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SYNTHESIS, CHARACTERIZATION & ANALGESIC ACTIVITY OF IMIDAZOLO-AZATIDINONE DERIVATIVES

Surwase Kumar Pralhadrao* Research Scholar, Oriental College of Pharmacy and Research, Oriental University, Indore.

Niharika Gokhale Oriental College of Pharmacy and Research, Oriental University, Indore.

Abstract

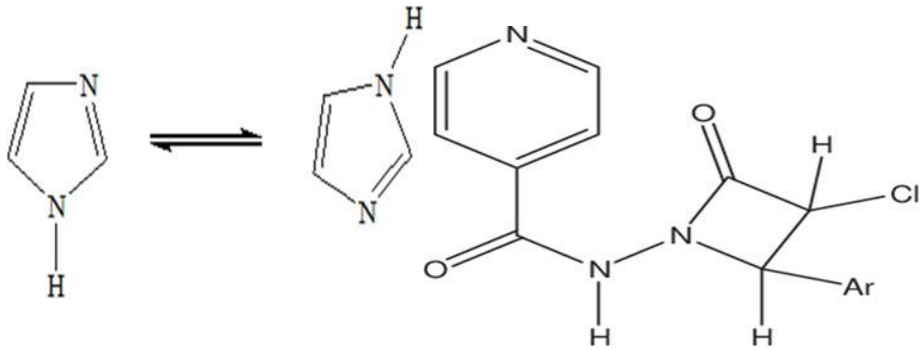
New series of 2-Azatidinone (4a-j) were synthesized, the synthesized the structure of these new derivative were confirmed using spectral methods. Starting from Imidazole We prepared by using Benzil, Aldehyde (Acetaldehyde) in Acetamide and Glacial acetic acid then treated with chloro methyl ethyl acetate then converted into hydrazide by using hydrazine hydrate, then schiff bases were synthesized using different aldehyde in ethanol.The synthesis of the designed compounds has been successfully achieved. Purity and characterization were confirmed by determination of physical properties (melting point, & Rf values) and 1H-NMR sp.

Keywords: Imidazole, Benzil, Aldehyde (Acetaldehyde), Acetamide, Glacial acetic acid, Schiff Base, 2-Azatidinone.

INTRODUCTION

Imidazole is a planer five-member heterocyclic ring with 3C and 2N atom and in ring N is present in 1st and 3rd positions. The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. Being a polar and ionisable aromatic compound, it improves pharmacokinetic characteristics of lead molecules and thus used as aremedy to optimize solubility and bioavailability parameters of proposed poorly soluble lead molecules. Imidazole derivatives have occupied a unique place in the field of medicinal chemistry. The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery. Imidazole drugs have broadened scope in remedying various dispositions in clinical medicines (1). Numerous methods for the synthesis of imidazole and also their various structure reactions offer enormous scope in the field of medicinal

chemistry. Infectious microbial disease causes worldwide problem, because microbes have resisted prophylaxis or therapy longer than any other form of life. In recent decades, problems of multidrug-resistant microorganisms have reached an alarming level in many countries around the world. Resistance of anti-microbial agents such as β-lactam antibiotics, macrolides, quinolones and vancomycin etc. and different species of bacteria causes increased important global problem. Imidazole and its derivatives are reported to be physiologically and pharmacologically active and find applications in the treatment of several diseases. Medicinal properties of imidazole include anticancer, b-lactamase inhibitors,20- HETE (20- Hydroxy-5,8,11,14-eicosatetraenoic acid) synthase inhibitors, carboxypeptidase inhibitors, hem oxygenase inhibitors, antiaging agents, anticoagulants, anti-inflammatory, antibacterial, antifungal, antiviral, antitubercular, antidiabetic and antimalarial(2).



