Some Novel Series of 3-Alkylidene 3-Phenylthio-3-Chloro Azetidine -2-One Were Synthesized by The Staudinger Ketene-Imine Cycloaddition and Evaluation of Their Biological Activity

Thakor Patel[1], Bhavik Hemant Kumar Panchal[2], Shanta Raj Lakshmi[2]

[1]*Shiva performance materials Pvt ltd, Shiva Building, Sarabhai compound, Sarabhai Road, vadiwadi, Vadodara, Gujarat, India.*
[2]*Department of Chemical science, Parul institute of applied sciences, Parul university, Vadodara, Gujarat, India.*
Email: tdpatel59@gmail.com

KEYWORDS
Anti-inflammatory, Analgesic, Ulcerogenic activities and toxicity studies.

ABSTRACT
Various series of 3-alkylidene 3-Phenylthio-3-Chloro azetidine -2-one(5) synthesized by reaction of 1- (4'-Methoxyphenyl)-3-chloro-3-phenylthio-4,4-diethoxycarbonylazetidin-2-one (4) with thionyl chloride taking MDC as solvent while synthesis of Compound 4 carried out the reaction between phenyl thio acetic acid and N-(4-Methoxyphenyl)-1,1-diethoxycarbonylimine (2) while compound second synthesized by staring material diethyl keto malonate and p-anisidine. these newly synthesized compound were evaluate using different analytical tools These compounds were also evaluated for their anti- inflammatory and analgesic activities.

1. Introduction

Heterocycles have amazing therapeutic qualities, which are why they are found in many different medications, such as antibiotics, anti-inflammatory, and antibacterial ones. A Chloro azetidine fusedaromatic heterocyclic ring have their own significant pharma activities. With this in mind, a novel series of thio substituted chloro azetidine series of compounds synthesized with anti-inflammatory and analgesic qualities and Structure of all newly synthesized molecules determine using (IR,1H NMR,13C-CMR, Mass Spectrometry).

Chemistry

The reaction sequence lading to the synthesis of various derivatives of 3-alkylidene 3-Phenylthio- 3-Chloro azetidine -2-one was manufacture by reacting with 1-(4'-Methoxyphenyl)-3-chloro-3- phenylthio-4,4-diethoxycarbonylazetidin-2-one (4) and thinyl chloride using methylene dichlorideas solvent. compound (4) was synthesized by reaction of N-(4-Methoxyphenyl)-1,1- diethoxycarbonylimine with phenyl thio acetic acid in presence of POCl3 and TRIETHYLAMINE ae around 0°c using methylene dichloride as solvent and followed by the reaction of di ethyl keto malonate and p-anisidine using aromatic hydrocarbon benzene as solvent.

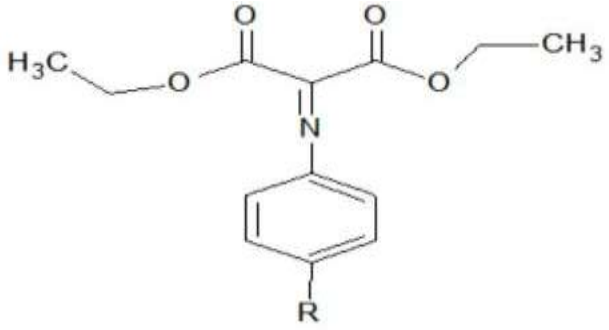
Experimental

All melting points are uncorrected and are expressed in degree(°C), using melting point SMP3 . IR spectra were recorded as KBr disks using shimadzu FT-IR 8400 using KBr disks. H NMR spectra were recorded using Bruker system AL 300 (300 MHz) and tetramethylsilane (TMS) as internal standard. C NMR spectra were recorded using Bruker system AL 300 (300 MHz) and tetramethylsilane (TMS) as internal standard C NMR.

