N-Substituted-2-butyl-5-chloro-3*H*-imidazole-4-carbaldehyde Derivatives as Anti-tumor Agents Against Ehrlich Ascites tumor Cells *In Vivo*

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Abstract: A new series of *N*-substituted 2-butyl-5-chloro-3*H*-imidazole-4-carbaldehyde derivatives were synthesized by using the different bioactive heteroaralkyl halides with 2-butyl-4-chloro-1*H*-imidazole-5-carbaldehyde in presence of powdered potassium carbonate in DMF medium. These compounds were screened for their antitumor activity. Our results show that treatment of imidazole derivatives inhibit proliferation EAT cells, decreases the ascites volume and increases the survivability of the animals *in vivo*. These compounds also inhibited the cellular proliferation of HUVEC cells *in vitro* by MTT assay. Further, these compounds could induce apoptosis, which is evident by the nuclear condensation of imidazole derivatives treated EAT cells *in vivo* by the cytological analysis. We have identified that pyrrolidine substituted imidazole derivative as potent anti-tumor compound. These inhibitors could represent as promising candidates for anticancer therapies, where the formation of peritoneal malignant ascites is a major cause of morbidity and mortality.

Key Words: Antitumor activity, HUVEC, imidazole derivative, EAT cells.

INTRODUCTION

Imidazole derivatives in general are known to possess a diverse range of pharmacological activity. Imidazole nucleus is an important pharmacophore in drug discovery [1]. Since it is commonly encountered in drugs that display diversity of pharmacological activities such as anti-inflammatory [2], histamine-H3 antagonist [3], antioxidant [4], gastroprotective [5], antitumoral and antiparasitic. It is well-known that cysteinyl moiety share common properties with imidazole functionalities [6] as was recently reported farnesyl protein transferase inhibitors were developed taking in advantage this characteristic [7]. An imidazole ring is part of some antitumor drugs such as dacarbazine [(5-(3,3-dimethyl-1-triazenyl) imidazole-4-carboxylic acid amide] and imuran [6-(1-methyl-4-nitroimidazolyl-thio)- purine] [8] and the tumor growth inhibiting l-carboxymethyl-2-iminoimidazolidine (cyclocreatine) [9]. Temozolomide is an alkylating cytostatic drug that finds increasing application in the treatment of melanoma, anaplastic astrocytoma and glioblastoma multiforme [10]. DuP 753 (losartan) is a nonpeptide angiotensin II antagonist, which is an orally active antihypertensive agent [11]. 2-Butyl-4-chloro-1*H*-imidazole-5-carboxaldehyde, is a major active metabolite and one of the key intermediate of DuP 753 (losartan). Previously, we have reported the microwave-assisted synthesis and X-ray crystal structure of 2-*N*-butyl-4-chloro-5-imidazolaldehyde [12], synthesis of a series of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-substituted δ^2-isoxazoline derivatives their antifungal activity [13] and group II phospholipase A_2 enzyme inhibitory activity [14]. In the preliminary work, herewith we discuss the synthesis of *N*-substituted 2-butyl-5-chloro-3*H*-imidazole-4-carbaldehyde derivatives and their antitumor activity, where imidazole derivatives could decrease EAT cell number, ascites volume and could increase survivability of mice. These imidazole derivatives could reduce human umbilical vein endothelial cells (HUVEC's) cell number.

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MATERIALS

Swiss albino mice were obtained from Central Animal Facility, Department of Zoology, University of Mysore, Mysore, India. MTT assay kit was procured from Merck. HUVECs, endothelial growth medium (EGM-2) and Fungizone were obtained from Cambrex Bioscience, Walkersville, USA. All other reagents were of highest analytical grade.

METHODS

Chemistry

IR (KBr) spectra were recorded on a Jasco FT/IR-4100 Fourier transform infrared spectrometer, ^{1}H NMR were recorded on Shimadzu AMX 400, spectrometer by using $CDCl_3$ as solvent and TMS as an internal standard (Chemical shift in ppm). Elemental analyses were obtained on a vario-EL instrument. Thin layer chromatography (TLC) was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck). All extracted solvents were dried over anhydrous Na_2SO_4 and evaporated with a BUCHI rotary evaporator. Reagents were obtained commercially and used as received the expanded version of DMF i.e. present as, *N*,*N*-dimethyl formamide (DMF).

