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Original Article

IN VIVO ANTICANCER EVALUATION OF (2-AMINO-3, 4, 5-TRIMETHOXYPHENYL) (6-METHOXY-1H-INDOL-3-YL) METHANONE

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ABSTRACT

Objective: The design and development of combretastatin A-4 analogues as anticancer agent has always attracted the attention of scientific community involved in anticancer research. The aim of the present work was to investigate the *in vivo* cytotoxic activity of a novel CA-4 analogues i.e., (2-amino-3, 4, 5-trimethoxyphenyl) (6-methoxy-1H-indol-3-yl) methanone.

Methods: The in vivo cytotoxic activity of test compound was examined using Human Tumor Xenograft model on MCF-7 cancer cell line in Balb/c mice.

Results: The test compound exhibited excellent cytotoxic activity against MCF-7 (0.013 µM), and colon HT-29 (0.143 µM), slightly higher than CA-4 activity. Relative Tumor Volume (RTV) value was found to increase rapidly in control group A during the period of 1 to 18 d indicating the higher growth rates of tumor. On the other hand group B and C showed slower growth rate of tumor as a result of treatment with CA-4 and test compound, respectively**.** The group B treated with CA-4 (5 mg/kg) showed significant inhibition in tumor growth during day 5 to 18 with %TGI in range of 47.24%-62.31% as compared to control group. The group C treated with test compound (5 mg/kg) caused significant inhibition in tumor growth during day 5 to 18 with %TGI in range of 55.66%-75.93% as compared to control group. The % survival value was found 100% indicating that CA-4 and test compound were nontoxic at the dose of 5 mg/kg under experimental conditions.

Conclusion: The parameters of *In vivo* anticancer evaluation i.e., relative tumour volume and % tumor growth inhibition showed better anticancer potential of test compound. It was further supported by non-toxic nature of test compound as indicated by 100% survival value determined at the dose of 5 mg/kg during *in vivo* studies.

Keywords: Combretastatin A-4, *In vivo***,** Human Tumor Xenograft model, Aroylindole derivative

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INTRODUCTION

Cancer is a group of diseases characterized by unregulated proliferation of cells in which a group of cells become abnormal with uncontrolled division, invade and destroy surrounding healthy tissue, including organs [1-5]. According to World Cancer Report 2014, cancer is a leading cause of death worldwide, accounting for 14 million new cases and 8.2 million deaths in 2012. The number of new cases is expected to rise by about 70% over the next 2 decades. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 million within the next 2 decades. Cancer scenario in India is quite alarming with nearly 25 lakh patients in the country currently suffering from the deadly disease and every year there is an increment of 8,50,000 new cancer cases being diagnosed and about 5,80,000 cancer related death occurs every year in India [3]. The main hindrance in the successful tumor chemotherapy i.e., high degree of adverse effects like bone marrow depression, alopecia, kidney damage, peripheral neuropathy, cardio toxicity, hepatotoxicity and development of resistance in tumor cells against cytotoxic agents call for an urgent need to develop some safer and effective treatment options. Recently, Combretastatin A-4 (CA-4) inhibitors have shown promise as anti-tumor agents.

CA-4 is a low molecular weight natural product isolated from the bark of the South African tree *Combretum caffrum* and binds to the colchicine site of tubulin. CA-4 has been found to be a potent cytotoxic agent, which strongly inhibits the tubulin polymerization by binding to the colchicine site (fig. 1) [6-8]. A number of CA-4 analogues such as CA-4P, AVE8062 (ombrabulin), OXi4503, ABT-751 (E7010), EPC2407 (Crolibulin), BNC-105P, T138067 etc are in different stages of clinical trials [9-11]. Microtubules has been identified as fascinating and well established molecular targets for anticancer therapy because microtubule polymerization dynamics can prominently influence crucial processes, such as mitosis and cell signaling [12-15].