N-(4-Methoxyphenyl)-1,1-diethoxycarbonylimine (2)

A mixture of diethyl keto malonate (0.7 g, 1 mmol) and p-anisidine (0.5 g, 1 mmol) was refluxed in dry benzene on a heating mental using a Dean-Stark apparatus. The reaction was monitored by TLC. After 4-5 h, when there was no spot left corresponding to the starting materials, benzene was removed under reduced pressure and the crude product 2a (1.0 g, 94%) thus obtained, as a liquid was used as such for thesubsequent reactions. It showed following spectral data: IR ($CHCl_3$): 1675, 1630, 1515, 1510 cm^{-1}; ^{1}H-NMR ($CDCl_3$) : 1.4 (q, 3H, $COOCH_2CH_3$), 1.5 (t, 3H, $COOCH_2CH_3$), 3.8 (s, 3H, OCH_3), 4.25 (q, 2H,

$COOCH_2CH_3$), 4.45 (2H, $COOCH_2CH_3$), 6.97 (dd, 4H, AB pattern, aromatic protons). (2H, $COOCH_2CH_3$), 6.97 (dd, 4H,AB pattern, aromatic protons).



Average Mass: 248.25456 Da

Molecular Formula: $C_{13}H_{14}NO_4$

Composition: C(62.89%) H(5.68%) N(5.64%) O(25.78%)

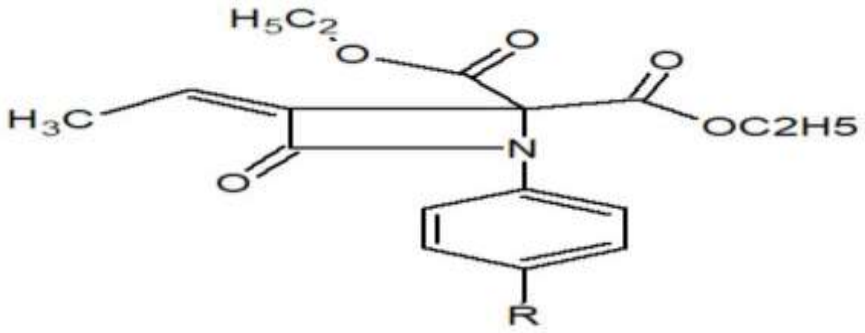
General procedure of azetidine-2-one (3)

N-(4-Methoxyphenyl)-3-ethylidene-4,4-diethoxycarbonyl azetidin-2-one 3a

To solution of 2-butenoicacid((0.45 g, 1.5 mmol), imine 2 (0.5 g, 1 mmol) and triethylamine (0.54 g, 3 mmol, 0.75 ml) in 80 mL dry methylene chloride was added dropwise under nitrogen atmosphere at 0ºC,a solution of phosphorus oxychloride ($POCl_3$) (0.41 g, 0.24 mL, 1.5 mmol) in 20 mL of dry methylene chloride with constant stirring. The reactant was stirred overnight at room temperature. The completion of reaction was monitored by TLC.

After the completion, the contents were washed successively with 1N HCl (30 ml), water (3x30 ml , 5% $NaHCO_3$ (30 ml) and brine (30 mL). The organic layer was separated and dried over anhydrous Na_2SO_4.The solvent was removed under reduced pressure and the crude product was purified by columnchromatography using silica gel eluting with 10% ethyl acetate : hexanes. Solvent evaporation furnishedpure -lactam 3a (0.66 g, 60%). Its structure was confirmed on the basis of following spectral data: m.p.

: 90–92°C ; IR ($CHCl_3$) : 1758, 1740 cm^{-1} ; ^{1}H-NMR ($CDCl_3$) : 1.2 (t, 6H, J = 7 Hz, 2x$COOCH_2CH_3$),2.2(q, 3H, CH_3),3.77 (s,3H,OCH_3), 4.40 (q, 4H, J = 7 Hz, 2x$COOCH_2CH_3$), 6.10 (q, 1H, C_{-3}-H), 6.8-7.5 (dd, 4H, aromatic protons)



Molecular Formula: $C_{17}H_{18}NO_5$

Average Mass: 316.32852 Da

Composition: C(64.55%) H(5.74%) N(4.43%) O(25.29%)

