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Anti-inflammatory and analgesic activities of imidazolyl triazolo hydroxamic acid derivatives

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Hydroxamic acids are directly related with cancer and its progression. Long term exposure with inflammatory responses, dysplasia develops which leads to cancer. Metastasis of cancer and expression of transient potential receptor ankyrin-1 are known to cause severe pain. Here, we explored the possibility of developing newer hydroxamic acid derivatives as anti-inflammatory and analgesic agent. Animals were administered with 100 mg/kg dose of the synthesized imidazolyl triazolo hydroxamic acid derivatives (FP1-FP12) and 50 mg/kg dose of standard diclofenac sodium. Carrageenan induced rat paw edema and Eddy's hot plate methods were considered for anti-inflammatory and analgesic activities. Among all the synthesized molecules, FP10 and FP4 were the most effective anti-inflammatory and analgesic agent, respectively. The activity profile of remaining molecules as anti-inflammatory agents was as follows: FP4>FP9> FP8> FP2 and as analgesic activity profile was FP10>FP3>FP8 >FP11 >FP2 > FP12. Presence of ethyl-benzyl and furan groups in linker portion of the structure minimized both the anti-inflammatory and analgesic activities. Results have shown that compounds with electron releasing groups considerably enhance both anti-inflammatory and analgesic activities.

Keywords: Antitumor,Carrageenan, Chemotherapy, Diclofenac Hydroxamic acid, Inflammation, Pain

Cancer is one of the major public health problems worldwide and it is the second leading cause of death in the United States. The global demographic characteristics predicted that by 2025 annually there will be about 420 million new cancer cases. In 2018, worldwide about 18 million cases of cancer were recorded out of which about 9.5 million were in men and about 8.5 million in women. Among all the cancers the commonest cancer is prostate cancer (1.28 million), female breast cancer (2.09 million), colorectal cancer (1.1 million), stomach cancer (1.03 million) and non-melanoma skin malignancies (1.04 million). In 2018, globally about 9.6 million deaths were estimated in cancer which shows that the second leading cause of deaths is cancer and about 1 in 6 deaths are due to cancer.

Considerable research has gone into curing cancer including drugs based hydroxamic acid derivatives. HDAC (Histone deacetylase) inhibitor the most promising target in combating various etiological factors of cancer. HDAC enzyme has been isolated into different classes as a zinc dependent and NAD (Nicotinamide adenine dinucleotide) subordinate. These catalysts were a part of multiprotein structures, catalyzing the removal of acetyl gathering from a lysine store on protein, including histone[1]. HDACI (Histone deacetylase inhibitor) was incited cell cycle capture and development hindrance which was related to the transcriptional enactment of p21WAF1/CIP1 (cycling-subordinate kinase inhibitor 1 or CDK-cooperating protein)[2], p27KIP1 (a cell cycle administrative protein that connects with cycling-CDK2 and CDK4, repressing the cell cycle movement at G1), GADD45 (development capture and DNA harm), restraint of cyclin A, cyclin D, and thymidylate synthetase[3,4]. Here, synthesized molecules (FP1-FP12) and imidazolyl-1,2,4-triazolo group were fused with hydroxamic acid part in the terminal and phenyl/ortho hydroxyl phenyl group in the receptor surface recognition part by considering the SAHA (suberoylanilide hydroxamic acid) as standard molecule[5,6] and the structure of SAHA. Previous study has shown that long-term inflammation leads to development of dysplasia[7]. Analgesic activity associated with COX/LOX inhibitors[8,9] and different prostaglandins was released by COX produce tumor promoters[10,11]. COX-2 enzyme converts procarcinogen to carcinogen and modulates angiogenesis and inflammation[12]. Overexpression of COX-2 is associated with human breast and colorectal proliferation[13].Hydroxamic acids are directly related to cancer and its progression. Long term exposure with inflammatory responses, dysplasia develops

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which leads to cancer. Also, metastasis of cancer and expression of transient potential receptor ankyrin-1 cause severe pain. As previously synthesized imidazolyl-triazolo hydroxamic acid derivatives (FP1-FP12) showed good inhibition of histone deacetylase enzyme and MCF-7 breast cancer cell line[14],so in this manuscript we explored the anti-inflammatory and analgesic efficacies of these synthesized molecules.

Materials and Methods

Chemicals

Carrageenan was purchased from Sigma-Aldrich Chemie, India. The standard drug used diclofenac sodium was procured from JB Chemicals and Pharmaceuticals, Mumbai, India. All chemicals used were of analytical grade and purchased from Merck, India.