Imidazole 2-Azatidinone

Pain, fever and inflammation have been associated with the mankind since the beginning; non-steroidal anti-inflammatory drugs (NSAIDs) are the first choice of drugs in the treatment of pain in the degenerative inflammatory joint disease. NSAIDs usually block the action ofcyclooxygenase-1 (COX-1) andcyclooxygenase-2 (COX-2). It was believedthat blocking (COX-2) will lead to the antipyretic analgesic and anti-inflammatoryresults. Despite the efficiency in defeating pain and inflammation NSAIDs including ibuprofen have some limitations such as dyspepsia, symptomatic, complicated gastric and duodenal ulcers due to blocking (COX1) activity. Mostly common NSAIDs differ in their relative inhibitory potency against both iso forms of COX: COX-1 and COX-2. The maximum extent of damage is usually caused by NSAIDs that are favoured COX-1inhibitors and having a free carboxylic group such as Ibuprofen, Ketoprofen. COX-1 and COX-2 have a similar catalytic activities and structures, but COX-2 has valine instated of isoleucine at positions 523. Valine is smaller than isoleucine by methyl group. These substitutions cause a larger and more flexible substrate channel and a secondary internal pocket of theblocker binding site of COX-2 which isn't observed in COX-1. COX-2 selective blockers have structures which occupy the additional pocket, so providing NSAIDswith larger pockets will provide more selectivity towards COX-2 enzyme and masking the COOH group will provide less local damage on the mucosa of stomach.

Azetidin-2-ones had attracted the attention of many researchers to investigate this skeleton

due to its multiple potential against several activities especially because of the antibacterial characteristics of cephalosporins and penicillin. In the recent years the interest was focused on the modification and synthesis of β-lactam ringto have compounds with diverse pharmacological activities like blockers of prostate specific antigens, thrombin, cholesterol absorption, human cytomegalo- virus protein, human leukocyte cysteine protease and elastase. As a consequence, the interest of theorganic chemists in the synthesis of many new β- lactam derivatives remains high. Some of these derivatives also had beenfound to be active moderately several kinds of microbialinfection (3).

Materials & Method:

Melting points are determined on a thermal melting point apparatus (Thermometer), and they are uncorrected. Completion of reaction and purity of all compounds are checked on TLC plates using Methanol: Acetic acid: Ether: Benzene (05:15:60: 20). as the mobile phase and visualized under iodine vapor.IR spectra were recorded on Jusco.

Raw material and characterization:

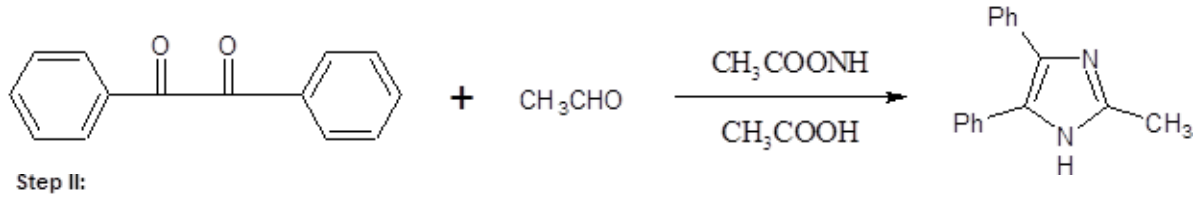
Table no. 1: Physical properties of raw material

Sr. No.	Name of chemical	Molecular formula	Mol. Wt.	M.P.	B.P.	Solubility
1	Benzil	C14H10O2	210.23	94.08	-	Methanol
2	Acetaldehyde	C2H4O	44.05	-	20.02	Ethanol, ether, benzene, acetone, chloroform, toluene.
3	Acetamide	C2H5NO	59.06	79	-	Ethanol, benzene, glycerol, chloroform.
4	Glacial acetic acid	CH3COOH	60.52	16.6	-	Water, ethanol, acetone
5	Imidazole	C3N2H4	68.07	90		Water
6	Chloro methyl ethyl acetate	C2H5OCL	108.52	-	56	Alcohol, diethyl ether
7	Ethanol	C2H6O	46.07	-	78.05	Water, ether
8	Hydrazine hydrate	N2H4	32.04	-	114	Water
9	4- Chloro benzaldehyde	C7H5ClO	140.56	47	214	-
10	4- Methyl benzaldehyde	C8H8O	120.15	-	204	-