Table 01

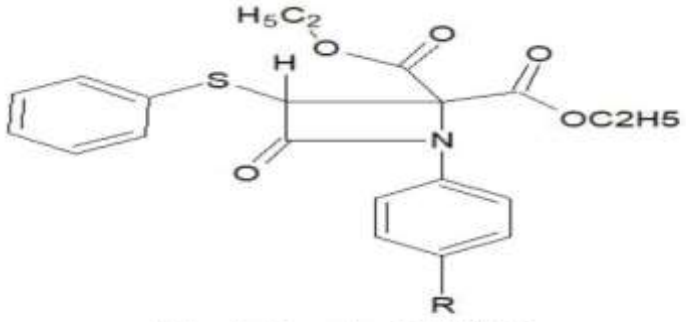
COMP	R	MP (°C)	YIELD IN (%)	RECRYSTALIZATION	MOLECULAR FORMULA	MOLECULAR WEIGHT	ELEMENTAL ANALYSIS							
							C		H		N		O	
							Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
(3)1	H	90	56	BENZENE	C17H19NO5	317.33	64.29	64.20	5.99	6.03	4.41	4.38	25.21	25.17
2	CL	93	45	XYLENE	C17H18NO5CL	351.78	57.99	58.03	5.12	5.15	3.98	4.00	22.74	22.70
3	Br	99	47	XYLENE	C17H18NO5Br	396.23	51.49	51.55	4.54	4.60	3.53	3.56	20.19	20.25
4	F	108	39	DMSO	C17H18NO5F	335.32	60.84	60.90	5.37	5.30	4.18	4.15	23.86	23.92
5	NO2	90	66	DMSO	C17H18N2O7	362.33	56.30	56.12	4.97	5.00	7.73	7.73	30.91	30.99
6	CH3	108	63	TOLUENE	C18H21NO5	331.36	65.19	65.24	6.34	6.40	4.23	4.28	24.14	24.17
7	OCH3	92	59	E/ACETATE	C18H21NO6	347.36	62.18	62.21	6.05	6.00	4.03	3.99	27.64	27.72
8	CN	89	48	BENZENE	C18H18N2O5	342.35	63.09	63.00	5.26	5.30	8.18	8.13	23.37	23.31

1-(4'-Methoxyphenyl)-3-phenylthio-4,4-diethoxycarbonylazetidin-2-one (4)

To solution of phenylthioacetic acid (0.45 g, 1.5 mmol), imine 2a (0.5 g, 1 mmol) and triethylamine (0.54g, 3 mmol, 0.75 mL) in 80 mL dry methylene chloride was added dropwise under nitrogen atmosphere at0ºC, a solution of phosphorus oxychloride ($POCl_3$) (0.41 g, 0.24 mL, 1.5 mmol) in 20 mL of dry methylenechloride with constant stirring. The reactant were stirred overnight at room temperature. The completionof reaction was monitored by TLC. After the completion, the contents were washed successively with 1NHCl (30 mL), water (3x30 mL), 5% $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was separatedand dried over anhydrous Na_2SO_4.

The solvent was removed under reduced pressure and the crude product was purified by column chromatography using silica gel eluting with 10% ethyl acetate : hexanes. Solvent evaporation furnishedpure -lactam 3b (0.66 g, 60%). Its structure was confirmed on the basis of following spectral data: m.p.

: 94–95°C ; IR ($CHCl_3$) : 1758, 1740 cm^{-1} ; ^{1}H-NMR ($CDCl_3$) : 1.2 (t, 6H, J = 7 Hz, 2x$COOCH_2CH_3$), 3.77 (s,3H,OCH_3), 4.40 (q, 4H, J = 7 Hz, 2x$COOCH_2CH_3$), 5.20 (s, 1H, C_3-H), 6.8-7.5 (m, 9H, aromatic protons); ^{13}C-NMR ($CDCl_3$) : 13.8, 14.16, 55.20, 62.11, 62.76, 72.4, 113.9, 121.4, 127.3, 129.32, 129.5, 130.3, 133.9, 157.3, 161.3, 165.5, 165.8; Anal. Calcd. for $C_{22}H_{23}O_6NS$: C, 61.53; H, 5.36; N, 3.26; Found: C, 61.40; H, 5.29; N, 3.21.