General Procedure for the Synthesis of *N*-substituted 2-butyl-5-chloro-3*H*-imidazole-4-carbaldehyde Derivatives

Equimolar mixtures of 2-butyl-4-chloro-1 *H* -imidazole-5-carbaldehyde and aryl/alkyl halides, were dissolved in DMF and 3 equivalents of powdered potassium carbonate

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and catalytic amount of ter-butylammonium bromide were added. The reaction mass was stirred at room temperature overnight till the reaction completed, which was monitored by tlc. After completion of the reaction, the reaction mass was poured into 10 volumes of water, the compounds were extracted in ethyl acetate (6 volumes x 3), the combined organic layer was washed with water, distilled completely. Using appropriate mixture of solvent like n-hexane, ethylacetate as eluent in silica gel column, pure product was separated.

Synthesis of 2-Butyl-5-chloro-3-[2-(2-methyl-4-oxo-4*H*-pyrido[1,2-a]pyrimidin-3-yl) ethyl]-3*H*-imidazole-4-carbaldehyde 2a

2a was synthesized by using **1** (100 mg, 0.536 mmol), 3-(2-chloroethyl)-2-methylpyrido[1,2-a]pyrimidin-4-one (119 mg, 0.536 mmol) and powdered potassium carbonate (111 mg, 0.804 mmol) in 4 mL of DMF. The product obtained was oily.

IR ν_{max} (KBr) cm^{-1}: 3030, 2926, 2890, 1680, 1712, 1595.

^{1}H NMR $CDCl_3$: δ0.93 (t, 3H, CH_3), 1.37 (m, 2H, CH_2), 1.68 (m, 2H, CH_2), 2.10 (s, 3H, Py-CH_3-), 2.61 (t, 2H, CH_2), 3.22 (t, 2H, CH_2), 4.08 (t, 2H, CH_2), 5.48 (d, 1H, CH), 5.99 (t, 1H, CH), 6.82 (t, 1H, CH), 7.45 (d, 1H, CH), 9.89 (s, 1H, CHO).

Elemental analysis for $C_{19}H_{21}ClN_4O_2$: C, 61.21; H, 5.68; Cl, 9.51; N, 15.03 Found C, 61.17; H, 5.61; Cl, 9.54; N, 15.0.

Synthesis of 2-butyl-4-chloro-1-(2-(6,7,8,9-tetrahydro-2-methyl-4-oxo-4*H*-pyrido[1,2-a]pyrimidin-3-yl)ethyl)-1*H*-imidazole-5-carbaldehyde 2b

2b was synthesized by using **1** (100 mg, 0.536 mmol) and 3-(2-chloroethyl)-6,7,8,9-tetrahydro-2-methylpyrido[1,2-a]pyrimidin-4-one (121.5 mg, 0.536 mmol) and powdered potassium carbonate (176 mg, 1.608 mmol) in 4 mL of DMF. The product obtained was oily.

IR ν_{max} (KBr)cm^{-1}: 3016, 2895, 1709, 1690, 1580.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.94 (t, 3H, -CH_3), 1.22-1.36 (m, 4H, -CH_2-), 1.57-1.65 (quintet, 2H, -CH_2-), 1.73-1.8 (quintet, 2H, -CH_2-), 1.82-1.9 (quintet, 2H, -CH_2-), 2.07 (s, 3H, Py-CH_3-), 2.55 (t, 2H, Imid-CH_2-), 2.7 (t, 2H, Py-CH_2-), 3.82 (t, 2H, -N-CH_2-), 4.20 (t, 2H, Imid-N-CH_2-), 9.66 (s, 1H, -CHO).

Elemental analysis for $C_{19}H_{25}ClN_4O_2$: C, 60.55; H, 6.69; N, 14.87 Found C, 60.55; H, 6.54; N, 14.87.

Synthesis of 2-Butyl-5-chloro-3-(2-piperidin-1-yl-ethyl)-3H-imidazole-4-carbaldehyde 2c

2c was synthesized by using **1** (100 mg, 0.536 mmol), 1-(2-chloroethyl)piperidine monohydrochloride (98.7 mg, 0.536 mmol) and powdered potassium carbonate (176 mg, 1.608 mmol) in 4 mL of DMF. The product obtained was oily.

IR ν_{max} (KBr) cm^{-1}: 2915, 2890, 1709, 1690, 1605.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (t, 3H, -CH_3), 1.10-1.15 (m, 4H, -CH_2-), 1.52-1.68 (m, 6H, Pip-CH_2-), 2.89 (t, 2H, Imid-CH_2-), 3.24 (t, 4H, Pip-N-CH_2-), 3.82 (t, 2H, N-CH_2-), 4.12 (t, 2H, Imid-N-CH_2-), 9.58 (s, 1H, -CHO).

Elemental analysis for $C_{15}H_{24}ClN_3O$: C, 60.49; H, 8.12; N, 14.11 Found C, 59.16; H, 7.74; N, 14.92.

