IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2020 Vol.:19, Issue:2

Synthesis of Novel Anti-Inflammatory Benzamide Derivatives Utilizing Non-Catalyst and Non-Solvent Smiles Rearrangements

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ISSN 2349-7203
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S. K. Mohite[1], P. M. Somade[2], A.R.Chopade[1]*, V. R. Dharanguttikar[1], S. A. Thorat[1]

[1]Rajarambapu College of Pharmacy, Kasegaon, Sangli, Maharashtra, India

[2]Krishna Institute of Medical Sciences, Karad, Maharashtra, India

Submission: 25 August 2020
Accepted: 31 August 2020
Published: 30 September 2020

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Docking study, benzamide derivatives, Anti-inflammatory activity

ABSTRACT

In this analysis, by synthesizing the sequence of N-substituted n-{[4-(pyrimidine-2-ylsulfamoyl) phenyl] carbamothioyl} benzamide derivatives of 2C (2C1-2C4) moiety, benzamide derivatives of 2C (2C1-2C4) moiety were planned and synthesized by Smiles rearrangement mechanism to develop a potential anti-inflammatory drug. Synthesized compounds have been docked with lipoxygen-3 soybean anti-inflammatory activity complex receptors (PDB Code-1IK3). Compounds 2C demonstrated powerful anti-inflammatory receptor activity against lipoxygenase-3 soybean complex receptors (PDB Code-1IK3). These 2C synthesized compounds were confirmed using IR, NMR, and MASS by spectral characterization. These compounds adopted the OECD Guideline 425 up and Down Method of Acute Oral Toxicity Analysis, which showed that these compounds were non-toxic. After an *in-vivo* and *in-vitro* study of the effects of anti-inflammatory activity, these compounds demonstrated promising usage compared to standard medicines. Hence present study described a novel method for synthesizing benzamide derivatives, in a single step with direct synthesis without any catalyst or nonsubsequent reagents.

INTRODUCTION:

Chronic inflammatory disease is a medical disorder characterized by chronic inflammation, described primarily by new connective tissue formation as a prolonged and persistent pro-inflammatory state. The local response of living mammalian tissues to injury due to any agent is known as inflammation. To suppress or restrict the spread of injurious agents, it is a body defense reaction, accompanied by the removal of necrosis cells and tissues. The most commonly prescribed, safe, and cost-effective pharmacological treatment of rheumatologic and inflammatory disorders is conventional non-steroidal anti-inflammatory drugs (NSAIDs) [1]. In addition, to treat mild to severe pain, this class of medications is commonly used. There are restrictions on medicinal usage for most commonly used non-steroidal anti-inflammatory drugs (NSAIDs) since they cause gastrointestinal and renal side effects that are inseparable from their pharmacological activities. As potential donor ligands of transition metal ions, compounds containing carbonyl and benzamide groups occupy a significant role among organic reagents. Among these thiourea derivatives are ligands that are potentially very versatile[2]. Thiourea derivatives' oxygen, nitrogen, and sulfur donor atoms give a range of bonding possibilities. A wide variety of biological activity is demonstrated by both the ligands and their metal complexes, including anti-inflammatory[3]. Some carbamothioyl derivatives resist bacterial growth and cell division Benzamide is the powerful anti-inflammatory agent used for many years to treat or prevent systemic inflammatory infections. In this study, a novel benzamide synthesis of 2-chloro-N-{[4-(pyrimidine-2-ylsulfamoyl) phenyl] carbamothioyl} benzamide was synthesized and tested for anti-inflammatory sensitivity tests.

Thus benzamide derivatives were further studied in the Insilco-pharmacology analysis for the synthesis by docking process where the novel thiourea ligands were docked on the receptor. The docking process was carried out on the lipoxygenase-3 soybean anti-inflammatory activity complex receptor (PDB Code-1IK3)[4]. To investigate their anti-inflammatory function, we have synthesized 4 new thiourea derivatives carrying urea, amide, and sulphonamide groups. Via spectral characterization using IR, NMR, and Mass, all compounds were confirmed. These studies indicate that the compounds are non-toxic and have shown promising anti-inflammatory effects.