Aroylindole derivative possessing three major structural elements i.e., ring A (trimethoxyphenyl ring), ring B (substituted phenyl ring with indole) and the keto bridgehead linker are structural analogs of CA-4 exhibited potent growth inhibition in several cancer cell lines due to their excellent antitumor and antivascular properties [16-21]. A series of novel 2-amino-3, 4, 5-trimethoxyaroylindole derivatives were synthesized and evaluated for their anticancer potential using *in vitro* methods [13]. The substitution of 2-amino-3, 4, 5-trimethoxyaroylindole derivatives at R_6 with methoxy group *i.e.,* (2-amino-3, 4, 5-trimethoxyphenyl)(6-methoxy-1H-indol-3-yl) methanone 2 (fig. 1) exhibited excellent cytotoxic activity against MCF-7 (0.013 µM), and colon HT-29 (0.143 µM), slightly higher than CA-4 activity [22]. The aim of these studies was to investigate the *in vivo* cytotoxic activity of (2-amino-3, 4, 5-trimethoxyphenyl)(6-methoxy-1H-indol-3-yl) methanone 2 using Human Tumor Xenograft model on MCF-7 cancer cell line in Balb/c mice.

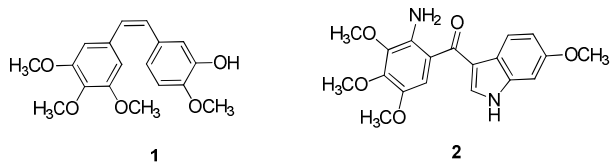


Fig. 1: Structure of Combretastatin A-4 (1) and 2-amino-3, 4, 5-trimethoxyphenyl) (6-methoxy-1H-indol-3-yl) methanone (2)

MATERIALS AND METHODS

In vivo testing is an experimental evidence *i.e.,* a candidate drug can reach its predictable target in a living system, has the desired physiological effect and has no undesirable side effects. The *in vivo* cytotoxic activity of compound was investigated using Human Tumor Xenograft model on MCF-7 cancer cell line in Balb/c mice.

Animals weighing 20-25 g were randomly divided into three groups (6 in each group) for the experiment and tumors were induced by subcutaneous injection in their dorsal region. The first group (A) was control group dosed thrice a week for four week subcutaneously with 7 µL/g of vehicle (0.9% NaCl), did not received any treatment. The second group (B) received the reference compound (CA-4) thrice a week for four week subcutaneously with the dose of 5 mg/kg body weight. The third group was treated with test compound [(2-amino-3, 4, 5-trimethoxyphenyl) (6-methoxy-1H-indol-3-yl) methanone] 2 thrice a week for four week subcutaneously with dose of 5 mg/kg body weight. Both test compound and reference compound were dissolved in vehicle.

RESULTS AND DISCUSSION

The *in vivo* cytotoxic activity of compound was examined using Human Tumor Xenograft model on MCF-7 cancer cell line in Balb/c mice. The weight of Balb/c mice in experimental duration was measured at various time intervals as mentioned in table 1 and fig. 2a. Tumor Volume of control group A (table 2 and fig. 2b), group B (table 3 and fig. 2c) and group C (table 4 and fig 2d) was measured.

Table 1: Average animal body weight (grams) data of BALB/c mice

Weeks	Days	A	B	C
0.0	1	23.6	23.5	22.3
0.7	5	23.7	23.2	22.4
1.3	9	24.0	23.0	22.6
1.7	12	24.2	23.0	22.8
2.1	15	24.4	23.2	22.9
2.6	18	24.6	23.4	23.2

Number of BALB/c mice in each group n = 6

Table 2: Tumor volume in group A of BALB/c mice

Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	mean±SD
0.00	1	0.06	0.06	0.06	0.07	0.05	0.04	0.06±0.01
0.71	5	0.46	0.55	0.49	0.55	0.26	0.23	0.42±0.14
1.29	9	1.32	1.44	1.48	1.46	0.98	1.00	1.28±0.23
1.71	12	1.91	2.22	2.25	2.16	1.94	1.80	2.05±0.19
2.14	15	4.49	4.73	4.77	4.63	4.58	4.20	4.57±0.21
2.57	18	8.00	7.48	8.40	8.38	8.30	7.37	7.99±0.46