Molecular Formula: $C_{22}H_{23}NO_6S$
Average Mass: 398.4522 Da
Composition: C(63.30%) H(5.06%) N(3.52%) O(20.08%) S(8.05%)

TABLE 02

COMP	R	MP (°C)	YIELD IN (%)	RECRYSTAIIZATION	MOLECULAR FORMULA	MOLECULAR WEIGHT	ELEMENTAL ANALYSIS							
							C		H		N		O	
							Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
(4)1	H	90	60	BENZENE	C21H21NO5S	399	63.16	63.12	5.26	5.33	3.51	3.55	20.05	20.15
2	CL	93	56	TOLUENE	C21H20NO5SCl	433.9	58.08	58.00	4.61	4.55	3.23	3.25	18.44	18.36
3	Br	98	59	DMSO	C21H20NO5SBr	478.35	52.68	52.63	4.18	4.15	2.93	2.98	16.72	16.66
4	F	105	48	DMSO	C21H20NO5SF	417.45	60.37	60.30	4.79	4.79	3.35	3.42	19.16	19.23
5	NO2	102	69	TOLUENE	C21H20N2O7S	444.45	56.70	56.55	4.50	4.42	6.30	6.25	25.20	25.30
6	CH3	99	70	XYLENE	C22H23NO5S	413.48	63.85	63.82	5.56	5.50	3.39	3.33	19.35	19.34
7	OCH3	95	72	MDC	C22H23NO6S	429.48	61.47	61.38	5.36	5.32	3.26	3.23	22.35	22.40
8	CN	110	50	MDC	C22H20N2O5S	424.46	62.20	62.15	4.71	4.65	6.60	6.70	18.85	18.90

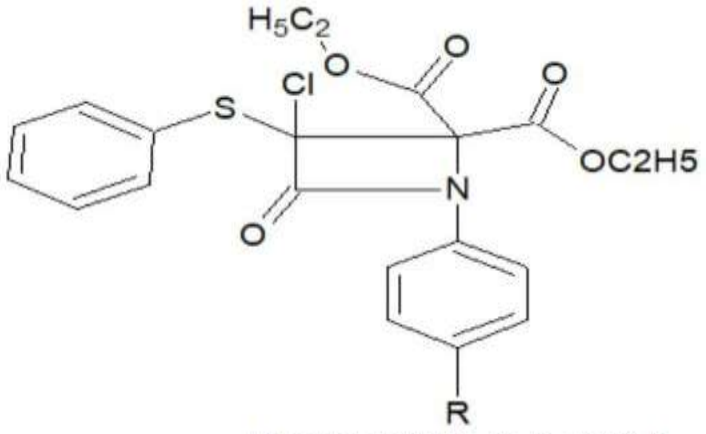
1-(4-Methoxyphenyl)-3-Chloro-3-phenylthio-4,4-diethoxycarbonylazetidin -2-one(5)

To a well stirred solution of α- phenylthio-β-lactam 4 (0.9g, 2mmoles)in 50 ml dry methylene Chloride

, under nitrogen at 0 °C , was added a solution of sulfuryl Chloride (SO_2Cl_2) (0.39g , 2mmol,0.2ml) in 10 ml dry methylene Chloride in 10 minutes contents were stirred for additional half hour . The progressof reaction was monitored by TLC. Solvent evaporation followed by column chromatography on silica gel using ethylacetate : hexanes(1:10) yielded pure β- lactam 3c (1.0g , 75%) ,IR: 1760,1720 cm^{-1}

,^{1}HNMR ($CDCl_3$) δ1.26 (t,6H, $2XCOOCH_2CH_3$),3.78(s,3H,OCH_3), 4.35(q,4H, $2XCOOCH_2CH3$),6.83- 7.73 (m,9H,aromatic protons) . ^{13}CNMR($CDCl_3$) δ:13.94, 29.70,55.459 , 63.37 , 113.97, 120.99 121.32

,127.36, 128.96, 129.15, 129.32,130.31, 130.47, 136.42 , 157.35, 159.15, 163.55



Molecular Formula: $C_{21}H_{19}ClNO_5S$

Average Mass: 432.89726 Da

Composition: C(58.26%) H(4.42%) Cl(8.19%) N(3.24%) O(18.48%) S(7.41%)