MATERIAL AND METHOD

Requirements

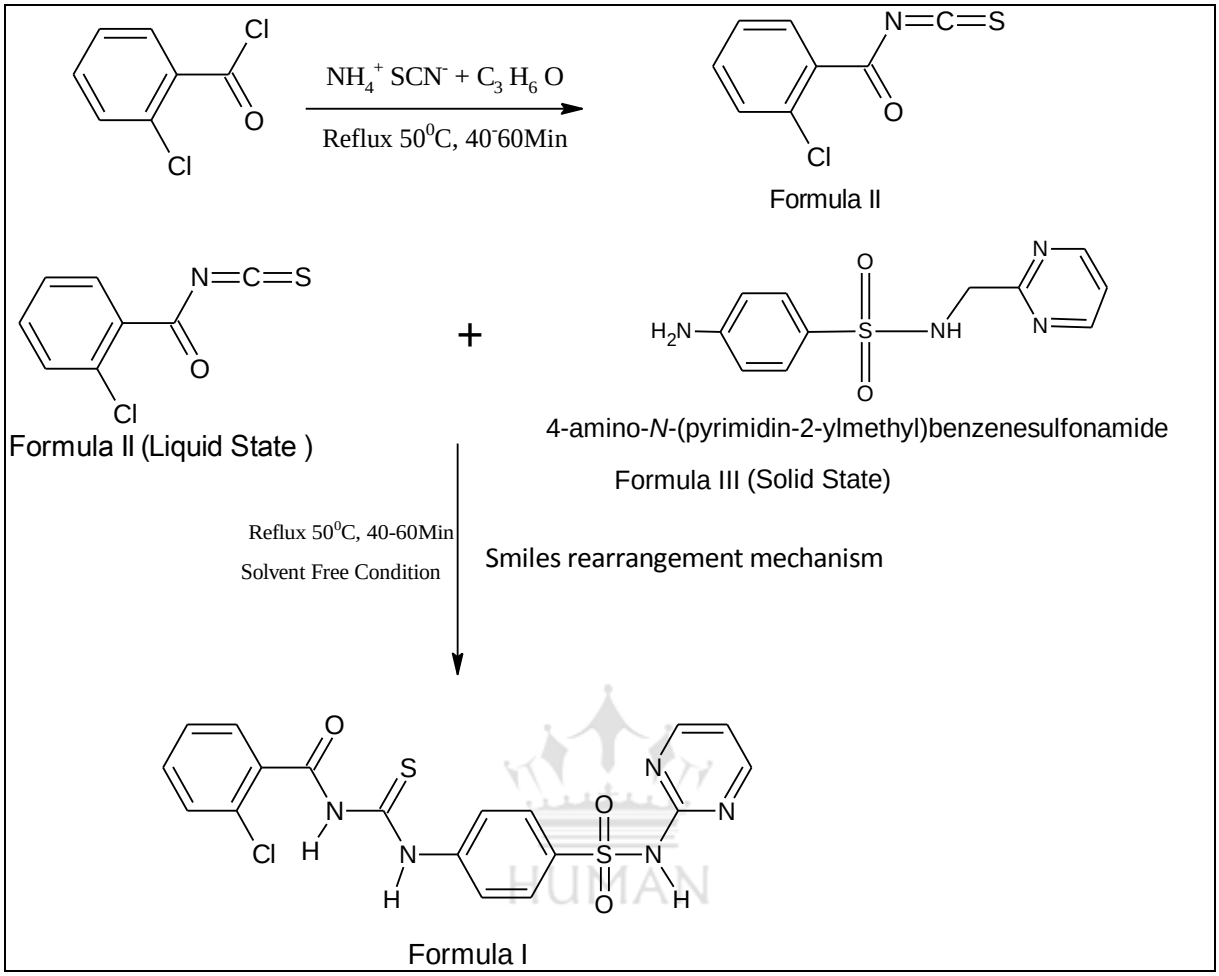
All chemical reagents and solvents were procured from commercial sources, purified and dried using standard procedures from literature whenever required the reagents were purchased from 2-chloro benzoyl chloride, ammonium thiocyanate, and acetone were purchased from Research laboratory, Mumbai.4-Nitro benzoyl chloride, 4-chloro benzoyl chloride were purchased from Marck laboratory, Mumbai and the 4-methoxy benzoyl chloride, Dichloro (isothiocyanatocarbonyl) sulfanium were purchased from S.D. Fine-chem. limited, Mumbai. The chemical required for TLC mobile phase such as Toluene Acetic acid was issued from the Research Laboratory of Rajarambapu College of Pharmacy, Kasegaon. Also, some other chemical required for animal activity studies such as CMC and Carrageenan was collected from the same research laboratory. Melting points were determined by the open capillary tube method and are uncorrected. TLC was used to assess the course of the reaction and the purity of the intermediate and the final compounds were confirmed by applying a single spot on the TLC plate (Silica gel G) using various solvents such as toluene: acetone system. TLC plates were visualized using an iodine chamber. IR spectra were recorded using ATR JASCO FTIR-4600. H1NMR spectra were performed in DMSO solution using 300 MHz Bruker spectrometers indicating chemical shift value on ppm scale and TMS was taken as an internal reference. Mass spectra were recorded on Pesciex (model no. API 2000) software analyst 1.4.2. Q1MS Q1/AUTO INJECTION