Number of BALB/c mice in each group n=6, SD: Standard deviation

Table 3: Tumor volume in group B of BALB/c mice

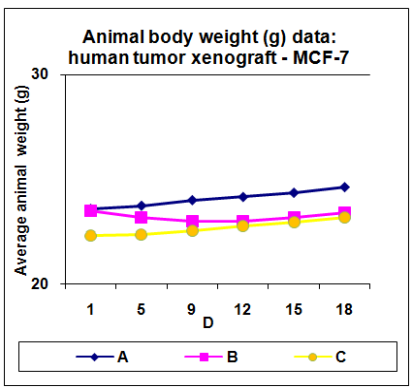
Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	mean±SD
0.00	1	0.06	0.06	0.06	0.06	0.09	0.06	0.07±0.01
0.71	5	0.24	0.29	0.29	0.24	0.18	0.16	0.23±0.05
1.29	9	0.54	0.55	0.55	0.53	0.56	0.42	0.53±0.05
1.71	12	0.90	0.90	0.91	0.93	1.01	0.90	0.93±0.04
2.14	15	2.28	2.55	2.53	2.24	2.29	1.69	2.26±0.31
2.57	18	4.36	4.62	4.36	3.95	4.31	3.86	4.24±0.29

Number of BALB/c mice in each group n=6, SD: Standard deviation

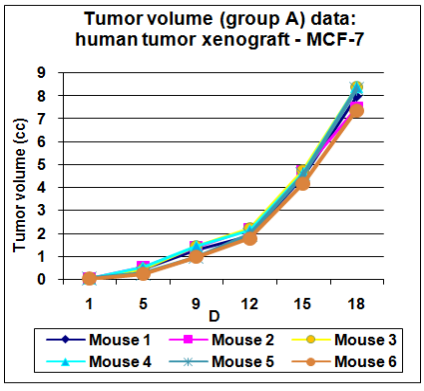
Table 4: Tumor volume group C of BALB/c mice

Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	mean±SD
0.00	1	0.06	0.07	0.07	0.06	0.06	0.06	0.06±0.01
0.71	5	0.10	0.20	0.21	0.24	0.16	0.22	0.19±0.05
1.29	9	0.32	0.32	0.33	0.30	0.38	0.41	0.34±0.04
1.71	12	0.60	0.52	0.52	0.56	0.56	0.66	0.57±0.05
2.14	15	1.18	1.41	1.63	1.79	1.79	1.95	1.63±0.28
2.57	18	3.34	3.85	4.04	4.47	4.16	4.39	4.04±0.41

Number of BALB/c mice in each group n=6, SD: Standard deviation



(a)



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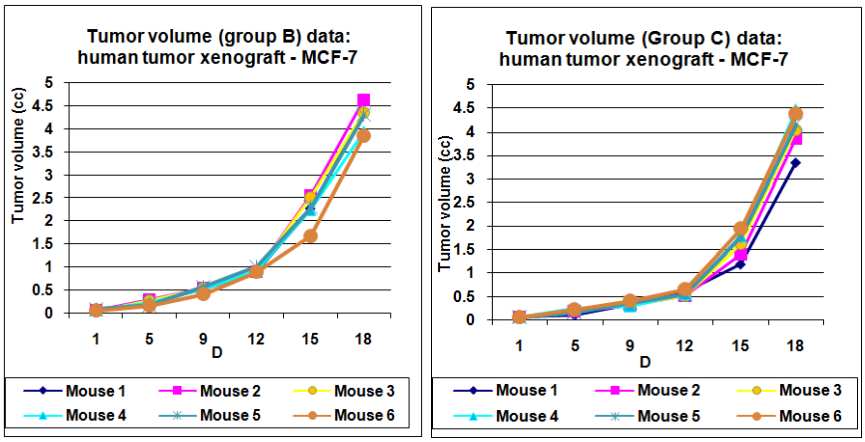

