	% ILS Life span	Increase in Body weight grams	Cancer cell count mlx10^6 Cells/ml	PCV %
G 1	>30 days	1.88±0.47	-	14.30±2.41
G 2	47%	6.26±1.80^{a**}	2.25±0.52^{a**}	31.53±4.48 ^{a**}
G 3	89%	2.52±0.36 ^{b**}	1.30±0.38 ^{b**}	19.76±2.57 ^{b**}
G 4	69%	5.29±1.86 ^{b*}	1.70±0.44 ^{b*}	25.88±3.16 ^{b*}
G 5	77%	6.13±0.69 ^{b**}	1.24±0.21 ^{b**}	21.73±2.18 ^{b**}

*G1=Normal control; G2=Tumor control; G3=Positive control; G4=Low dose treatment control; G5=High dose treatment control. All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G_1) at $p < 0.001$ *b – Values are significantly different from cancer control (G_2) at $p < 0.01$ **b – Values are significantly different from cancer control (G_2) at $p < 0.001$*

The average life span of animals was calculated as 47% in DAL tumor control group. In 5 and 10 mg/kg of test drug groups the increase in lifespan was found to be 69% and 77% respectively. These obtained values were found to be significant (P<0.001) when compared to cancer control group. In case of 5-FU treated group, the average life span was found to be 89%. Thus it is a potent antiproliferative agent. The test drug was also found to be antiproliferative, by the significant (P < 0.01, P < 0.001) reduction of increase in body weight in animal with test drug dose of 5 and 10mg/kg body weight. Also a significant (P < 0.001) reduction of packed cell volume and viable tumor cell count were also found at the same dose. The results are given in table 1.

RBC, Hb level, Platelet count and packed cell volume were found to be reduced significantly (P < 0.001) in cancer control group and became normal after treatment with test drug of dose 5 and 10 mg/kg body weight. In contrast, the WBC count increased significantly (P < 0.001) in DAL control group and became normal by treatment with test drug at dose 5 and 10mg/kg body weight. 5-FU at the dose of 20 mg/kg body weight which is used as the standard produced good results in all the above mentioned hematological parameters. The results are given in table 2.

Biochemical Parameters such as Total cholesterol,

triglycerides, Aspartate Amino Transferase, Alanine Amino Transferase and Alkaline Phosphatase were found to be significantly (P<0.001) increased in DAL control group. Test drug at doses 5 and 10 mg/kg body weight reversed these changes. Standard 5-FU treatment also produced similar results. The results are given in table 3.

Table. 2: Effect of test drug on Hematological parameters

Treatment	Total WBC cells/mlx10³	RBC Count Mill/cumm	Hb gm/dl	Platelets Lakhs/cu.mm
G 1	9.73 ± 0.85	3.15 ±1.15	12.45 ± 2.36	3.35 ±0.73
G 2	13.87 ±1.72 a**	1.65 ±0.95a**	7.65 ± 1.63a**	1.86±0.57 a**
G 3	10.13 ±1.43 b**	2.27 ±1.30 b**	11.6 ±1.52 b**	2.46 ±0.71 b**
G 4	12.60 ± 1.68 b*	2.18 ± 0.68 b*	9.45 ±1.64 b*	1.83 ±0.38 b*
G 5	11.30 ±2.05 b**	2.74 ± 0.40 b**	10.80 ±1.47 b**	2.45 ±0.49 b**

*G1=Normal control; G2=Tumor control; G3=Positive control; G4=Low dose treatment control; G5=High dose treatment control. All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G_1) at p < 0.001; *b – Values are significantly different from cancer control (G_2) at p < 0.01; **b – Values are significantly different from cancer control (G_2) at p < 0.001*

Table. 3: Effect of test drug on biochemical parameters

Treatment	Cholesterol (mg/dl)	TG (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G 1	101.40±2.5	126.67±4.8	39.50±1.7	39.60±1.5	129.56±2.8
G 2	143.51±3.2 a**	209.46±5.1a**	89.6±2.3a**	62.68±2.6a**	241.65±3.7a**
G 3	111.82±3.7 b**	158.23±3.6 b**	57.70±1.1 b**	42.69±1.3b**	166.74±2.7b**
G 4	128.18±2.7 b*	189.94±2.1 b*	79.45±2.6b*	57.18±2.7 b*	203.05±3.6 b*
G 5	122.30±3.2 b**	171.35±2.7 b**	72.48±1.4 b**	44.22±1.5b**	191.53±2.4 b**

*G1=Normal control; G2=Tumor control; G3=Positive control; G4=Low dose treatment control; G5=High dose treatment control. All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G_1) at p < 0.001; *b – Values are significantly different from cancer control (G_2) at p < 0.01; **b – Values are significantly different from cancer control (G_2) at p < 0.001*

The tumor cells can utilize the ascitic fluid and thus the ascitic fluid will increase to meet the nutritional requirement of tumor cells [28]. The tumor bearing group showed a rapid increase in ascitic fluid volume. The test groups treated with 5 and 10 mg/kg body weight inhibited the tumor volume and viable tumor cell count. Also, the test drug treated group showed significant increase in lifespan which also shows value of anticancer drugs [29]. Myelosuppression and anemia occurs usually on association with cancer chemotherapy [30]. Decreased RBC or Hb level is the main cause for anemia. But this can occur due to iron deficiency or hemolytic or myelopathic conditions [31]. In test treated group, Hb level, RBC and WBC count were brought back to normal levels. This indicates that the test drug-have protective action on haematopoietic system.