Chemistry

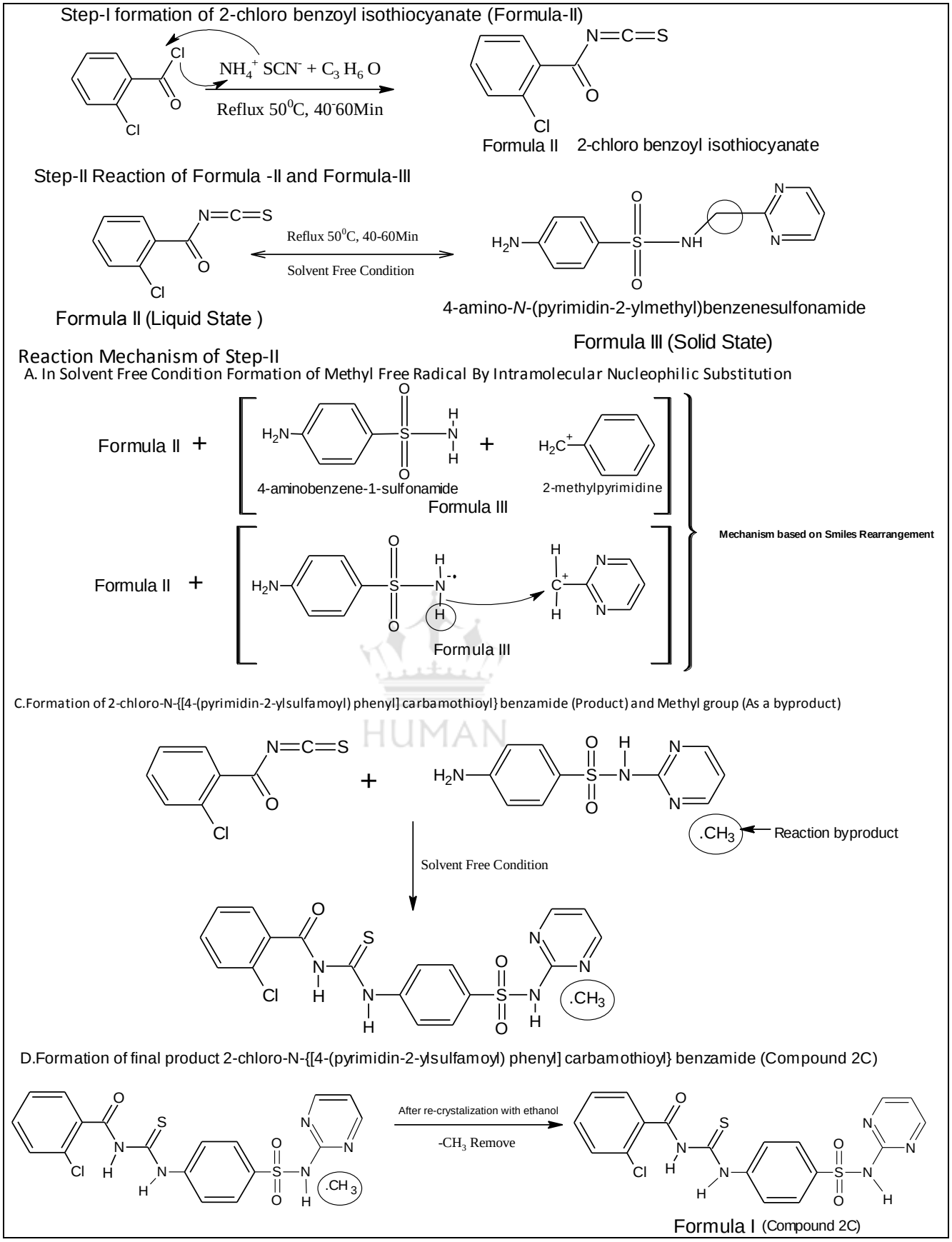
Synthesis of the desired compounds 2Cwas achieved by allowing a solution of aroyl chloride and ammonium thiocyanate in acetone in a molar ratio of 1:1:2 in 500 mL of the round bottom flask and then they said mixture was subjected for reflux at 50^{o}C for 40 to 60 min to obtain. After completion of the reaction (confirmed by TLC), the reaction mixture was cooled to room temperature and the precipitate was filtered off. And collect the freshly prepared solution of aroylisothiocyanate derivative (primary reactant) [5].

4-Amino-N-pyrimidin-2-yl methyl benzenesulfonamide (secondary reactant) was added to aroylisothiocyanate derivative in a molar ratio of 2:4 was the mixture was reflux for 40 to 60 min at50^{o}C. Then the product was monitored by TLC, the resulting precipitate was collected

by filtration and the product was recrystallized in butanol to give the pure product as shown in Scheme.[6]



Scheme 1.a: - General reaction for synthesis of benzamide derivative



Scheme 1.b: - Reaction Mechanism According To Smiles Rearrangement

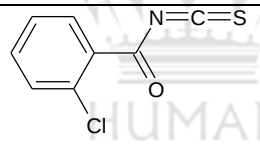
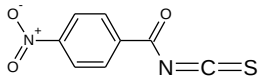
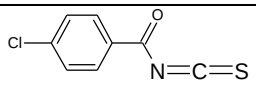
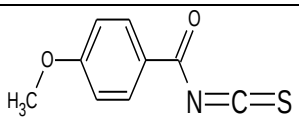
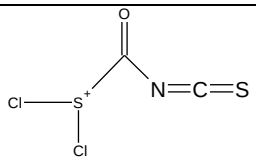
EXPERIMENTAL PROTOCOLS

Synthesis

a. Preparation of intermediate compound i.e. Formula II (substituted benzoyl isothiocyanate compound):

2-chloro benzoyl chloride, ammonium thiocyanate, and acetone in a molar ratio of 1:1:2 were added in 500 mL of round bottom flask and then said mixture was subjected for reflux at 50^{o}C for 40 to 60 min to obtain 2-chloro benzoyl isothiocyanate. Similarly, 4-Nitro benzoyl isothiocyanate, 4-chloro benzoyl isothiocyanate, 4-methoxy benzoyl isothiocyanate, or dichloro (isothiocyanatocarbonyl) sulfanium were prepared[7-8].