Synthesis of 2-butyl-4-chloro-1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-imidazole-5-carbaldehyde 2d

2d was synthesized by using **1** (100 mg, 0.536 mmol), 1-(2-chloroethyl) pyrrolidine (71.6 mg, 0.536 mmol) and powdered potassium carbonate (176 mg, 1.608 mmol) in 4 mL of DMF. The product obtained was oily.

IR ν_{max} (KBr) cm^{-1}: 2985, 2900, 2840, 1705, 1615.

^{1}H NMR(400 MHz, $CDCl_3$): δ 0.97 (t, 3H, -CH_3), 1.2-1.42 (m, 4H, -CH_2-), 1.61-1.71 (m, 4H, Pyrr-CH_2-), 2.40-2.65 (m, 6H, CH_2-), 3.44 (t, 2H, N-CH_2-), 4.28 (t, 2H, Imid-N-CH_2-), 9.64 (s, 1H, -CHO).

Elemental analysis for $C_{14}H_{22}ClN_3O$: C, 59.25; H, 7.81; N, 14.81 Found C, 59.16; H, 7.74; N, 14.92.

Synthesis of 2-Butyl-5-chloro-3-(2-morpholin-4-yl-ethyl)-3*H*-imidazole-4-carbaldehyde 2e

2e was synthesized by using **1** (100 mg, 0.536 mmol), 1-(2-chloroethyl) morpholino monohydrochloride (98.7 mg, 0.536 mmol) and powdered potassium carbonate (176 mg, 1.608 mmol) in 4 mL of DMF. The product obtained was oily.

IR ν_{max} (KBr) cm^{-1}: 2948, 2901, 2825, 1720 (-CHO), 1625(-C=N-).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (t, 3H, -CH_3), 1.14-1.38 (m, 4H, -CH_2-), 2.76 (t, 2H, -CH_2), 2.1-2.2 (m, 4H, Pyrr-CH_2), 3.20 (t, 2H, CH_2-), 4.03 (t, 4H, 2X-CH_2-), 4.21 (t, 2H, Imid-N-CH_2-), 9.61 (s, 1H, -CHO).

Elemental analysis for $C_{14}H_{22}ClN_3O_2$: C, 56.09; H, 7.40; N, 14.02 Found C, 55.75; H, 7.23; N, 14.24.

Synthesis of 2-Butyl-5-chloro-3-(2-piperazin-1-yl-ethyl)-3*H*-imidazole-4-carbaldehyde 2f

2f was synthesized by using **1** (100 mg, 0.536 mmol), 1-(2-Chloroethyl)piperazine (795 mg, 0.537 mmol) and powdered potassium carbonate (111 mg, 0.804 mmol) in 4 mL of DMF. The product obtained was oily.

IR ν_{max} (KBr) cm^{-1}: 3460, 2910, 2840, 1712, 1610(-C=N-).

^{1}H NMR $CDCl_3$: δ0.96 (t, 3H, CH_3), 1.39 (m, 2H, CH_2), 1.71 (m, 2H, CH_2), 2.59 (t, 2H, CH_2), 2.68 (t, 4H, 2XCH_2), 2.75 (t, 4H, 2XCH_2), 3.22 (t, 2H, CH_2), 4.11 (t, 2H, CH_2), 5.45 (s, 1H, NH), 9.86 (s, 1H, CH).

Elemental analysis for $C_{14}H_{23}ClN_3O$: C, 56.27; H, 7.76; Cl, 11.86;N, 18.75: O, 5.35 Found C, 56.15; H, 7.68; Cl, 11.75;N, 18.69: O, 5.38.

Biology

In Vivo Tumor Growth

In vivo culture of EAT cells and isolation of EAT cells from peritoneum cavity was done as reported earlier [15]. In

brief, EAT cells (5X10 ^{6} cells /mouse) were injected intraperitoneally (i.p) into 8 weeks old Swiss albino mice. The animals showed a significant increase in the body weight over the growth period and the animals succumbed to the tumor burden 14-16 days after implantation. The number of cells increased over the 10 days of growth with the formation of 6-7 ml of ascites fluid.

In Vivo Treatment of Imidazole Derivatives 2(a-f)

To determine whether imidazole derivatives **2(a-f)** inhibit tumor growth *in vivo,* imidazole derivatives **2(a-f)** (100 mg/kg body weight/i.p) was injected into the EAT bearing mice intraperitoneally using 26 gauge needle on every alternate day starting 6 days of tumor implantation and growth of the tumor was monitored by taking the body weight of the animals on every day. Control mice were injected with 0.2 ml of saline (i.p) on every alternate day. Each treatment consisted of at least 5 mice and each experiment was repeated thrice. After giving each dose (imidazole derivatives + 200 µl of 0.9% Saline), animals were sacrificed and the EAT cells along with ascites fluid were harvested. The set of animals were used to study the survivability after treatment until their death.