A tumor in human body or animal can affect the liver functions. So elevated level of total cholesterol, triglyceride, AST, ALT and ALP in serum indicates the liver damage. In this study, the test drug treated group showed a significant reversal of these changes and these parameters became normal. This also indicates the antiproliferative nature

of the test compound [N-[3-chloro-2-oxo-4-(2-hydroxyphenyl)-4-oxoazetidin-1yl]-2-(2-methyl-1H-benzimidazol-1-yl)acetamide]. The tumor induced animal group exhibited the toxic effects of tumor. Evaluation of biochemical parameters revealed that these toxic effects became normal in the serum of the test drug treated group. This clearly shows the antiproliferative effect of the test compound [N-[3-chloro-2-oxo-4-(2-hydroxyphenyl)-4-oxoazetidin-1yl]-2-(2-methyl-1H-benzimidazol-1-yl)acetamide].

Conclusion

In conclusion, the test compound [N-[3-chloro-2-oxo-4-(2-hydroxyphenyl)-4-oxoazetidin-1yl]-2-(2-methyl-1H-benzimidazol-1-yl)acetamide] exhibited potent antiproliferative activity against Dalton's Lymphoma Ascitic in female Swiss albino mice. Derived, biochemical and haematological parameters were evaluated. All the results strongly support the antiproliferative activity of the test compound.

Acknowledgement

The authors are thankful to everyone who assisted in this work

Conflict of interest:

None

References

1. Walia, R., Hedahitulla, M., Naaz, S.F., Iqbal, K., Lamba, H.S., 2011. Benzimidazole derivatives- An Overview. Int. j. pharm. chem. biol. sci. 1 (3), 565-74.
2. Soderlind, K.J., Gorodetsky, B.,Singh, A.K., Bachur, N.R., Miller,G.G., Lown, J.W., 1999. Bis-benzimidazole anticancer agents: Targeting human tumour helicases. Anticancer Drug Des. 14, 19–36.
3. Kapuriya, K., Ganure, A., Davda S., Kitawala, M., Topiya, H., 2013. Benzimidazole: A promising Lead for Anticancer Drug Design. UJP. 02(03), 57-62.
4. Kumar, K., Awasthi, D. Lee, S.Y., Cummings, J.E., Knudson, S.E., Slayden, R.A., Ojima, I., 2013. Benzimidazole- based antibacterial agents against *Francisella tularensis*. Bioorg. Med. Chem. 21, 3318–3326.
5. Mentese, E., Bektas, H., Ulker, S., Bekircan, O., Kahveci, B., 2014. Microwave-assisted synthesis of new benzimidazole derivatives with lipase inhibition activity. J. Enzyme Inhib. Med. Chem., 29, 64–68.
6. Velík, J., Baliharová, V., Fink-Gremmels, J., Bull, S., Lamka, J., Skálová, L., 2004. Benzimidazole drugs and modulation of biotransformation enzymes. Res. Vet. Sci., 76, 95–108.
7. Janupally, R., Jeankumar, V.U., Bobesh, K.A., Soni, V., Devi, P.B., 2014. Structure-guided design and development of novel benzimidazole class of compounds targeting DNA gyrase B enzyme of *Staphylococcus aureus*. Bioorg. Med. Chem., 22, 5970–5987.
8. Tonelli, M., Paglietti, G., Boido, V., Sparatore, F., La Colla, P.,Loddo, R., 2008. Antiviral activity of benzimidazole derivatives. I. Antiviral activity of 1-substituted-2-[(benzotriazol-1/2-yl) methyl] benzimidazoles. Chem. Biodivers., 5, 2386–2401.
9. Vitale, G., Corona, P., Loriga, M., Carta, A., Paglietti, G., Loddo, R, 2010. Styryl benzimidazoles. Synthesis and biological activity. Med Chem., 6(3), 70–78.
10. Tonelli, M., Simone, M., Tasso, B., Novelli, F., Paglietti, 2010. Antiviral activity of benzimidazole derivatives. II. Antiviral activity of 2-phenylbenzimidazole derivatives. Bioorg. Med. Chem., 18, 2937–2953.
11. Vitale, G., Corona, P., Loriga, M., Carta, A., Farci, P., Marongiu, M.E. 2012. 5-Acetyl-2-arylbenzimidazoles as antiviral agents. Eur. J. Med. Chem., 53(4), 83–97.
12. Tonelli, M., Novelli, F., Tasso, B., Sparatore, F., Sanna, G., Giliberti, G., 2014. Antiviral activity of benzimidazole derivatives. III. Novelanti-CVB-5, anti-RSV and anti-Sb-1 agents. Bioorg. Med. Chem., 22, 4893–4909.
13. Ke, Y., Zhi, X., Yu, X., Ding, G., Yang, C., Xu, H., 2014, 2015. Combinatorial synthesis of benzimidazole-azo-phenolderivatives as antifungal agents. Comb. Chem. High Throughput Screen, 17, 89–95. Molecules, 20 15222
14. Achar, K.C.S., Hosamani, K.M., Seetharamareddy, H.R., 2010. *In vivo* analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. Eur. J. Med. Chem., 45, 2048–2054