Table No. 1: Characterization data of Intermediate compound (benzoyl isothiocyanate compound)

Intermediate Name	Structure	Boiling point	Percentage Yield (% Yield)
2-chloro benzoyl isothiocyanate		198-210^{0}C	91.20%
4-Nitro benzoyl isothiocyanate		170-180^{0}C	90.75%
4-chloro benzoyl isothiocyanate		185-210^{0}C	65.96%
4-methoxy benzoyl isothiocyanate		150-170^{0}C	69.52%
Dichloro (isothiocyanatocarbonyl) sulfanium		120-130^{0}C	69.45%

b. Preparation of final compound 2C(Formula-I):

2-chloro benzoyl isothiocyanate (primary reactant) and 4-Amino-N-pyrimidin-2-ylmethyl benzenesulfonamide (secondary reactant/formula-III) in a molar ratio of 2:4 were added in 500 mL of the round bottom flask and the said mixture was then subjected for reflux at 50^{o}C for 40 to 60 min to obtain 2-chloro-N-{[4-(pyrimidin-2-ylsulfamoyl) phenyl] carbamothioyl} benzamide (compound 2c). The compound (2C) was further purified by the chromatography and re-crystallization technique. The purity of the synthesized compound (2C) was checked by TLC using the mobile phase Toluene – Acetic acid (7:3) as shown in Scheme. Similarly, compound 2c-1, 2c-2, 2c-3, 2c-4 were prepared by changing the primary reactant[9].

(2c):- Synthesis of (2-chloro-N-{[4-(pyrimidin-2-ylsulfamoyl) phenyl] carbamothioyl} benzamide): Yield: 97.75**%**, mp200-210^{0}C. IR (KBr): 2938, 1691, 1531, 1438, 1164, 835 cm-1. 1H NMR (400 MHz, DMSO-d6): 12.71 (s, 1H, NH), 11.95 (s, 1H, NH), 8.43,8.41,8.17 (t, J ¼ 7.7, 1.2 Hz, 2H, H2, H6), 7.96,7.52,7.50 (d, J ¼ 8.5 Hz, 2H, sulfamoylphenyl), 7.47 (dd, J ¼ 8.5 Hz, 2H, sulfamoylphenyl), 7.40 (s, 1H, NH2), 6.94(dd, J ¼ 8.5 Hz, 2H, sulfamoylphenyl) MS (70 eV): m/ z ¼ 391.89 [Mþ_]. Anal.Calcd for C18H14ClNO3S2: C, 55.17; H, 3.6; N, 3.57.Found: C, 67.91; H, 7.44; N, 18.89.

(2C1):-Synthesis of 4-nitro-*N*-{[4-(pyrimidin-2-ylsulfamoyl) phenyl] carbamothioyl} benzamide.

Yield: 97.00 **%**, mp 203-205^{0}C. IR (KBr): 2900, 1644, 1577, 1440, 1164, 838 cm^{-1}. 1H NMR (400 MHz, DMSO-d6): 12.72 (s, 1H, NH), 11.71 (s, 1H, NH), 7.99 (dd, J ¼ 7.7, 1.2 Hz, 2H, H2, H6), 7.91 (d, J ¼ 8.5 Hz, 2H, sulfamoylphenyl), 7.86 (d, J ¼ 8.5 Hz, 2H, sulfamoylphenyl), 7.66 (t, J ¼ 7.7 Hz, 1H, H4), 7.55 (t, J ¼ 7.7 Hz, 2H, H3,\H5), 7.42 (s, 2H, NH2). MS (70 eV): m/ z ¼ 458.47 [Mþ_]. Anal. Calcd for $C_{18}H_{14}N_6O_5S_2$: C, 51.56; H, 6.63; N, 12.03. Found: C, 67.91; H, 7.44; N, 18.89.

(2C2):-Synthesis of 4-chloro-*N*-{[4-(pyrimidin-2-ylsulfamoyl) phenyl] carbamothioyl} benzamide.

Yield: 68.96 **%**, mp 199-205^{0}C. IR (KBr): 1671, 1590, 1434, 1145, 831 cm^{-1}. 1H NMR (300 MHz, DMSO-d6): 8.38(dd, J ¼,2H,2CH),8.00 (s, 1H, NH), 7.89 (dd, J ¼ 7.7, 1.2 Hz, 2H, H2, H6), 7.68(dd J¼,2.5 Hz,2H,H2,H6), 7.45 (dd, J ¼ 8.5 Hz, 2H, sulfamoylphenyl),6.74(dd J¼,2.5 Hz,2H,H2,H6),6.58(s,1H,CH),4.0 (dd, 1H, NH). MS (70 eV): m/ z ¼ 447.91 [Mþ_].

Anal. Calcd for $C_{18}H_{14}ClN_5O_3S_2$: C, 52.38; H, 5.75; N, 11.75. Found: C, 67.91; H, 7.44; N, 18.89.