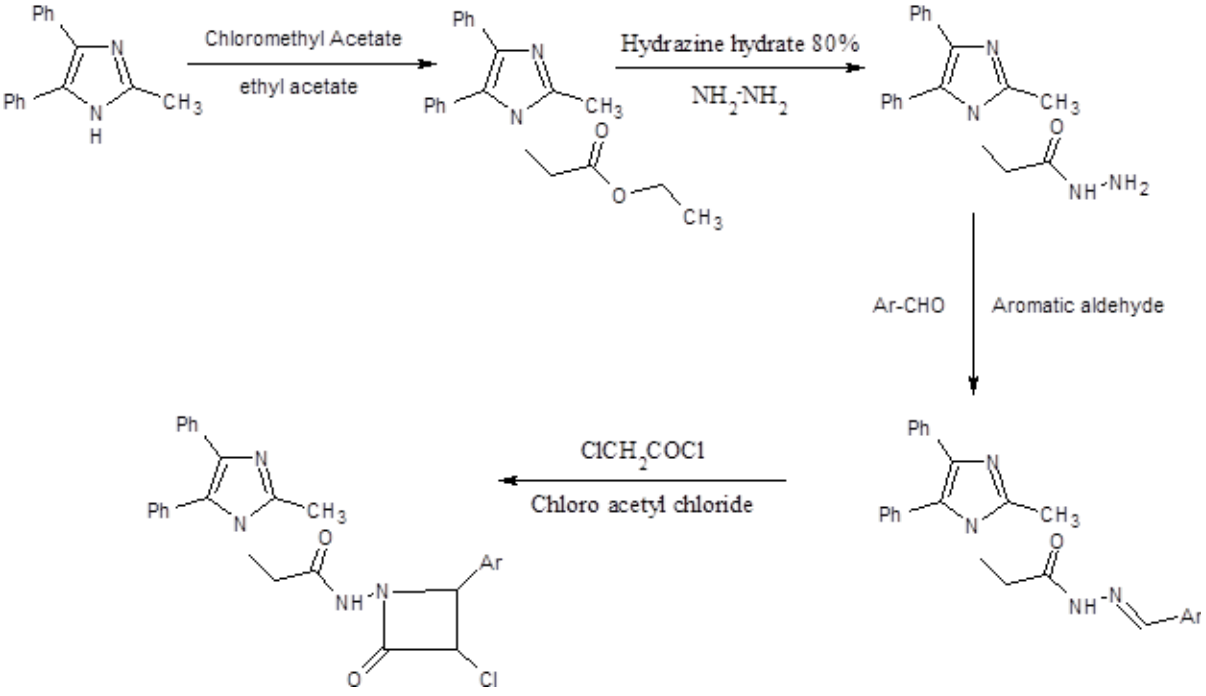
11	4- Hydroxy benzaldehyde	C7H6O2	122.12	-	310	-
12	4- Bromo benzaldehyde	C7H5BrO	185.02	57	-	-
13	Benzaldehyde	C7H6O	106.12	-	179	-
14	Anisaldehyde	C8H8O2	136.15	-	248	-

Scheme:

Step I:



Step II:



Synthetic method and physical data of synthesized compounds:

Step I-Synthesis of 2-(2-methyl, 4,5 diphenyl)-1H-Imidazole).

Benzil (0.01mole, 2.10gm), Acetaldehyde (0.01mole, 0.44ml) in Acetamide (0.01mole, 5gm) and Glacial acetic acid (0.01mole, 5gm) were dissolved. The reaction mixture was stirred for 27min at 350wolt (level5) power in microwave. This solution was poured into ice for formation of crystals. Recrystallized by the of dissolving it in acetone. Product was filtered

dried and stored intightly closed container in normal temperature.