TABLE 03

COMP	R	MP (°C)	YIELD IN (%)	RECRYSTAIIZATION	MOLECULAR FORMULA	MOLECULAR WEIGHT	ELEMENTAL ANALYSIS							
							C		H		N		O	
							Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
(5)1	H	88	45	MDC	C21H20NO5ClS	433.9	58.08	58.03	4.61	4.70	3.23	3.25	18.44	18.44
2	CL	83	43	MDC	C21H19NO5Cl2S	468.35	53.81	53.76	4.06	4.10	2.99	3.00	17.08	17.00
3	Br	84	40	ACETONE	C21H19NO5ClBrS	512.8	49.14	49.19	3.71	3.75	2.73	2.75	15.60	15.56
4	F	88	45	ACETONE	C21H19NO5ClFS	451.89	55.77	55.89	4.20	4.25	3.10	3.15	17.70	17.78
5	NO2	94	50	Ethyal acetate	C21H19N2O7ClS	478.9	52.62	52.70	3.97	4.00	5.85	5.90	23.39	23.44
6	CH3	97	52	Methanol	C22H22NO5ClS	447.93	58.94	59.00	4.91	4.93	3.13	3.18	17.86	17.90
7	OCH3	90	55	Methanol	C22H22NO6ClS	463.93	56.91	56.99	4.74	4.80	3.02	3.10	20.69	20.72
8	CN	103	38	EDC	C22H19N2O5ClS	458.91	57.53	57.63	4.14	4.17	6.10	6.14	17.43	17.50

Series of 3 ,4 and 5 were tested for their acute toxicity as well as their anti-inflammatory and analgesic properties.

Albino Charles-Foster strain rats of either sex—but not pregnant females—and between the ages of6 and 9 months, weighing between 100 and 120 g, were used in the experiment. The animals were provided with unlimited access to food (chaw pallet) and water. Propylene glycol was used to dissolve the test Molecules. For the comparison of the anti-inflammatory and analgesic activity, indomethacin and phenylbutazone were used as reference drugs.

Anti-inflammatory activity against carrageenan-induced rat 's paw oedema

This study was carried out in accordance with the methodology used by Winter et al. Three groups of six rats each were created using the terms "control," "drug treated," and "standard drug." Each ratreceived 0.05 ml of a freshly made suspension of carrageenan (1% in 0.9% saline) injected under theplanter aponeurosis of the right hind paw.

Animals from drug-treated groups and the standard drug group, respectively, received test Moleculesand the standard drug orally one hour prior to the carrageen injection. A plethymometer was used tomeasure each rat's paw volume before and after it was treated with carrageenan for 1 and 3 hours. Utilizing the following formula, the percent anti-inflammatory activity was calculated.

Oedema inhibition percentage: (1-Vt/Vc) 100

Where, for the drug, treated, and control groups, respectively, Vt and Vc represent the volume of oedema. The results were statistically analyzed.

study of acute toxicity Using the Smith et al. [1960] method, the test Molecules' acute toxicity (ALD50) was examined in albino mice. Different doses of the test substances were orally administered to various animal groups. After giving the drug for 24 hours, the percent mortality in each group was noted. ALD50 was calculated using the information gathered.

Analgesic activity

This activity was completed using Berkowitz et al.'s method. This approach is based on the test Molecule's ability to counteract the pam syndrome in mice caused by phenyl quinone. After giving the test substance to groups of five mice orally, 0.25 ml of a 0.02% solution of phenylquinone in ethanol (5%) was injected intraperitoneally. Following the injection of an irritant, each mouse's number of writhes was counted for 5 min (between 5 and I 0 min). The percentage of protection in comparison to control was used to measure the analgesic effect.