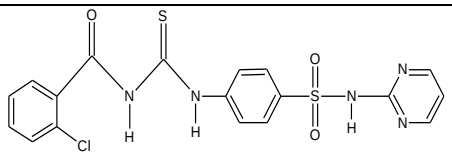
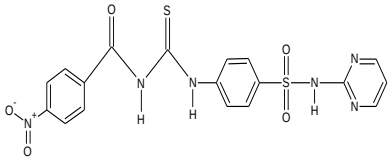
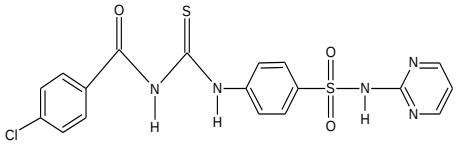
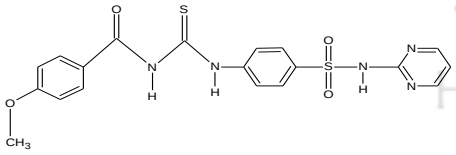
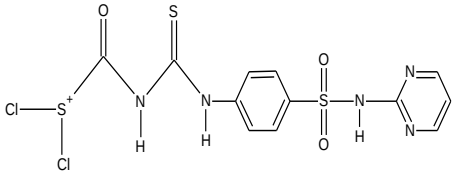
(2C3):- Synthesis of 4-methoxy-*N*-{[4-(pyrimidin-2-ylsulfamoyl) phenyl] carbamothioyl} benzamide.

Yield: 70.60**%**, mp 200-210^{0}C. IR (KBr): 2938, 1658, 1581, 1139, 835 cm^{-1}.1H NMR (400 MHz, DMSO-d6): 12.72 (s, 1H, NH), 11.71 (s, 1H, NH), 7.99 (dd, J ¼ 7.7, 1.2 Hz, 2H, H2, H6), 7.91 (d, J ¼ 8.5 Hz, 2H, sulfamoylphenyl), 7.86 (d, J ¼ 8.5 Hz, 2H, sulfamoylphenyl), 7.66 (t, J ¼ 7.7 Hz, 1H, H4), 7.55 (t, J ¼ 7.7 Hz, 2H, H3,\H5), 7.42 (s, 2H, NH2). MS (70 eV): m/ z ¼ 443.5[Mþ_]. Anal. Calcd for $C_{19}H_{17}N_5O_4S_2$: C, 54.8; H, 6.3; N, 11.84. Found: C, 67.91; H, 7.44; N, 18.89.

(2C4):-Synthesis of dichloro ({[4-(pyrimidin-2-ylsulfamoyl) phenyl] carbamothioyl} carbamoyl) sulfanium.

Yield: 72.52 **%**, mp 180-200^{0}C. IR (KBr): 1697, 1538, 1402, 829 cm^{-1}.1H NMR (400 MHz, DMSO-d6): 12.72 (s, 1H, NH), 11.71 (s, 1H, NH), 7.99 (dd, J ¼ 7.7, 1.2 Hz, 2H, H2, H6), 7.91 (d, J ¼ 8.5 Hz, 2H, sulfamoylphenyl), 7.86 (d, J ¼ 8.5 Hz, 2H, sulfamoylphenyl), 7.66 (t, J ¼ 7.7 Hz, 1H, H4), 7.55 (t, J ¼ 7.7 Hz, 2H, H3,\H5), 7.42 (s, 2H, NH2). MS (70 eV): m/ z ¼ 439.33 [Mþ_]. Anal. Calcd for $C_{12}H_{10}Cl_2N_5O_3S_3$: C, 42.41; H, 6.23; N, 10.3. Found: C, 67.91; H, 7.44; N, 18.89.

Table No. 2: Characterization data of final compound

Compound	Structure	Primary reactant	Secondary reactant	Melting Point	Percentage Yield (% Yield)
2c		2-chloro benzoyl isothiocyanate	4-Amino-N-pyrimidin-2-ylmethyl benzenesulfonamide	200-210ºC	97.75%
2c-1		4-Nitro benzoyl isothiocyanate		203-205ºC	97.00%
2c-2		4-chloro benzoyl isothiocyanate		199-205ºC	68.96%
2c-3		4-methoxy benzoyl isothiocyanate		200-210ºC	70.60%
2c-4		Dichloro (isothiocyanato carbonyl) sulfanium		180-200ºC	72.52%

Molecular Docking:

Software Methodology

The BioPredicta (VLifeMDS 4.1, Pune) program was used in the binding pocket of the structure of antimicrobial receptors to dock potential inhibitors (Ligand). In the protein binding sites selected by the user, VLifeMDS has given a facility to dock different ligands. VLifeMDS has provided both rigid (no torsional flexibility for both a protein and a ligand) and flexible (torsional flexibility for a rigid protein-ligand) molecular docking. Less

inaccurate poses were found to be created by BioPredicta and 85% of binding models had an RMSD of 1.4A0 or less from a co-crystallized native structure[10].

A variety of new benzamide derivatives have been synthesized characterized and identified as potential anti-inflammatory agents in the past. 2C (2C1-2C4) benzamide derivatives were modeled using VlifeMDS 4.6 software in the current analysis.

Ligand Preparation

In VlifeMDS, all ligand molecules are drawn in 2D format and then transformed into 3D, which is easier for further docking. By using temple-based alignment techniques, all molecules of ligands are synchronized. Energy minimization of batch optimized ligands optimizes a series of molecules where the energy of all molecules is determined by Force Field batch minimization for that phase by the parameter set as Entering 10000 as a Maximum number of cycles, 0.01 as Convergence Criterion, 1.0 as Constant (medium dielectric constant, 1). From the Force, Field drop-down chart, pick MMFF. The MMFF atomic charges are chosen automatically for the MMFF Force Field.