Step II-Synthesis of 2-(2-methyl, 4,5 diphenyl)-1H-Imidazole-1-yl) acetate.
To the solution of compound, I (0.01mol, 2.3gm) in (5ml) ethanol (0.01mol, 1.085gm) of chloromethyl ethyl acetate was added and reaction mixture kept in microwave for 15 min (level5). This solution was poured in ice for crystal formation. Recrystallized by the of dissolving it in acetone. Product was filtered dried and stored in tightly closed container in normal temperature.

Step III- Synthesis of 2-(2-methyl, 4,5 diphenyl)-1H-Imidazole-1-yl) acetahydrazide.
To the solution of compound II (0.01mol, 3.2gm)in (15ml) ethanol,(0.01 mole, 0.32gm) of hydrazine hydrate(80%) was added. The reaction mixture was stirred at room temperature overnight. On the next day, the dark yellow precipitate was filtered with suction filtration and washed with cold (5ml) ethanol, the solvent was removed under vacuum and the crude product was washed with ether under stirring(4).

Step IV- General Procedure for the Schiff's bases compounds (3a-f).
To a stirred solution of compound 2(0.01mole, 2.9gm) in (30ml) ethanol, various aromatic aldehydes (0.01mole) were added, after which the mixture was heated at 450wolt for 30min until the completion of the reaction (TLC monitoring using ethyl acetateand n-hexane 3:1 ratio). The combination was chilled to normal lab temperature. A residue were poured on crushed ice, The solid crystals gained and splashed using water then recrystallization by using water and ethanol.4 (a-f) (5).

Step V-General procedure for the 2-Azetidinones compounds (4a-f)
To a solution of all Schiff bases compounds (0.001mole) in anhydrous 1,4-dioxane, chloro acetyl chloride(0.0015 mole ,0.169g) were added drop wise in a period of 20 min at 0-5.The mixture of reaction was stirred at room temperature for 3 hours. The solution was heated under reflux for 5 hours and then the solvent were vaporized by low pressure conditions. The solid product were washed by using (10ml) water, filtered off, dried and recrystallized from absolute ethanol. To afford the corresponding Schiff base derivatives of 3-chloro-4-oxoazetidin.5(a-f) (6).

5a: N-(2(-4-Chloro-2formylbenzylidene)-3-chloro-4-oxoazetidin-1-yl)-2-(2-methyl-4,5-diphenyl-1H-Imidazole-1-yl) acetamide
Dark yellow crystals yield 33.08 %; mp.88-92 ^{0}C, IR (KBr, m, cm^{-1}): 3029-(Ar-CH) 3064 (Ar-CH) 2916, 2848 (CH_2-CH) 1681 (-C=O) 3400(-NH) 1325 (-N Imidazole) 1660 (-C=O Azatadine) 779,773 (-Cl) 1288(- CN Azatadine)

5b: N-(2(-4-Methoxy-2formylbenzylidene)-3-chloro-4-oxoazetidin-1-yl)-2-(2-methyl-4,5-diphenyl-1H-Imidazole-1-yl) acetamide
Faint yellow crystals, yield 36.042 %; mp.69-73^{0}C,IR (KBr, m, cm-1): 3029-(Ar-CH) ,3064

(Ar-CH) ,2916, 2848 (CH2-CH) 1681 (-C=O) ,3400(-NH) ,1325 (-N Imidazole) ,1660 (-C=O Azatadine) ,2785(-CH_3) ,1288 (- CN Azatadine).

5c: N-(2(-4-Hydroxy-2formylbenzylidene)-3-chloro-4-oxoazetidin-1-yl)-2-(2-methyl-4,5-diphenyl-1H-Imidazole-1-yl) acetamide

Yellow crystals, yield 68.41 %; mp.94-98^{0}C, IR (KBr, m, cm-1): 3029-(Ar-CH), 3064 (Ar-CH) ,2916, 2848 (CH2-CH) ,1681 (-C=O), 3400(-NH) ,1325 (-N Imidazole), 1660 (-C=O Azatadine) ,3317(-OH), 1288 (- CN Azatadine)

5d: N-(2(-4-Bromo-2formylbenzylidene)-3-chloro-4-oxoazetidin-1-yl)-2-(2-methyl-4,5-diphenyl-1H-Imidazole-1-yl) acetamide.