TABLE 04

COMP	R	Anti-inflammatory activity		Analgesic Activity		Acute toxicityALD50mg/Kg p.o.
		DOES (MG/KG p.o)	%Oedemainhibitor relation	DOES (MG/KG p.o)	% protection	
(3)1	H	50.00	9.80	50.00	8.90	>1200
2	CL	50.00	24.65	50.00	11.19	>1200
3	Br	50.00	19.33	50.00	17.80	>1200
4	F	50.00	20.50	50.00	15.53	>1200
5	NO2	50.00	13.56	50.00	10.30	>1200
6	CH3	50.00	22.33	50.00	11.80	>1200
7	OCH3	50.00	23.44	50.00	9.27	>1200
8	CN	50.00	18.33	50.00	9.27	>1200
(4)9	H	50.00	21.5	50.00	11.20	>1200
10	CL	50.00	16.80	50.00	8.99	>1200
11	Br	50.00	20.60	50.00	11.50	>1200
12	F	50.00	24.20	50.00	16.70	>1200
13	NO2	50.00	39.50	50.00	43.00	>1200
14	CH3	50.00	38.90	50.00	39.50	>1200
15	OCH3	50.00	40.50	50.00	37.00	>1200
16	CN	50.00	32.00	50.00	28.50	>1200
(5)17	H	50.00	34.54	50.00	28.30	>1200
18	CL	50.00	33.23	50.00	27.32	>1200
19	Br	50.00	32.35	50.00	27.34	>1200
20	F	50.00	30.50	50.00	32.00	>1200
21	NO2	50.00	38.35	50.00	37.70	>1200
22	CH3	50.00	37.45	50.00	37.33	>1200
23	OCH3	50.00	41.33	50.00	37.91	>1200
24	CN	50.00	33.50	50.00	35.00	>1200