Protein Selection

Protein selection for docking studies is dependent on many variables, i.e. X-ray diffraction should decide the structure and the resolution should be between 2.0-2.5Ao, it should contain co-crystallized ligands; no protein breaks in its 3D structure should be found in the selected protein[11].

Protein Preparation

The RCSB protein data bank (http:/www.rcsb.org/pdb) obtained the X-ray crystal structures of the protein viz., lipoxygenase-3 soybean complex (PDB Code- lIK3) with a resolution of 1.8 Å (for antimicrobial activity). The protein preparation wizard of the VlifeMDS suite was used to prepare protein after collection. By deleting the crystallographically observed water molecules (water without H bonds), correcting the errors in the PDB file, optimizing hydrogen bonds, the proteins were individually preprocessed. Using MMFF force field value, energy minimization with root mean square deviation (RMSD) value of 0.30Å was finally achieved after assigning charge and Protonation state[12].

Molecular Docking

Using GRIP Batch docking using Biopredicta software, the binding of the ligand molecule to the protein molecule was analyzed. Software to find the correct conformation of the ligand (with the rotation of the bonds, the molecule structure is not rigid) and configuration (with the rotation of the entire molecule, the molecule structure remains rigid) to obtain the minimum structure of energy. Choose the necessary cavity number for the docking to be carried out. The parameters used for the docking were selected as the 30o phase size of the Exhaustive and input Rotation Angle by which the ligand is rotated for different poses. Input Number of Placements as 30 and Ligand Wise Results as 5 for each ligand to obtain 5 top poses[13].

Animal Studies:

Acute Toxicity Study:

Animals: Animals In this study, Wistar rats (150-200 g) and Swiss albino mice (18-30 g) of either sex were purchased from the National Institute of Biosciences Animal House, Pune. Standard rodent pellets (National Institute of Biosciences, Pune, and Maharashtra) and water were maintained under standard laboratory conditions (12 h light / dark cycles at 22 ± 2 ⁇ C) and fed to the animals. For the monitoring and supervision of animal experiments, the protocol was approved by the Committee (Reg. No. 1290 / PO / Re / S/09 / CPCSEA 19/01/17)[14].

Acute Toxicity Study: Method for acute oral toxicity- Up and Down: The test procedure defined following the OECD Guideline for estimating the acute oral toxicity of a new chemical compound (such as 2c) has been adopted. The acute toxicity test consists of a single ordered progression of the dose under which the animals are dosed once at a time at intervals of at least 48 hours. Until beginning the dosing, all animals fasted overnight and the dosing was started with a dose of 175 mg/kg. Test compound 2c was administered orally with 0.5 percent CMC in the form of suspension.175mg / kg b.w. was obtained by the first animal. The dose and subsequent doses increased by a factor of 3.2 to 2000 mg/kg b.w. During the first 30 minutes after dosing, the animals were examined individually, regularly during the first 24 hours (with special emphasis during the first 4 hours) and daily for a total of 14 days thereafter. All signs of toxicity during the time must be registered. It was verified that up to 1500mg / kg b.w/w was safe for this synthesized 2C compound[15].

***In-vivo* Anti-Inflammatory Activity:**

Carrageenan-induced paw edema method: Increase in the rat hind paw linear circumference induced by plantar injection of the phlogistic agent used for measurement of acute inflammation. Three groups of each as control, standard, and test (compound 2c). All these groups are to be kipped for fasting overnight and only allowed water ad labium. The test rats (n = 6) were received the synthesized compound 2c at dose 20 mg/kg, oral. While the standard rats ware received a dose of Aceclofenac (10 mg/kg, oral). One hour after treatment, 0.1 ml of carrageenan (1%, w/v in normal saline) administered into the sub-plantar tissue of the right hind paw of both test and standard groups. The linear paw circumference was measured for all the animals before starting the dosing procedures such as before injecting the phlogistic agent (carrageenan). After injecting, the carrageenan linear paw circumference was measured for the three groups such as control, test, and standard. The group's linear paw circumference was recorded after an interval of every 30 minutes for each group up to 4 hours by using the cotton thread method. Anti-inflammatory activity was determined by analyzing the reduction in edema size and calculating % inhibition of edema. A mean reduction in edema when compared with control and an increased percentage inhibition in the treated groups is an indication of anti-inflammatory activity[17].

RESULTS AND DISCUSSION

a. The principle involved in molecular rearrangement for the synthesis of the benzamide derivative:

Formula-II and Formula-III reaction is carried out under reflux condition (i.e. 50^{o}C for 40 to 60 minutes) in our initial foray into the wide range of unexplored substrate structures viable for Smiles rearrangement. Scientifically interpreted as the heating of the solid-liquid process of synthetic reagents at continuous temperature without any solvent, the molecules are subjected to intra-molecular nucleophilic replacements under the condition that the Formula-III molecule is ionized into two species to form the final product[18]. The use of this mechanism facilitated the synthesis of a variety of substrates that were easily modified at key points of elaboration in substrate design. The substrates incorporate an amino functional group to lend resonance stabilization to the proposed Nucleophile. This is disposed of appropriately to the ring such as to proceed through pyrimidine ring by Intra-molecular nucleophilic substitutions, which are favored by Smiles rearrangements. In general, a modular approach was established through a reaction of an array of available substituted

sulfonamide that allowed substituent effects to be examined while maintaining a consistent distance between the Nucleophile carbanion and the electrophonic ring atom[19].

b. Molecular Docking on Anti-Inflammatory Receptor:

Lipoxygenase-3 soybean complex (PDB Code- lIK3): The docking score of compound 2c was-70.00shown minimum dock score than other compounds shown in Table 3. Indicate that the designed compounds have a good binding affinity to the anti-inflammatory receptor of the lipoxygenase-3 soybean complex (PDB Code- lIK3). The pose obtained by docking results is given in Table 2 and the pose of molecular docking represents Figure1a which shows the interaction between ligand and receptor[20].