Yellow crystals, yield 31.60 %, mp.78-82^{0}C; IR (KBr, m, cm-1): 3029-(Ar-CH) 3064 (Ar-CH) 2916, 2848 (CH2-CH) 1681 (-
C=O) 3400(-NH) 1325 (-N Imidazole) 1660 (-C=O Azatadine) 560(-Br) 128 (-CN Azatadine)

5e: N-(2(-2(-2formylbenzylidene)-3-chloro-4-oxoazetidin-1-yl)-2-(2-methyl-4,5-diphenyl-1H-Imidazole-1-yl) acetamide. Yellowish brown crystals, yield 35.04 %; mp.80-85^{0}C,IR (KBr, m, cm-1): 3029-(Ar-CH) 3064 (Ar-CH) 2916, 2848 (CH2-CH)1681 (-C=O) 3400(-NH) 1325 (-N Imidazole) 1660 (-C=O Azatadine), 1288 (-CN Azatadine)

5f: N-(2(-2(-2Formyl-5methoxybenzylidene)-3-chloro-4-oxoazetidin-1-yl)-2-(2-methyl-4,5-diphenyl-1H-Imidazole-1-yl) acetamide.

Dark Yellow sticky matter; yield 43.37 %, mp.68-70^{0}C;IR (KBr, m, cm-1): 3029-(Ar-CH) 3064 (Ar-CH) 2916, 2848 (CH2-CH)1681 (-C=O) 3400(-NH) 1325 (-N Imidazole) 1660 (-C=O Azatadine) 2785 (-OCH_3), 1288 (- CN Azatadine)

Table no. 2: Analytical and physicochemical data of the synthesized compounds 5(a-f).

Comp. Code	Mole. Formula	Mole. Wt.	M.P.	Yield (%)
5a	$C_{28}H_{22}O_3N_4Cl_2$	532	88-92	33.08
5b	$C_{29}H_{25}O_3N_4Cl$	512	69-73	36.04
5c	$C_{28}H_{23}O_4N_4Cl$	514	94-98	68.41
5d	$C_{28}H_{22}O_3N_4BrCl$	577	78-82	31.60
5e	$C_{28}HO_{24}N_4Cl$	812	80-85	35.04
5f	$C_{29}H_{25}O_4CL$	472	68-70	43.37

Analgesic activity

All synthesized compounds 5(a-f) 120 mg was dissolved in distilled water immediatelybefore used orally, glacial acetic acid diluted in distilled water to provide 0.06% solution for intraperitoneal injection, pentazocine and normal saline. The mice were divided into three groups containing six animals (n = 6) in each group (control, standard, and test group). The test drug synthesized compounds 5(a-f) 120 mg/kg and normal saline 25 ml/kg was administered orally 2 h prior. Standard drug pentazocine 10 mg/kg was administered

intra-peritoneal 15 minprior to the experiment. Significant analgesia of pentazocine occurs between 15 and 30 min.(7)

The study was conducted after getting approval from institution Animal ethical committee (IAEC). (CPCSEA Approval number 1942/PO/Re/S/17/ CPCSEA/2019/02/01/01) Albino mice of either sex of average weight 30-50 g aged 8-12weeks were used in experiments. The albino mice were bred in central animal house of Pravara Rural College of Pharmacy, Loni.

The study was done in Department of Pharmacology. Animals were acclimatized to the laboratory conditions for at least 1 h before testing and were used during experiments. The doses of drugs were based on the human daily dose converted to that of mice according to Pagetand Barnes (1962).