Trypan Blue Assay

EAT cells were treated with different concentrations of imidazole derivatives **2(a-f)** for various time periods (0-8 h). Cell viability was assessed by mixing aliquots of cell suspension with 0.4% trypan blue. Cells that picked up the dye were considered to be dead.

Acridine Orange / Ethidium Bromide Staining

Nuclear staining was performed according to the method of Srinivas *et al*. [16]. EAT cells either treated or untreated with imidazole derivatives **2(a-f)** *in vivo* were smeared on a glass slide and fixed with methanol: acetic acid (3:1) and air-dried. The cells were hydrated with PBS and stained with a mixture (1:1) of acridine orange and ethidium bromide (4 µg/ml) solutions. The cells were immediately washed with PBS and viewed under a Leitz –DIAPLAN fluorescent microscope.

Cell Culture and In Vitro Treatment of Imidazole Derivatives 2(a-f)

HUVEC cells were cultured in a 96-well plate at 80% confluency and incubated in presence and absence of imidazole derivatives (100uM) cell viability was measured by means of MTT (dimethylthiazol-diphenyl tetrazolium bromide) assay [18] and percentage of cell death was calculated as 100X(1- viability of treated endothelial cells/viability of untreated endothelial cells).

RESULTS

Chemistry

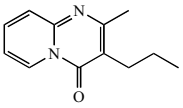
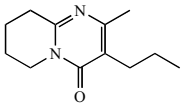
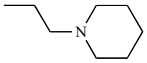
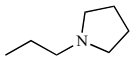
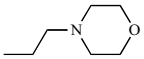
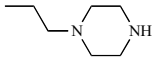
The condensation reaction of different bioactive heteroaralkyl halides with 2-butyl-4-chloro-1*H*-imidazole-5-carbaldehyde in presence of powdered potassium carbonate in DMF gave a good yield in the ratio of 80-85 % with high purity. All the synthesized compounds were characterized by IR spectra, ^{1}H NMR spectra and elemental analyses. The Infrared Spectra of all the synthesized compounds showed a band in the region of 1710-1720 cm^{-1}, which is due to carbonyl stretching frequency of –CHO (aldehyde) group and C-H stretching frequency of aldehyde group was observed at 2890 cm^{-1}. Compounds **2a** and **2b** showed a band in the region of 1685-1690 cm^{-1} which indicates the keto group present on the pyrimidine group. The band in the region 1585-1595 cm^{-1} may be due to C=N group. Aromatic C-H stretch was observed in the region of 3000-3030 cm^{-1}. ^{1}H NMR spectrum of all compounds showed a singlet for one proton in the region 9.5-9.9 ppm, which indicates the presence of –CHO group. Two triplets were observed for two protons each in the region 4-4.5 and 3.2-3.5 ppm that indicates the –CH_2-CH_2 attached to imidazole ring nitrogen atom. Compounds **2a** and **2b** showed a singlet in the region 2-2.1 ppm, which is due to methyl group present on pyrimidine ring. In compound **2e** a singlet was observed at 5.45 ppm, which is due to –NH group of piperazine ring. All other substituents are observed in the expected region. The formation of the product was further confirmed by CHNS elemental analyses **(**Table **1** and Scheme **1**).

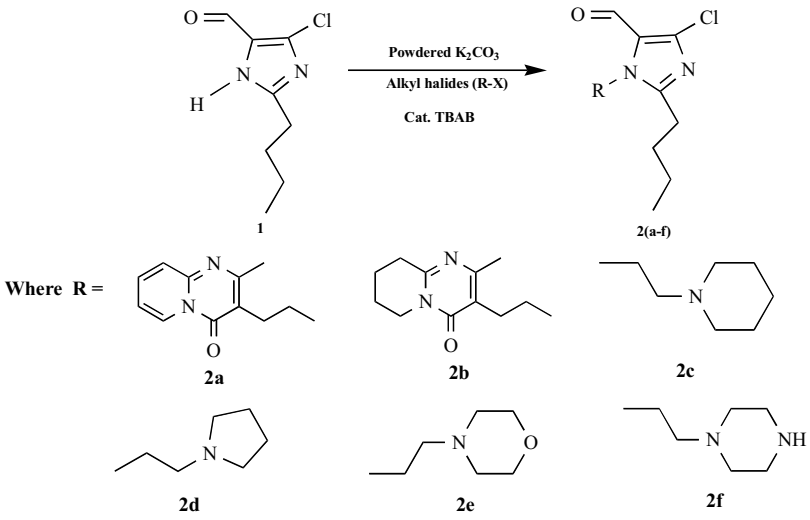
Biology

Imidazole Derivatives 2(a-f) Inhibit Proliferation EAT Cells and Increases the Survivability of the Animals In Vivo