All the imidazolyl-triazolo hydroxamic acid derivatives (FP1-FP12) considered for the anti-inflammatory and analgesic activities were previously synthesized and established by different analytical tools[14].

Animals

Wister albino rats of either gender weighing 130-170 g was obtained. The animals were divided into several groups of six animals each. All the animals were kept under standard ambient environment of temperature (22±3°C) and relative humidity of 50±5%. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 h light/dark. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal. All experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC).

Preparation of test compounds

Synthesized molecules (FP1-FP12) and the reference drug (diclofenac sodium) were prepared as a suspension in 1% Tween 80. Control group was administrated with 0.1 mL of Tween 80 suspension orally. The reference group received a dose of 50 mg/kg suspension of diclofenac sodium while the test groups were given 100 mg/kg of synthesized compounds.

Acute toxicity study

The acute toxicity study was carried out by OECD guidelines to calculate the successful dose of the test compounds. Wister albino rats of either sex weighing between 130-170 g were divided into several groups of 6 animals each. Animals were starved for 12 h before the experiment. All test animals (including those that die during the test or were removed from the study for animal welfare reasons) should be contingent to necropsy. On the day of the experiment, animals were treated with sample molecules to different groups in an increasing order of 10,50, 100, 250, 500 and 1000 mg/kg body wt. by oral feeding. The test animals were observed continuously for 3 h for any behavioural and autonomic profiles, followed by every 30 min check for next 4 h and finally for next 24 h or till death. As per this oral toxicity experiment, it was detected that in the highest dose of 1000 mg/kg body wt., all test animals were found to be safe. Hence, $1/10^{th}$ of the highest tolerated dose, 100 mg/kg body wt. was chosen for anti-inflammatory and analgesic activity experiments[16].

Anti-inflammatory activity

Anti-inflammatory activity of the synthesized molecules (FP1-FP12) was done by carrageenan induced rat paw edema method. The animals were divided into 14 groups of six each[17]. After 1.0 h of oral administration, acute inflammation was initiated by 0.1 mL, 1% w/v aqueous suspension of carrageenan, which was directly injected in the right hind paw subplanter region of each rat[18]. A cross mark was applied on the malleolus of leg to facilitate subsequent readings[19]. The paw volume was measured by plethysmometer with 30 min, 2and 4 h interval after the carrageenan administration[20]. The % inhibition was calculated by newbould formula[21,22] % Inhibition = $(1 - V_t/V_c) \times 100$ where $V_t$and $V_c$are the mean change in paw volume of treated and control rats, respectively[23].

Analgesic activity

Analgesic activity of the synthesized molecules (FP1-FP12) was done by Eddy's hot plate method. Animals were individually placed on a hot plate maintained at a constant temperature (55°C) and the reaction of animals such as paw licking or jump response (whichever appears first) was taken as the end point[24]. A cut-off time of 15 s was taken as maximum analgesic response to avoid any injury of the paws[25]. The reference group was administered with a dose of 50 mg/kg of the suspension of diclofenac sodium (standard)[26]. The reaction time for each animal was noted on the hot plate at 30, 60, and 90 min after the drug administration[27,28].

Statistical analysis

The results were expressed as mean ± SEM and were statistically validated using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. The probability value with 0.05 or less was considered statistically significant. Factual examination was performed by Graphpad Prism software version 7.0, GraphPad Software Inc. USA.

Results and Discussion

In this study, anti-inflammatory activity of synthesized molecules (FP1-FP12) was evaluated against carrageenan induced rat paw edema method. The response of this experiment was recorded for 30 min, 2 h and 4 h[29,30]. The outcomes showed that most of the synthesized molecules have significant anti-inflammatory effects (P<0.05-0.0001)[31,32]. In case of inhibition of inflammatory responses at 30 min, 2h and 4 h intervals, % inhibitions of diclofenac sodium were 63.12, 64.23 and 61.85 followed by FP10 and FP4 with % inhibitions 53.78, 51.86, 50.48 and 50.24, 49.20, 50.97, respectively. Among the other molecules, the activity profile was as follows FP4>FP9> FP8> FP2. The result was reported in (Fig. 1A) and Table 1. As per the statistical result, r^2 value of this experiment is 0.8467. Analgesic activity of synthesized molecules (FP1-FP12) was evaluated against diclofenac sodium using eddy's hot plate method[33,34]. The response of this experiment was recorded for 30 min, 60 min and 90 min[35]. The outcomes showed that many sample molecules have significant analgesic effects (P<0.05-0.0001)[36]. At 30 min, % inhibition of diclofenac sodium was 110.11, FP4 was 63.29 and FP10 was 57.30. At 60 min interval, % inhibition of diclofenac sodium was 113.38, FP3 was 68.77, FP4 was 63.94, FP9 was 59.47, FP10 was 58.73 and FP11 was 55.76. At 90 min interval, % inhibition of diclofenac sodium was 117.40, FP4 was 66.66, FP11 was 61.48 and FP10 was 61.11. Among the other molecules, the activity profile was as follows FP8 >FP11 >FP2 >FP12. The result was reported in (Fig. 1B) and Table 2. As per the statistical result, r^2 value of this experiment was 09247.