Table No. 3: Summary of Molecular docking

Sr. No.	Molecule Name	Final Energy	Final GRMS	Dock score
1	2c	74.62	0.9415	-70.00
2	2c-1	68.86	0.8248	-67.33
3	2c-2	46.57	0.6439	-53.95
4	2c-3	83.19	1.1112	-50.19
5	2c-4	47.71	1.0858	-49.90

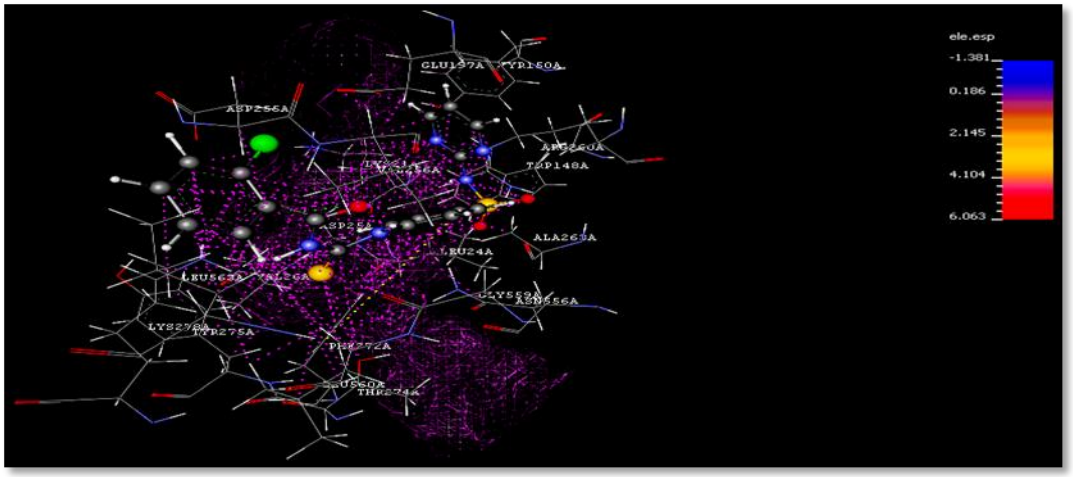


Figure No. 1a: 3D Dock poses of 2C against lIK3 receptor

All designed compounds adopt a very similar conformation at the lipoxygenase-3 soybean complex binding pocket, showing van der Waals binding with an amino acid of GLY559A, ASN556A, LYS21A, ASP25A, VAL28A, GLU197A, ARG260A, ASP255A, LYS278A, VAL256A, LEU 503A, LEU560A. Which result expressed in table 3 and poses are shown by 2D representation diagram (Figure 1b) [21].

Table No. 4: Interaction between compound 2c with Amino Acid

Amino acid	Atom of Ligand	Type of Interaction
ASP 25 A	5N	H-Bond Interaction
GLY 559 A	27C	VDW Interaction
ASN 556 A	28C	VDW Interaction
LYS 21 A	5N	VDW Interaction
VAL 26 A	16C,17C	VDW Interaction
GLU 197 A	8C	VDW Interaction
ARG 260 A	7C	VDW Interaction
ASP 255 A	25C	VDW Interaction
LYS 278 A	1N	VDW Interaction
VAL 256 A	25C,26C	VDW Interaction
LEU 563 A	25C,26C	VDW Interaction
LEU 560 A	26C,27C,28C	VDW Interaction