To determine the *in vivo* effect on cell growth, mouse mammary carcinoma cell line EAT was treated with imidazole derivatives **2(a-f)** (100 mg/kg body weight/i.p), during its growth period, 3 doses on every alternative day starting from 6 days after inoculation of tumor cells. Animal weights were monitored through out the course of treatment. Imidazole derivatives were not cytotoxic to the cells as verified by MTT assay and trypan blue dye exclusion method. In control EAT bearing mice there is gradual increase in body weight, which from day six attains an exponential phase of growth. A rise in body weight of about 8-9 g over 12 days period of tumor growth was observed. However, when treated mice with the imidazole derivatives **2(a-f)**, about 25.23%, 54.19%, 20.59 %, 61.84%, 19.96%, and 51.76% decrease in the body weight was observed respectively indicating the anti-tumor effect of imidazole derivatives **2(a-f)** (Table **2**). As shown in Fig. (**2**), imidazole derivatives **2(a-f)** treatment resulted in a dose-dependent inhibition of proliferation of EAT cells. Decrease in tumor growth also correlates with decrease in ascites volume of treated mice Fig. (**1**). The survivability of EAT cell-bearing mice (control) halved on the 12th day after implantation and no animal survived beyond 14th day. Imidazole derivatives **2(a-f)** treatment increased the survivality, where animals were alive beyond the 26th day. The survivability of **2d**, **2b**, **2f**, and **2a**, imidazole derivatives treated mice were found to be 26, 23, 21, 17days respectively, whereas for **2c** and **2e** imidazole derivatives treated mice survivability was 15 days, correlating with the anti-tumor effect of these imidazole derivatives. These results represent that imidazole derivative **2d** is the most effective inhibitor of tumor growth than other **2b**, **2f**, **2a**, **2c** and **2e**. However, **2b** and **2f** also showed significant effect on tumor growth. The results are listed in Table **2**.

Table 1. Physical Data of the Compounds 2(a-f)

Compound	R	R_f Value	Eluent	Yield (%)
2a		0.83	Benzene: Ethyl acetate 9:1	78
2b		0.74	Benzene: Ethyl acetate 9:1	56
2c		0.78	Benzene: Ethyl acetate 9:1	53
2d		0.80	Benzene: Ethyl acetate 9:1	60
2e		0.65	Benzene: Ethyl acetate 9:1	48
2f		0.75	Benzene: Ethyl acetate 9:1	76



Scheme 1.

Table 2. Effect of Compounds 2a-f on Tumor Growth Inhibition, and Survivability of the Animals *In Vivo*.

	Control	2a	2b	2c	2d	2e	2f
Tumor growth inhibition in%	___	25.23%	54.19%	20.59 %	61.84%	19.96%	51.76%
Survivability of the animals in days	14	17	23	15	26	15	21

The effect of compounds **2(a-f)** on the body weight (which corresponds to EAT cell number and ascites volume) of the EAT-bearing mice was verified and percentage of inhibition after treatment was calculated taking 100% growth in the case of untreated mice. Survivability of the untreated mice was 14 days, whereas increased survivability in treated mice was observed. All the experiments contain at least five animals and each experiment was repeated thrice. Results are mean values (n = 3).