Carrageenan-induced rat paw edema is used as working model of inflammation to develop newer

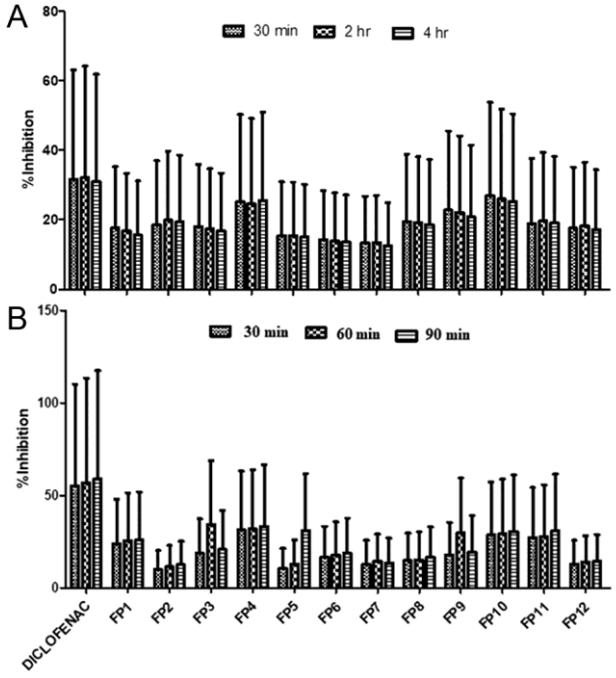

Fig. 1 — (A) Anti-inflammatory; and (B) Analgesic activities of FP1-FP12 and diclofenac sodium as reference standard

Table 1 — Effect of synthesized molecules (FP1-FP12) and diclofenac sodium on carrageenan induced rat paw edema

Compound	Mean changes in paw edema (mL) mean ± SEM			% Inhibition		
	30 min	2 h	4 h	30 min	2 h	4 h
Control	0.621±0.011	0.752±0.054	0.616±0.013	-	-	-
Diclofenac sodium	0.229±0.018	0.269±0.017	0.235±0.019	63.12±0.095	64.23±0.095	61.85±0.095
FP1****	0.402±0.026	0.501±0.021	0.424±0.023	35.26±0.011	33.37±0.010	31.16±0.019
FP2****	0.391±0.029	0.453±0.028	0.378±0.024	37.03±0.033	39.76±0.051	38.63±0.033
FP3****	0.398±0.037	0.491 ± 0.036	0.410±0.033	35.90±0.042	34.70±0.021	33.44±0.015
FP4****	0.309±0.038	0.382±0.0037	0.302±0.033	50.24±0.014	49.20±0.063	50.97±0.019
FP5***	0.429±0.011	0.520±0.014	0.430±0.009	30.91±0.032	30.85±0.013	30.19±0.026
FP6****	0.445±0.024	0.543±0.023	0.449±0.021	28.34±0.142	27.79±0.029	27.11±0.018
FP7**	0.455±0.022	0.549±0.019	0.462±0.020	26.73±0.008	26.99±0.005	25.00±0.007
FP8****	0.379±0.017	0.465±0.018	0.386±0.019	38.96±0.032	38.16±0.026	37.33±0.028
FP9****	0.338±0.022	0.420±0.021	0.360±0.026	45.57±0.041	44.14 ± 0.09	41.55 ± 0.09
FP10****	0.287±0.013	0.362±0.012	0.305±0.010	53.78±0.043	51.86±0.041	50.48±0.011
FP11****	0.387±0.031	0.456±0.034	0.380±0.032	37.68±0.035	39.36±0.048	38.31±0.028
FP12****	0.403±0.025	0.477 ± 0.030	0.404±0.031	35.10±0.038	36.56±0.034	34.41±0.022

[****, ***, **: significant at P<0.0001, P<0.001, and$P$<0.01 from control]

Table 2 — Analgesic activity of synthesized molecules (FP1-FP12) with diclofenac sodium on Eddy's hot plate method