15. Datar, P.A., Limaye, S.A., 2015. Design and Synthesis of Mannich bases of Benzimidazole Derivatives as Analgesic Agents. Anti-Inflamm. Anti-Allergy Agents Med. Chem, 14, 35–46.
16. Shingalapur, R.V., Hosamani, K.M., Keri, R.S., Hugar, M.H., 2010. Derivatives of benzimidazole pharmacophore: Synthesis, anticonvulsant, antidiabetic and DNA cleavage studies. Eur. J. Med. Chem., 45, 1753–1759.
17. Dona S.N., Suresh, K.A., Chakraborty, K., Valluru, L., 2018. *In vitro* Antiproliferative effect of Marine Macroalgae on different Human Cancer Cell lines. Int. j. pharm. drug. anal. 6 (2), 342-51.
18. Arul. K., Anton Smith. A., 2016. Evaluation of Anti Neoplastic Activity of a New 1, 2, 4 - Triazole Derivative Against Dalton's Lymphoma Ascitic in Mice. Transylvanian Review, 24(5), 478- 482.
19. Ecobichon, D.J., The basis of toxicology testing, 2nd ed. 43-60, (1997)
20. Jaslin, E.J., Padmaja, V., 2015. Anticancer activity of *Coleus vettiveroids* K.C Jacob against Dalton's ascitic lymphoma. International. Journal of trends in pharma. and life sciences., 1(5), 553-557.
21. Sathyanarayanan, L., Shinnathambi, Anilmozi, Chidhambranthan, N., 2006. Anticarcinogenic activity of *Leptadenia reticulata* against Dalton's ascitic lymphoma. Iranian Journal of Pharmacology and Therapeutics. 6, 133-136.
22. Mahender Mohan. V., Murugan., Geetha, K. M., Mahesh Kumar, K, 2018 Synthesis Of Bis - [2-(Chloroethyl) Amino] Acetamido - 4 - Substituted Phenyl Thiazole Derivatives as Possible Antioxidant and Alkylating Anticancer Agents. Int. j. pharm. drug. anal. 6(3), 352 – 360.
23. Jacqueline, M.H., Darius, J.N., Mathew, J.M., Ronald, D.B., 1998. Blood lipid profile in children with acute lymphoblastic leukemia. Cancer. 83(2), 379 – 384.
24. Ronald, A.S., 1995. Disease of white blood cells. Wildman's Clinical Interpretation of Laboratory Tests. 10th ed. 164.
25. Viroj, W., 2001. High serum alkaline phosphatase levels, a study in 181 Thai adult hospitalized patients. BMC Family Practice, 2(2), 293-309.
26. Intyre, M.C., Rosalki, S., (1991), Biochemical investigations in the management of Liver Disease. In: Oxford Text book of Clinical Hepatology, Oxford University Press, Chennai. 293-309.
27. Santhosh, K.H., Senthil, K.N., Reghu, C.H., 2007. Antitumor activity of Methanolic extract of *Hypericum hookerianum* on EAC cell line in swiss albino mice. J. Pharmacological. Sci. 103, 354- 359.
28. William, A.S., Hariharan, M., 2013. Production of Methioninase from *Serratia marcescens* isolated from soil and its anticancer activity against Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascitic carcinoma (EAC) in Swiss albino mice. Trop. J. Pharmaceut. Res. 5, 13, 699 – 704.
29. Bhaskar, V.H., Rajalakshmi, V., 2010. Antitumor activity of aqueous extract of *Biophytum sensitivum Linn*. Ann. Biol. Res. 1(3), 76-80.
30. Aravind, S., Joseph, M.M., Varghese, S., Balaram, P., Sreelekha, T.T., 2012. Antitumor and immune potentially activity of Polysacharide PS Tool isolated from the seed kernal of *Tamarindus indica*: An *in vivo* study in mice. Sci. World J. 1-14.
31. Patel, M.S., Antala, B.V., Dowerah, E., Senthilkumar, R., Lahlean, M., 2014. Antitumor activity of *Pogostemos benghalensis* Linn. on Ehrlich Ascites Carcinoma tumor bearing mice. J. Canc. Res. Therapeut. 4, 10, 1071 – 1075.