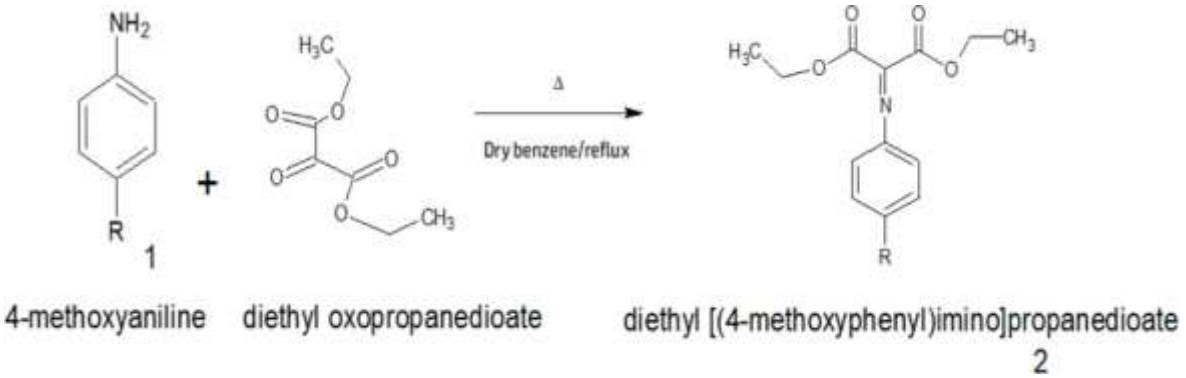
2. Results and Discussion

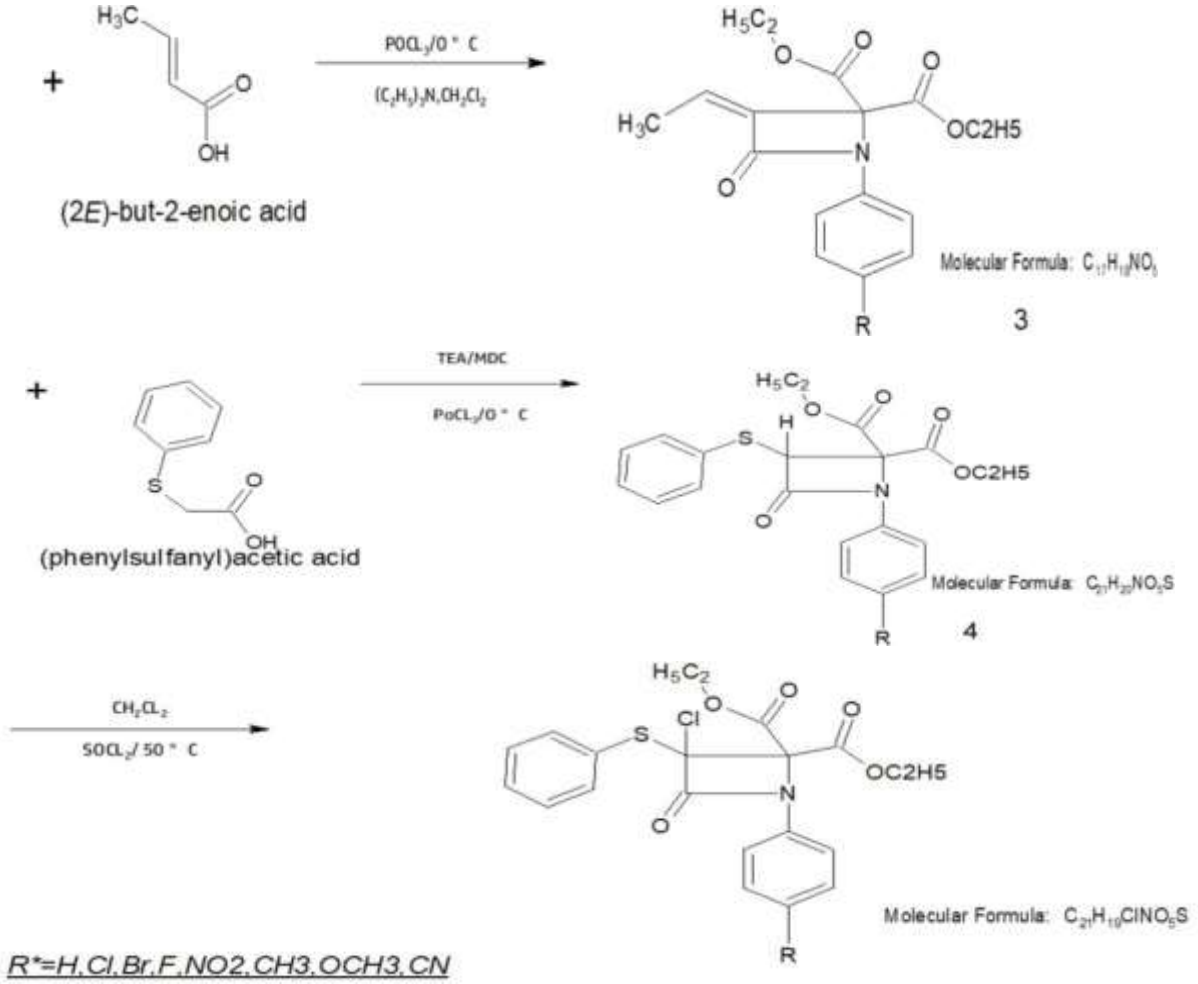
Recently created molecules were investigated for their ability to reduce inflammation in response tooedema caused by carrageenan. Every molecule was examined at an oral dosage of 50 mg/kg. Table4 displays the study's findings. ALI's series 3 ,4 and 5 molecules have demonstrated varying degreesof anti-inflammatory activity (9.80–42.00%). In the phenylbutazone comparison, the active molecules in this series, 5{21} and 5{22},5{23} and 4{13},4{14},4{15} were found to have more powerful anti-inflammatory activity. The molecule 5{23}&4{15}, which had a methoxy group ASAs Substitution molecules, demonstrated a 41.33% & 40.50% Respectively inhibition of oedema. The hydrogen as as subtitution containing molecule 3{1} had the lowest activity of 9.80%. The superior anti-inflammatory activity, demonstrated by molecules 4{15} 40.50% respectively and by 5{23} 41.33% at a dosage of of 50 mg/kg p.o. as compared to phenylbutazone .

Similarly, molecules of series 4 which containing No2&

-OCH3 group as substitution show highest analgesic properties such as 4{13} gives 43.00% and 4{14} gives 39.50% same as in series 5 molecules contain -OCH3 group as substitution gives highestanalgesic activities 5{23} gives 37.91 % of analgesic activities against standard.

REACTION SKIM





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