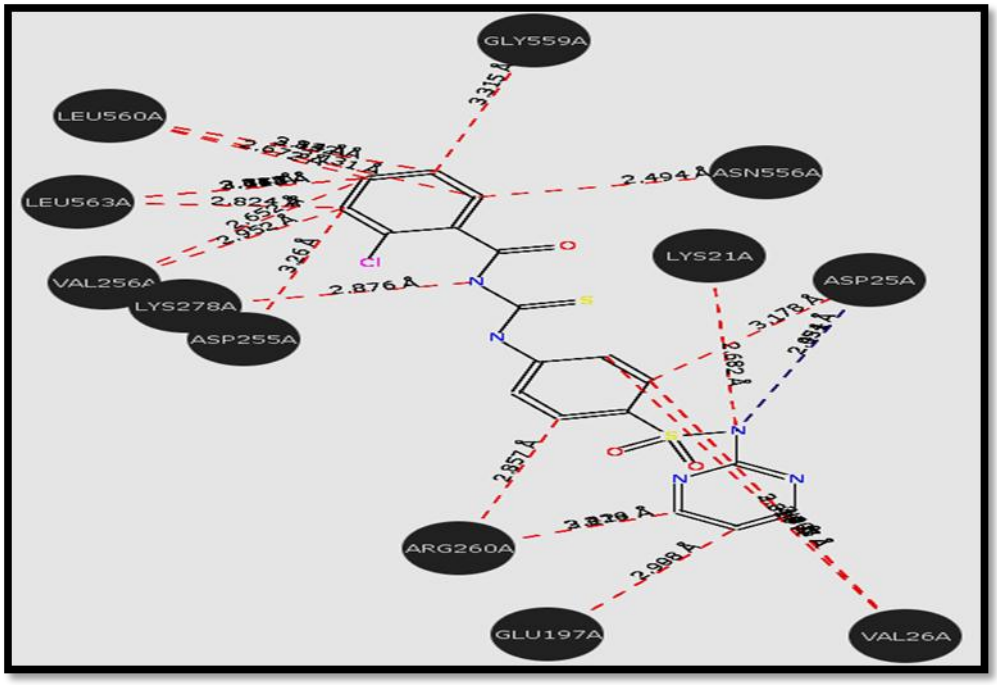


Figure No. 1b: 3D Dock poses of 2C against lIK3 receptor

c. *In-Vivo* Anti-Inflammatory Activity:

Compounds 2C exhibiting diverse *In-Vivo* activity were evaluated for in vivo anti-inflammatory potential at a dose of 20 mg/kg to carrageenan-induced rat paw edema method and the findings are summarized in Table 4. The anti-inflammatory activity of all compounds observed was less than that of the reference drug, with positive control of Aceclofenac. With the cotton thread process, the paw volumes were registered at 0 min to180 minutes. The paw volume registered just before the injection of carrageenan was regarded as the initial volume (V_0). The percent increase in paw volume was calculated by the following formula, where Vt indicates volume after injection of carrageenan at different intervals[22].

$$\% \textbf{ Anti} - \textbf{inflammatory activity} = [(\mathbf{V_t} - \mathbf{V_o})\mathbf{V_o}] \times \mathbf{100}$$

Like other test drugs, the production of the second phase of edema has been suppressed, which is due to their ability to bind COX. The percentage increase in paw volume at 150 min was therefore regarded as a measure of inflammation induced by prostaglandin. Of the research compounds, 2C was found to have potent anti-inflammatory activity with a percentage increase of 22.09-58.17 at 150 minutes, while Aceclofenac showed a percentage increase of 26.89-60.99 at the same dosage. With a percentage increase, compounds 2C3 and 2C4 were less potent than those that are in line with *in-vivo* assay findings[23].

Table No. 5: Comparative % inhibition

Sr. No.	Compound	% Rise in Paw Volume						
		0 Min	30 Min	60 Min	90 Min	120 Min	150 Min	180 Min
1.	Control	0.0	48.97	51.37	53.92	60.65	72.12	48.95
2.	Standard (aceclofenac)	0.0	26.89	26.99	28.21	41.95	60.99	43.36
3.	Compound (2c)	0.0	22.09	25.57	32.60	40.67	58.17	42.06

The results are expressed as mean ± SEM (n = 6). The statistical significance of difference across the groups was determined using ANOVA followed by Dunnett's multiple comparisons test. Table 4 clearly shows similar results between Aceclofenac and compound 2c. **** P < 0.0001 vs. control (one-way ANOVA, Dunnett's multiple comparisons)[24].

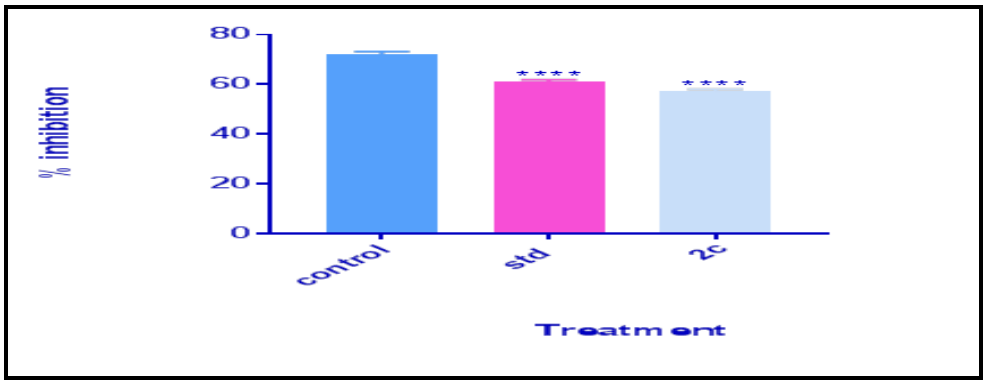


Figure No. 2: % rise in paw volume at 150 min for the Control, STD, and Test compounds 2C

CONCLUSION:

The study concluded that benzamide derivatives (i.e. 2-chloro-N-{[4-(pyrimidine-2-ylsulfamoyl) phenyl] carbamothioyl} benzamide) (compound 2C) had a higher activity receptor for soybean complex lipoxygenase-3 (PDB Code-1IK3). The significant usage of this benzamide derivative is that it was produced by rearranging smiles with intra-molecular nucleophilic substitutes and produces the most popular anti-inflammatory sensitivity agent. There is, therefore, a tremendous potential for the use of benzamide derivatives i.e. 2C as an inflammatory agent to be developed by this type of synthesis.

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