(c) **(d)**

Fig. 2: The *in vivo* cytotoxic activity of compound was investigated using Human Tumor Xenograft model on MCF-7 cancer cell line in Balb/c mice. (a) Plot of average animal body weight (g) data versus days of treatment of BALB/c mice. (b) Plot of tumor volume in group A versus day of treatment of BALB/c mice. (c) Plot of tumor volume in group B versus day of treatment of BALB/c mice. (d) Plot of tumor volume in group C versus day of treatment of BALB/c mice

The relative tumor volume (RTV) for group A, B and C for 18 d was measured using formula RTV = Tumor Volume on day of measurement/Tumor Volume on day 1. RTV value was found to increase rapidly in control group A during the period of 1 to 18 d indicating the higher growth rates of tumor (table 5 and fig. 3). On the other hand, group B and C showed slower growth rate of tumor as a result of treatment with CA-4 and (2-amino-3, 4, 5-trimethoxyphenyl)(6-methoxy-1H-indol-3-yl)methanone 2, respectively. The ratio of treated/control (T/C), an important parameter employed to quantify the treatment effects in drug screening tumor xenograft experiments was calculated for group A and group B (table 6 and fig. 4).

Table 5: Relative tumor volume data

Weeks	Days	Group		
		A	B	C
0.00	1	1.00	1.00	1.00
0.71	5	7.19	3.79	2.92
1.29	9	22.19	8.36	5.34
1.71	12	36.06	14.68	8.92
2.14	15	80.91	36.15	25.30
2.57	18	141.82	67.68	62.88

Tumor growth inhibition (% TGI) is one of parameter used to express the drug efficacy, can be calculated using the equation 100-(T/C × 100). The group B treated with CA-4 (5 mg/kg) showed significant inhibition in tumor growth during day 5 to 18 with %TGI in range of 47.24%-62.31% as compared to control group (table 7 and fig. 5). The group C treated with (2-amino-3, 4, 5-trimethoxy-phenyl)(6-methoxy-1H-indol-3-yl) methanone 2 (5 mg/kg) caused significant inhibition in tumor growth during day 5 to 18 with %TGI in range of 55.66%-75.93% as compared to control group.

The % survival value was found 100% indicating that CA-4 and (2-amino-3, 4, 5-trimethoxy-phenyl)(6-methoxy-1H-indol-3-yl) methanone 2 were nontoxic at the dose of 5 mg/kg under experimental conditions (fig. 6). As a result the substitution of 2-amino-3, 4, 5-trimethoxy-aroylindole derivatives at R_6 with methoxy group *i.e.,* (2-amino-3, 4, 5-trimethoxyphenyl)(6-methoxy-1H-indol-3-yl) methanone 2 exhibited excellent *in vitro* [22] and *in vivo* cytotoxic activity.

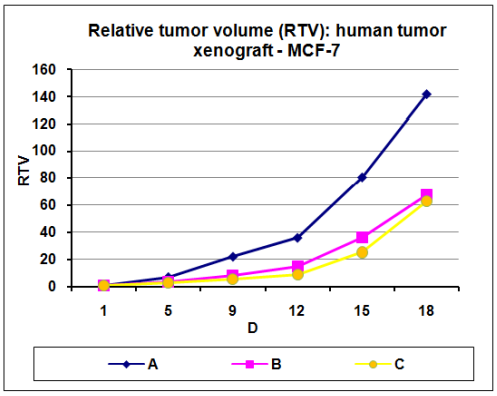

Fig. 3: Plot of RTV versus day of treatment of BALB/c mice

Table 6: T/C from RTV Data

Weeks	Days	Group	
		B	C
0.00	1	1.00	1.00
0.71	5	0.53	0.41
1.29	9	0.38	0.24
1.71	12	0.41	0.25
2.14	15	0.45	0.31
2.57	18	0.48	0.44