Compound	Reaction time after drug administration (s) mean ± SEM			% Inhibition		
	30 min	60 min	90 min	30 min	60 min	90 min
Control	2.67±0.014	2.69±0.017	2.70±0.019	-	-	-
Diclofenac sodium****	5.61±0.019	5.74±0.018	5.87±0.024	110.11±0.089	113.38±0.091	117.40±0.094
FP1****	3.95±0.024	4.07±0.018	4.10±0.014	47.94±0.011	51.30±0.014	51.85±0.018
FP2*	3.21±0.023	3.31±0.019	3.38±0.016	20.22±0.031	23.05±0.034	25.18±0.033
FP3****	3.67±0.023	4.54±0.019	3.83±0.017	37.45±0.038	68.77±0.019	41.85±0.016
FP4****	4.36±0.022	4.41±0.019	4.50±0.016	63.29±0.016	63.94±0.019	66.66±0.021
FP5***	3.24±0.024	3.39±0.022	4.37±0.017	21.35±0.032	26.02±0.013	61.85±0.026
FP6***	3.56±0.023	3.65±0.020	3.72±0.018	33.33±0.105	35.69±0.067	37.77±0.025
FP7**	3.36±0.022	3.48±0.024	3.43±0.018	25.84±0.009	28.99±0.014	27.03±0.018
FP8**	3.46±0.024	3.51±0.018	3.59±0.015	29.58±0.024	30.48±0.021	32.96±0.022
FP9****	3.62±0.021	4.29±0.018	3.76±0.015	35.58±0.037	59.47±0.090	39.25±0.011
FP10****	4.20±0.022	4.27±0.019	4.35±0.012	57.30±0.041	58.73±0.023	61.11±0.015
FP11****	4.12±0.021	4.19±0.017	4.36±0.015	54.30±0.029	55.76±0.038	61.48±0.025
FP12**	3.36±0.023	3.45±0.018	3.48±0.011	25.84±0.031	28.25±0.036	28.88±0.025

[****, ***, **, *: significant at $P<0.0001$, $P<0.001$, $P<0.01$, and $P<0.05$ from control]

anti-inflammatory agents. The development of edema in rat paw region after carrageenan injection is a biphasic event[37]. The initial phase is related to the histamine and serotonin release, then edema is maintained between the first and second phase by kinin, the second phase of edema is controlled by prostaglandin[38]. All the mediators appear to be dependent upon an intact complement system for their activation and release[39]. It has been shown that, in the early phase of the oedema, the dominant cells are polymorphonuclear leukocyte whereas in advanced stages dominant cells are mononuclear leukocyte[40].

Pain is complex process which is modulated by opiate, dopaminergic, noradrenergic and serotonergic systems[41]. The analgesic effect showed by the test and standard molecules, produced via central mechanisms involving receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain[42,43]. From the structural view, presence of 4-hydroxy-3-methoxy group in the imidazolyl-triazolo linker portion with the ortho hydroxyl phenyl group in surface recognition part in case of FP10 and 4-hydroxy-3-methoxy group in the imidazolyl-triazolo linker portion with the phenyl group in surface recognition part in case of FP4 were showed the good anti-inflammatory and analgesic activity, respectively as compared to the standard molecule diclofenac sodium.

Presence of ethyl- benzyl and furan groups in linker portion of the structure minimized both the anti-inflammatory and analgesic activities. Over all, the results of this study have shown that the presence of electron releasing group directly contribute to both anti-inflammatory and analgesic activity. These anti-inflammatory and analgesic activities indicate that the synthesized molecules conquer against various inflammatory responses, COX/LOX pathways, induction of pain and release of various tumor necrosis factors.

Conclusion

In this study we have made an attempt to develop a series of phenyl and ortho hydroxy phenyl- linked imidazolyl triazolo hydroxamic acid derivatives- as anti-inflammatory and analgesic agents. Accordingly. carrageenan induced rat paw edema anti inflammatory activity and Eddy's hot plate analgesic activity procedures were used to assess the anti inflammatory and analgesic activities using different concentrations of the synthesized molecules and diclofenac as standard molecule. Outcomes revealed that presence of 4-hydroxy-3-methoxy group in the imidazolyl-triazolo linker portion with the ortho hydroxyl phenyl group in surface recognition part in case of FP10 and FP4 support significant anti inflammatory and analgesic activity, respectively as compared to the standard molecule diclofenac sodium. This study has demonstrated that the presence of electron releasing group directly contribute to both anti-inflammatory and analgesic activity.

Conflict of interest

Authors declare no competing interests.

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