Result and discussion:

Azetidinone derivatives (5a-f) were prepared using the method summarized in scheme. First, Benzil and acetaldehyde was reacted with acetamide and glacial acetic acid whereby the corresponding 2-methyl, 4,5 diphenyl imidazole was obtained. 2-(2- methyl, 4,5 diphenyl)-1H-Imidazole-1-yl)as a compound (1) then treated with chloro methyl ethyl acetate produced 2-(2-methyl, 4,5 diphenyl)-1H-Imidazole-1-yl) acetate as a compound (II) on amination with hydrazine hydrate in absolute ethanol afforded 2-(2-methyl, 4,5 diphenyl)-1H-Imidazole-1-yl) acetahydrazide as a compound (III).the condensation reaction of compound (III) with various aromatic aldehydes yielded Schiff's bases compounds (4a-f). Finally, the compounds (4a-f) upon reaction with chloracetyl chloride afforded 2-Azetidinones compounds (5a-f).

Analgesic Activity

a) Eddy's Hot Plate Method:

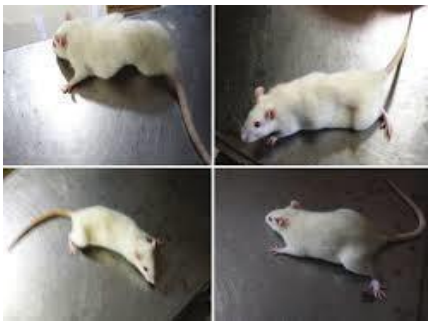
Thus, the latency period of all synthesized compounds 5(a-j) was significantly ($P < 0.05$) good when compared to control at time period 30-120 min, whereas the latency period of the standard was more significant ($P < 0.05$) when compared to synthesized compounds 5(a-f) at all-time intervals of experimentation [Tables 1, 2, 6]. (8)

Fig. 1: Eddy's Hot Plate Test on Mice

b) Writhing Method:

All synthesized compounds 5(a-f) which was given orally 2 h before intra-peritoneal injection of acetic acid significantly reduced the number of writhes. Significant inhibition of the writhing response was observed after the administration of synthesized compounds 5(a-f) 120mg/kg when compared to normal saline control group. The number of writhes of synthesized compounds 5(a-f) was less when compared to standard, whereas the number of writhes of standard drug (pentazocine) were less when compared to synthesized compounds 5(a-f) and normal saline. When compared to control, the percentage inhibition of synthesized compounds 5(a-f) was 56.39% and that of the standard was 84.35% [Table3] (9)

Fig. 2: Writhing Test on Mice



c) Tail Flick Method:

Thus, the mean reaction time of all synthesized compounds was significantly ($P < 0.05$)good as compared to control at time period 30-120 min, whereas the latency period of the standard was more significant ($P < 0.05$) when compared to all synthesized compounds at all-time intervals of experimentation [Tables 4,5] (10).

Fig. 3: Tail Flick Test on Mice

Table No.3: The analgesic activity of synthesized compound 5(a-f) in thermal pain model-Eddy's hot plate

Groups	0 min	30 min	60 min	90 min	120 min
Control	0.89+/0.07	1.08+/0.06	2.1+/-0.07	2.31+/-0.04	2.19+/-0.06
Standard	2.95+/0.11*	6.48+/0.06*	7.04+/-0.07	8.25+/-0.04*	10.21+/0.05*
5a	0.9+/-0.03	4.04+/-0.10*	6.01+/-0.08*	7.03+/-0.06*	5.44+/0.08*
5b	0.6+/-0.02	2.02+/0.03	5.03+/-0.07	5.08+/-0.04	4.34+/-0.06
5c	0.7+/-0.04	2.03+/0.05	4.02+/-0.05	6.07+/-0.06	6.03+/-0.04
5d	1.06+/0.07	5.32+/0.02	6.07+/-0.08	5.08+/-0.04	1.66+/-0.07
5e	0.9+/-0.08	3.09+/0.03	3.09+/-0.06	4.02+/-0.05	3.15+/-0.08
5f	1.8+/-0.04	4.07+/0.04	5.08+/-0.04	6.01+/-0.03	5.18+/-0.07

Table No. 4: The % analgesic activity of synthesized compound 5(a-f) and control when compared to standard – Eddy's Hot Plate:

Groups	0 min	30 min	60 min	90 min	120 min
Control	30.16	16.66	29.82	28	21.44
5a	30.50	62.34	85.36	85.21	53.28
5b	30.45	62.24	85.30	85.18	53.22
5c	30.25	62.14	85.16	85.06	53.07
5d	30.38	62.08	85.04	85.02	53.03
5e	28.57	61.40	83.42	84.28	52.34

5f	30.20	62.03	85.06	85.01	53.04

Table No. 5: The analgesic activity of synthesized compound 5(a-f) in visceral pain model-Writhing method:

Groups	**No. Of Wriths**	**% Of Inhibition**
Control	35.16+/-2.85	0
Standard	5.5+/-2.42	84.35
5a	15.33+/-2.16	56.39
5b	20.45+/-1.85	74.66
5c	28.36+/-1.92	83.22
5d	30.02+/0.68	86.65
5e	18.35+/-1.76	67.02
5f	19.35+/-2.04	70.93

Table No. 6: The analgesic activity in mechanical pain mode – Tail flick method:

Groups	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control	0.89+/-0.07	0.73+/-0.23	2.81+/-0.22	2.71+/-0.16	2.95+/-0.21
Standard	2.95+/0.11*	8.84+/0.12*	10.06+/0.13*	10.20+/0.07*	10.21+/0.0*
5a	0.9+/-0.03	2.03+/0.11*	6.14+/-0.16*	6.50+/-0.18*	5.53+/0.23*
5b	0.6+/-0.02	4.04+/0.10*	7.03+/-0.07	7.08+/-0.04	8.34+/-0.06
5c	0.7+/-0.04	6.02+/-0.03	5.02+/-0.05	8.07+/-0.06	6.03+/-0.04
5d	1.06+/-0.07	4.03+/-0.05	6.07+/-0.08	6.08+/-0.04	6.66+/-0.07
5e	0.9+/-0.08	3.32+/-0.02	7.09+/-0.06	7.02+/-0.05	8.15+/-0.08
5f	1.8+/-0.04	5.09+/-0.03	9.08+/-0.04	10.01+/-0.03	7.18+/-0.07

Table No.7: The % analgesic activity of synthesized compound 5(a-f) & control when compared with standard Tail flick method:

Groups	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control	30.16	8.25	27.93	26.36	29.47

5a	30.50	22.96	61.03	63.72	55.24
5b	30.35	22.76	61.08	63.50	55.64
5c	28.85	22.82	60.07	63.45	55.23
5d	30.58	22.98	61.05	62.78	54.65
5e	30.19	22.56	60.03	62.84	53.98
5f	30.28	22.26	60.02	60.89	54.86

Table No.8: The % analgesic activity of synthesized compound 5(a-f) when compared tocontrol-Eddy's hot plate method:

Groups	0 min	30 min	60 min	90 min	120 min
5a	0.34	45.68	55.54	57.21	31.84
5b	0.32	44.78	54.65	57.14	31.54
5c	0.28	45.56	55.58	56.85	30.94
5d	0.23	43.52	53.89	54.65	31.25
5e	0.33	41.98	54.87	55.68	31.34
5f	0.32	44.87	55.25	55.79	30.84

The experimental protocol conducted according to the guideline for the use and care of the experimental animals.

The test drug of all azetidine derivative shows significant analgesic activity when compared to that of control in all the three established experimental models of pain. The analgesic activity was maximum at 60 min and 90 min. The possible mechanism is due to decreasing the central sympathetic tone, increase in the release of β-endorphin and encephalinlevels in the spinal cord, increasing the angiotensin 1-7 levels and decreasing PGE2 and COX2.

Thus, to conclude, all azetidine derivative possibly exhibits its analgesic activity both by central analgesic activity (Eddy's hot plate and tail flick) through release of β-endorphin and encephalin and also peripheral analgesic action (writhing method) through inhibition of COX 2 and PGE2

Conclusion:

The 2-Azetidinone continue to be one of the most researched areas in medicinal chemistry, synthesis of some new substituents of 2-azetidinone has been described using conventional method by cyclo condensation of Chloro-acetyl chloride with Schiff base derivatives (5 a-f).

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