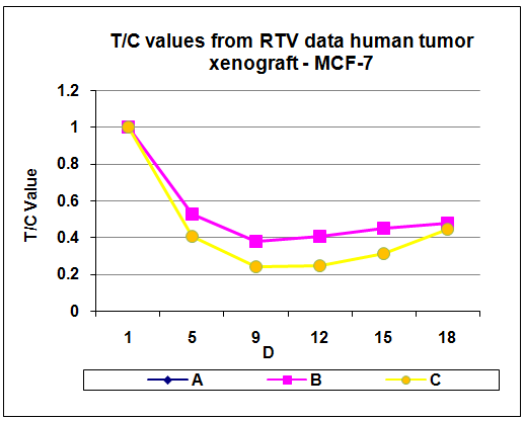

Fig. 4: Plot of T/C values versus days of treatment

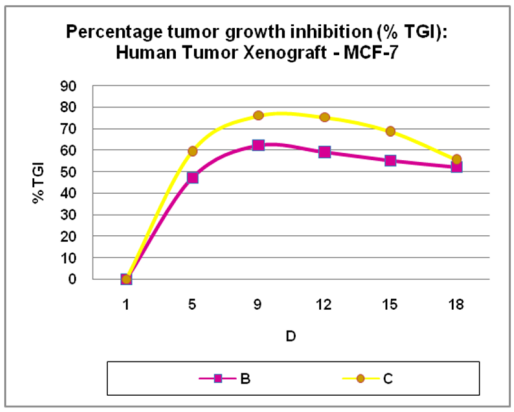

Fig. 5: Plot of percentage tumor growth inhibition (% TGI) versus days of treatment

Table 7: Percentage tumor growth inhibition (% TGI)

Weeks	Days	Group	
		B	C
0.00	1	0	0
0.71	5	47.24	59.43
1.29	9	62.31	75.93
1.71	12	59.28	75.25
2.14	15	55.32	68.73
2.57	18	52.28	55.66

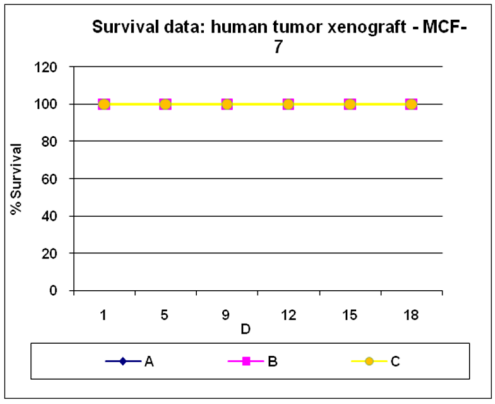

Fig. 6: Plot of % survival versus days of treatment

CONCLUSION

The *in vivo* cytotoxic activity of CA-4 analogues i.e., (2-amino-3, 4, 5-tri-methoxyphenyl)(6-methoxy-1H-indol-3-yl) methanone 2 plan text was investigated using Human Tumor Xenograft model on MCF-7 cancer cell line in Balb/c mice. The parameters of *In vivo* anticancer evaluation i.e., relative tumour volume and % tumor growth inhibition showed better anticancer potential of test compound. It was further supported by non-toxic nature of test compound as indicated by 100 % survival value determined at the dose of 5 mg/kg during *in vivo* studies.