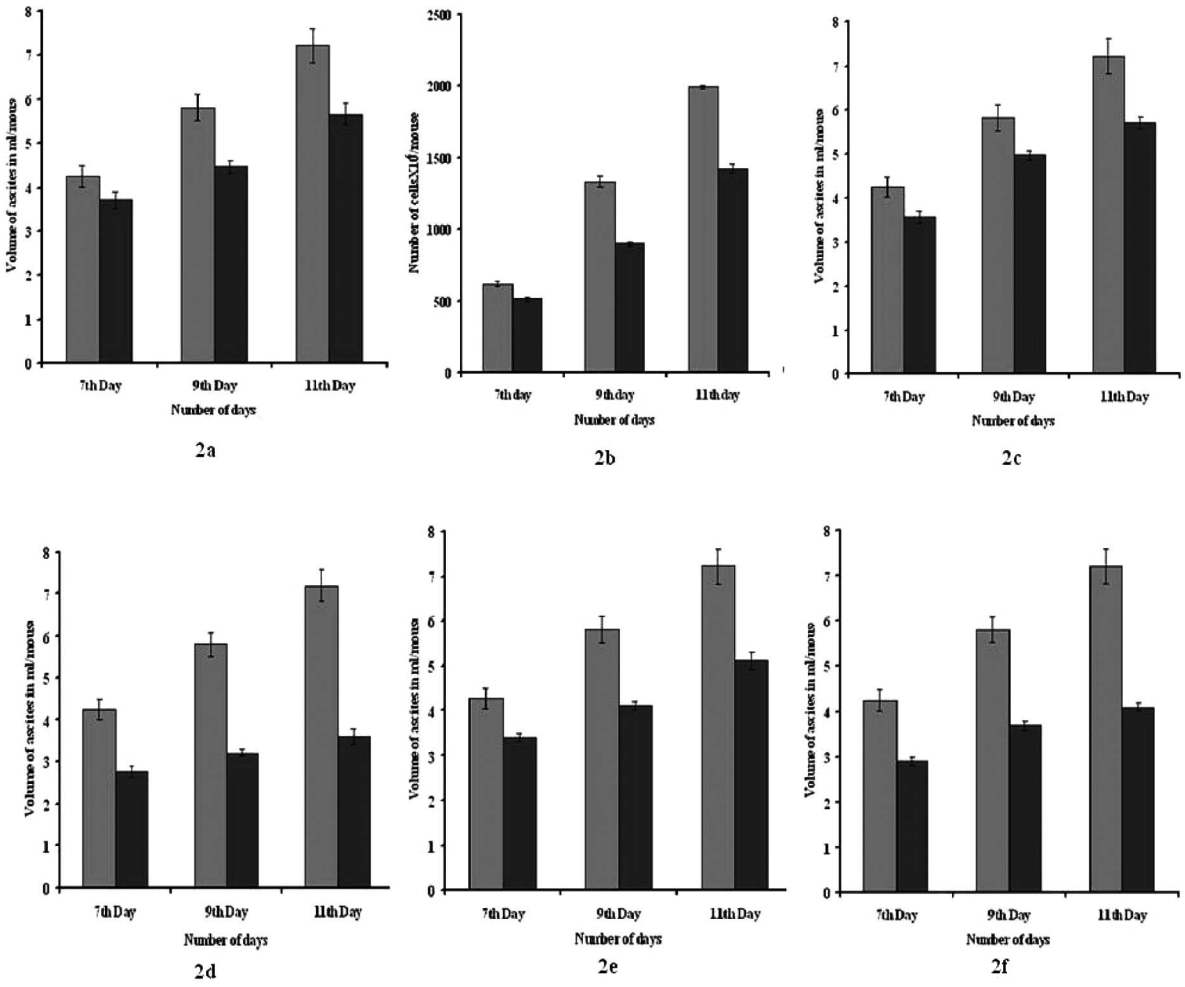


Fig. (1). Effect of imidazole derivatives 2(a-f) on ascites volume.

EAT bearing mice treated with or without imidazole derivatives were sacrified after giving each dose on alternative days, cells along with ascites fluid were harvested. Acsites volume was recorded. The above results are an average of three individual experiments.

Imidazole Derivatives 2(a–f) Induce Nuclear Condensation of EAT Cells

An attempt was made to find out whether the inhibition of proliferation of EAT cells by imidazole derivatives **2(a-f)** was due to induction of apoptosis in EAT cells. During *in vivo* studies, cytological analysis of acridine orange/ethidium bromide stained treated EAT cells (*in vivo*) showed nuclear condensation, which is an important feature of apoptosis as compared to the untreated EAT cells Fig. (**3**).

Imidazole Derivatives 2(a-f) Inhibit Growth of HUVEC's Cells **In Vitro**

We examined the effect of imidazole derivatives **2(a-f)** on the cellular proliferation of HUVEC cells by using the MTT assay. Significant dose dependent growth inhibition was observed when they were treated with imidazole derivatives **2(a-f)** for 24 h. imidazole derivatives **2(a-f)** showed significant growth inhibition at 100 μM. Fig. (**4**). These results suggest that imidazole derivatives **2(a-f)** potently reduce HUVECs cell number.

DISCUSSION

Our results showed on *in vivo* tumor inhibition that, imidazole derivatives **2(a–f)** have potent anti-tumor activity when compared with that of the parent molecule, where the parent molecule does posses minimal antitumor activity. (data not shown). However, we observed that imidazole derivative **2d** with the pyrrolidine moiety which is attached to imidazole ring nitrogen through ethyl group has shown the highest anti-tumor activity of 61.12%, whereas imidazole derivatives **2b** and **2f** with 6,7,8,9-tetrahydro-pyrido [1,2-a] pyrimidine-4-one and piperidine moieties respectively attached to imidazole ring nitrogen through ethyl group have shown moderate decrease in body weight (54 and 57% respectively). Imidazole derivatives **2a**, **2c** and **2e** bearing pyrido [1,2-a]pyrimidine-4-one, pipyridine and morpholine moieties on treatment showed 25, 20% and 19% decrease in body weight, respectively. These results correlate with the decrease in cell number and ascites volume to considerable amount. Treatment of imidazole derivatives **2a-f** also increased the survivability of the animals. Imidazole derivative