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CONFLICT OF INTERESTS

The authors confirm that this article content has no conflicts of interest

REFERENCES

1. Lawrence B, John L, Keith P, Goodman and Gilman"s. The Pharmacological Basis of Therapeutics. 11th ed. Mc. Graw-Hill Medical Publishing Division, New York, USA; 2006.
2. DeVita VT, Lawrence TS, Steven AR. Cancer-principles and practice of oncology. 8th ed. Wolters Kluwer/lippincott Williams and Wilkins, Philadelphia, USA; 2008.
3. World Cancer Report. WHO; 2014.
4. Mohammadian M, Mahdavifar N, Salehiniya H. Trend of incidence of gastric cancer in sistan and baluchestan province, iran. Asian J Pharm Clin Res, 2016;9:230-23.
5. Santhosh MP, Harshini AK. Knowledge and awareness about oral cancer among undergraduate dental students. Asian J Pharm Clin Res 2016;9:165-7.
6. el-Zayat AA, Degen D, Drabek S, Clark GM, Pettit GR, Von Hoff DD. *In vitro* evaluation of the antineoplastic activity of combretastatin A-4, a natural product from combretum caffrum (arid shrub). Anticancer Drugs 1993;4:19-25.
7. Rajak H, Dewangan PK, Patel V, Jain DK, Singh A, Veerasamy R, *et al*. Design of combretastatin A-4 analogs as tubulin targeted vascular disrupting agent with special emphasis on their cis-restricted isomers. Curr Pharm Des 2013;19:1923-55.
8. Tron GC, Pirali T, Sorba G, Pagliai F, Busacca S, Genazzani AA. Medicinal chemistry of combretastatin A4: present and future directions. J Med Chem 2006;49:3033-44.
9. Patel VK, Rajak H. Significance of amino group substitution at combretastatin a-4 and phenstatin analogs. Lett Drug Des Discovery 2016;13:943-51.
10. Patel VK, Singh Chouhan K, Singh A, Kumar Jain D, Veerasamy R, Singh Pawar R, *et al*. Development of structure activity correlation model on azetidin-2-ones as tubulin polymerization inhibitors. Lett Drug Des Discovery 2015;12:351-65.

11. Lu Y, Chen J, Xiao M, Li W, Miller DD. An overview of tubulin inhibitors that interact with the colchicine binding site. Pharm Res 2012;29:2943-71.
12. Attard G, Greystoke A, Kaye S, Bono JD. Update on tubulin-binding agents. Pathol Biol 2005;54:72-84.
13. Prinz H. Recent advances in the field of tubulin polymerization inhibitors. Expert Rev Anticancer Ther 2000;2:695-708.
14. Kingston DGI. Tubulin-interactive natural products as anticancer agents. J Nat Prod 2009;72:507-15.
15. Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. Curr Med Chem Anticancer Agents 2002;2:1-17.
16. Liou JP, Chang YL, Kuo FM, Chang CW, Tseng HY, Wang CC, *et al*. Concise synthesis and structure-activity relationships of combretastatin A-4 analogues, 1-aroylindoles and 3-aroylindoles, as novel classes of potent antitubulin agents. J Med Chem 2004;47:4247-57.
17. Liou JP, Mahindroo N, Chang CW, Guo FM, Lee SW, Tan UK, *et al*. Structure-activity relationship studies of 3-aroylindoles as potent antimitotic agents. Chem Med Chem 2006;1:1106-18.
18. Wu YS, Coumar MS, Chang JY, Sun HY, Kuo FM, Kuo CC, Chen YJ, *et al*. Synthesis and evaluation of 3-aroylindoles as anticancer agents: metabolite approach. J Med Chem 2009;52:4941-5.
19. La Regina G, Sarkar T, Bai R, Edler MC, Saletti R, Coluccia A, *et al*. New arylthioindoles and related bioisosteres at the sulfur bridging group. 4. Synthesis, tubulin polymerization, cell growth inhibition, and molecular modeling studies. J Med Chem 2009;52:7512-27.
20. La Regina G, Bai R, Rensen WM, Di Cesare E, Coluccia A, Piscitelli F, *et al*. Toward highly potent cancer agents by modulating the C-2 group of the arylthioindole class of tubulin polymerization inhibitors. J Med Chem 2013;56:123-49.
21. Zhao DG, Chen J, Du YR, Ma YY, Chen YX, Gao K, *et al*. Synthesis and structure-activity relationships of N-methyl-5,6,7-trimethoxylindoles as novel antimitotic and vascular disrupting agents. J Med Chem 2013;56:1467-77.
22. Patel VK, Rajak H. Synthesis, biological evaluation and molecular docking studies of 2-amino-3, 4, 5-trimethoxy-aroylindole derivatives as novel anticancer agents. Bioorg Med Chem Lett 2016;26:2115-8.

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