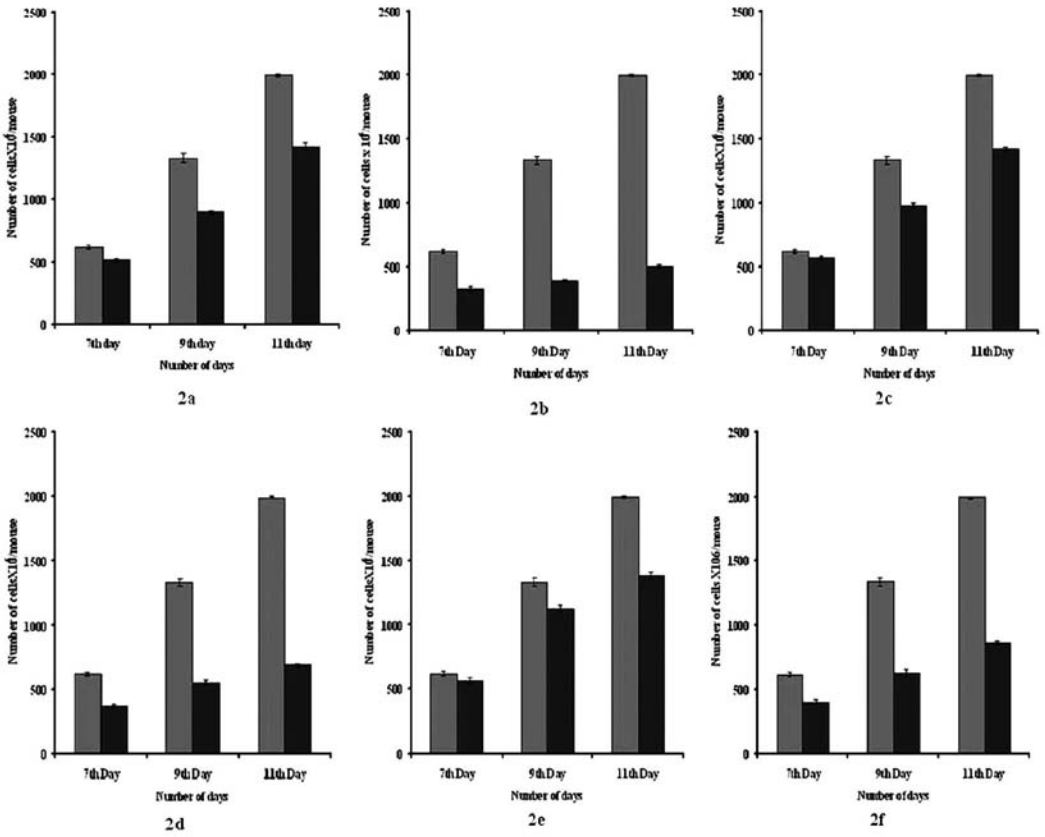


Fig. (2). Effect of imidazole derivatives 2(a-f) EAT cell number.

EAT bearing mice treated with imidazole derivatives **2(a-f)** were sacrificed after giving each dose on alternative days and cells along with ascites fluid were harvested. The number of cells per mouse was determined by counting the cells in hemocytometer. The above results are an average of three individual experiments.

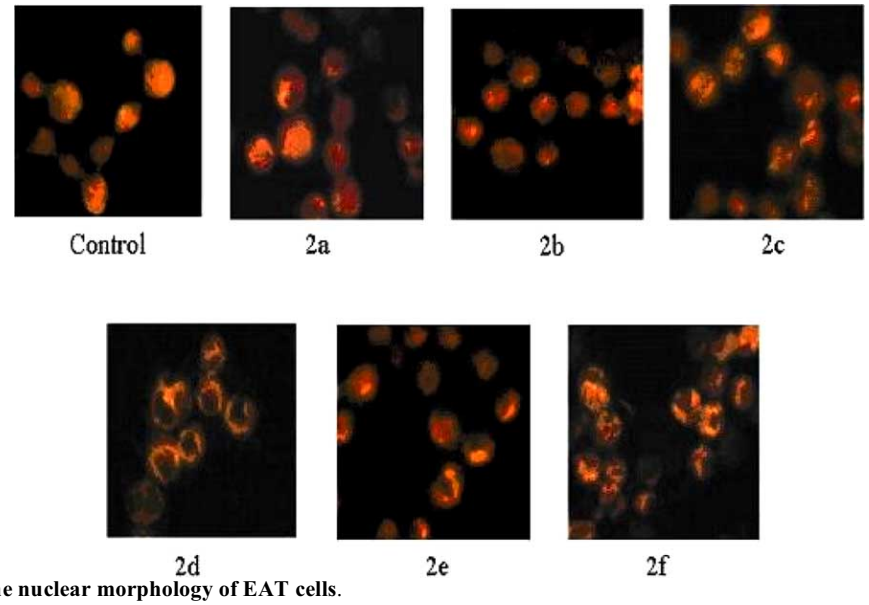


Fig. (3). Changes in the nuclear morphology of EAT cells.

Nuclear staining was performed according to the method of Srinivas *et al*. EAT cells either treated or untreated with imidazole derivatives 2**(a-f)** *in vivo* were smeared on a glass slide and fixed with methanol: acetic acid (3:1) and air-dried. The cells were hydrated with PBS and stained with a mixture (1:1) of acridine orange–ethidium bromide (4 μg/ml) solutions. The cells were immediately washed with PBS and viewed under a Leitz –DIAPLAN fluorescent microscope.

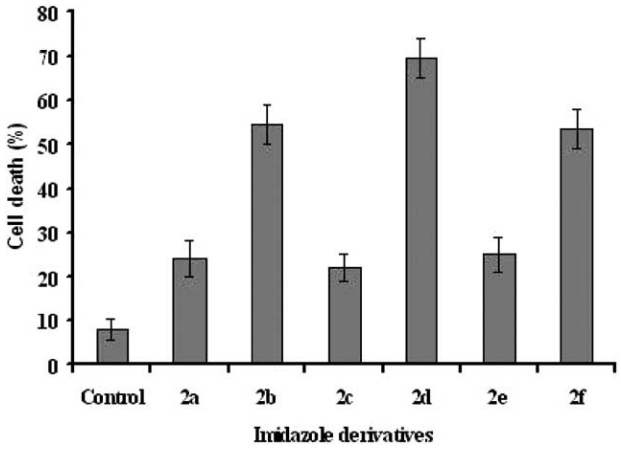


Fig. (4). Effect of imidazole derivatives 2(a-f) on proliferation of HUVECs cells.

HUVECs cells were grown to 80% confluence and were incubated in presence and absence of imidazole derivatives (100uM) cell viability was measured by means of MTT assay and expressed in % cell death. The above results are an average of three individual experiments.

2d treated animals were alive for 26 days and the number of animals which survived in this group was 86%, whereas imidazole derivatives **2b** and **2e** treated animals survived for 23 and 21 days respectively with 70% of the animals surviving. In the case of imidazole derivatives **2a**, **2c** and **2e** the percentage of animals that survived was 58%, whereas the animals were alive only for an average 15 days. These results elucidate that imidazole derivative **2d** with N-ethyl pyrollidine group, which is attached to imidazole nitrogen atom, possess more potent anti tumor activity. Despite variation in the tumor inhibition activity, all the compounds were able to induce the apoptosis where the mechanism of tumor growth inhibition was involved. Our results on nuclear condensation and formation of apoptotic bodies, showed that imidazole derivatives **2(a–f)** were inducing apoptosis in EAT cells. Our investigation on *in vitro* HUVECs cell proliferation showed that imidazole derivatives **2(a-f)** had potent growth inhibition effect as verified by MTT assay. The imidazole derivative **2d** that is of pyrrollidine moiety to imidazole ring has shown 70% growth inhibition at 100µM suggesting the possible role of pyrrollidine group. The relative cell number significantly plateaued at 10 µM and dropped off significantly and at 150 µM where there was almost no growth. But the imidazole derivatives **2b** and **2f** with pyrimidine 4-one and piperazine moiety to imidazole showed significant growth inhibition effect of 60% and 50% at 100µM respectively. The relative cell number significantly plateaued at 10uM and dropped off significantly at 200 µM where there was no growth. Imidazole derivatives **2a**, **2c** and **2e** could excrete moderate growth inhibitory effect on endothelial cells. Taken together such active imidazole analogs prove to be a potential anti-tumor factor, which could be further, developed and translated into a therapeutic regime for treatment of human cancer where formation of peritoneal malignant ascites is a major cause of morbidity and mortality. Further research to know the mechanism of inhibition and the modifications of the imidazole derivatives to improve the potency of this series are currently under progress in our lab.

CONCLUSION

Currently, a large variety of chemotherapeutic drugs are being used to treat cancer. Unfortunately, many compounds hold limited efficacy, due to problems of delivery and penetration and a moderate degree of selectivity for the tumor cells, thereby causing severe damage to healthy tissues. From our studies, it is clear that imidazole derivatives **2b**, **2d** & **2f** have potent antitumor effect as shown by the reduction in the EAT cell number, ascities volume *in vivo*. The above study shed light towards the identification of newer series of *N*- substituted 2-butyl-5-chloro-3*H*-imidazole-4-carbaldehyde as antitumor agents towards the cancer therapy.

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