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Insilico Drug Design, Synthesis and Evaluation of Anti-inflammatory Activity Pyrimidine Analogue

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A class of pyrimidine-based molecules was designed for their in silico study, synthesis, and testing for their in vitro anti-inflammatory evaluation. The compounds were tested in an in silico study against anti-inflammatory proteins like FAAH (PDB ID: 4DO3) by using two different software programmes, Ace-dock and Arguslab, and showed promising signs of being a possible drug candidate. In silico toxicity prediction was also done on these compounds. The drug-likeness screening was done to satisfy the Lipinsky rule of five. In our recent investigation, we focused on environment-friendly approaches to synthesising pyrimidine derivatives in the presence of an ethanolic potassium hydroxide solution. The Claisen-Schmidt condensation of acetophenone and various substituted benzaldehydes produces pyrimidine. The pyrimidine derivatives 2a-p and 3a-c were synthesized. The synthesised molecules were screened on the basis of an in silico study, and the molecules were selected and subjected to a check for their in vitro anti-inflammatory activity. A test called the albumin denaturation assay was used to see how much heat-induced protein denaturation could be stopped. The compounds that were synthesised and the standard drug, diclofenac sodium, both stopped protein denaturation at levels ranging from 100 to 500 ppm. Maximum inhibition of 68.59% was observed at the concentration of 100 ppm of compound 2d. Diclofenac sodium showed the maximum inhibition, which was 80.58% at a concentration of 100 ppm. It is concluded that 2d has the potential for further investigation for anti-inflammatory activity.

Keywords: Ace Dock; ArgusLab; Inflammation; In-silico screening; Protox-II; Pyrimidine.

Pyrimidine derivatives constitute a significant and strange class of heterocyclic drugs, widely recognized in both synthetic organic chemistry and pharmaceutical chemistry. Furthermore, because heterocycles are present in the RNA and/or DNA framework, pyrimidine derivatives have accumulated more attention[1]. Pyrimidine-structured heterocyclic compounds

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demonstrate an extensive array of biological activities, including anti-proliferative [2], antiviral [3], anticancer [4], anti-inflammatory [5], antibacterial [6], antifungal [7], and anti-tubercular qualities [8]. Furthermore, thiamine, riboflavin, barbitone, and folic acid are among the vitamins that contain the pyrimidine ring [9]. Antimetabolic pyrimidine Antineoplastic Medicines are well known for their comparable mode of action [10]. When these substances get into cells, they change into biological nucleotides because of an enzyme response in the pyrimidine metanolic pathway. To sum up, these processes can result in metabolites that block one or more enzymes involved in the production of DNA. This has the potential to induce apoptosis and destroy DNA [11].

Apart from their fundamental role in DNA and RNA, pyrimidine pharmacophores are widely used in chemistry and pharmacology to make antibiotics, antibacterial agents, heart-rate medications, agrochemicals, and veterinary goods [12]. Analogues of pyrimidine that have been demonstrated to be antagonistic, anti-parkinsonian, anti-conceptive, and inhibitors of platelet aggregation were found to be present in these derivatives (Figure 1) [13].

In silico screening refers to the computational stimulation process that aids in identifying compounds with a high probability of binding to therapeutic targets. We can now predict how a substance will behave in cells or throughout our body thanks to computational models that simulate biological processes and environments. These computational models emulate biological processes and environments, enabling us to predict how a compound behaves within cells or in the environment [14]. Molecular docking predicts the intermolecular complex formed between a drug and a receptor or enzyme, offering valuable insights into potential biological activities. Using scoring functions, it evaluates the binding affinity, predicts the binding strength, and estimates the energy of the resulting complex [15]. It has become a widely adopted approach for virtual screening to optimise lead compounds [16, 17]. It is principally employed in the precise estimation of the most advantageous binding modes and bio affinities between ligands and their receptors [18].

The great biological activity of pyrimidines has earned them a distinguished place in chemical and medicinal chemistry [19].

EXPERIMENTAL METHODS

In-silico study

Method for docking

Ligand selection

The abundance of synthetic pyrimidine derivatives and compounds discussed in the literaturesupported our decision to focus on this moiety for in-silico research[20]. Furthermore, we proceeded to design additional molecules in conjunction with our research.

Protein selection

The enzyme fatty acid amide hydrolase (FAAH) hydrolyzes anandamide, an endocannabinoid[21]. Epirazole inhibits the fatty-acid amide hydrolase (FAAH) enzyme, which degrades the endocannabinoid anandamide within cells, in a potent and specific manner. It is important to note that epirazole (see figure 2) specifically inhibits anandamide oxidation without compromising carrier-mediated uptake, leading to the accumulation of non-metabolised anandamide within neurons and ultimately prompting its release from the cells [22, 23].

Molecular Docking

Molecular docking of pyrimidine derivatives using various software, such as Arguslab and Ace-dock, The Protein Data Bank was used to get the structure of the protein [PDB ID-4DO3]. The structure was then deprotonated by getting rid of the heteroatoms and water molecules. The binding sites were established in the protein structure. Using the Chem-Draw software, the compounds' structures were shown. The structure geometry was minimized, and the structure was designated as a ligand. Ligand docking was carried out by generating a receptor grid, and the resulting docking score was documented [24-26].

ADME properties and Toxicity study

ADME properties lead to the transformation of a promising lead compound into a viable drug. To ensure the effective use of the lead molecule in human applications, it is essential to gain insights into its absorption, distribution, metabolism, and excretion characteristics. In this regard, we utilisedProtox-II, an accessible online

tool, for predicting the ADME characteristics of a few selected compounds. Mass, log P, hydrogen bond acceptor, and donar were among the properties that were predicted using Lipinsky's Rule of Five [27, 28].

The ligands' LD50, toxicological end points, and organ toxicities were predicted using the Protox-II service. It was established which chemicals were hepatotoxic, carcinogenic, immunotoxic, mutagenic, and cytotoxic[29].

Synthesis

Method-1: Two step synthesis of pyrimidine

Step 1: Greener synthesis of chalcone

A mixture of 0.01 mol of substituted benzaldehydes and 0.01 mol of acetophenone was combined with 10 mL of ethanol. While stirring, a 40% aqueous potassium hydroxide solution was added slowly, at a rate of 10 mL. The reaction was agitated at ambient temperature for a duration of three hours and observed using thin-layer chromatography with silica gel-G. Following overnight chilling, the product underwent filtration, cold water washing till reaching a neutral state, then acidification with weak hydrochloric acid if necessary. The chalcones, which were pale yellow in color, were collected, dried, and recrystallized from rectified spirit.

Step 2: Conventional synthesis of pyrimidine

In 10 mL of 95% ethanol, chalcone (0.01 mol) and urea (0.01 mol, 0.6 g) were mixed together. Ten milliliters of a 40% potassium hydroxide solution in water were added slowly while the

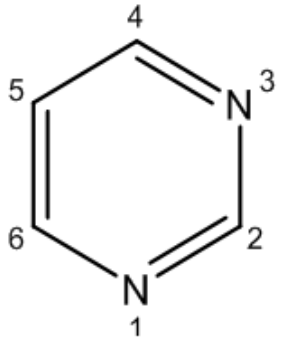

Fig. 1. Structure of pyrimidine(Drawn by using ChemBio Draw Ultra 14.0)

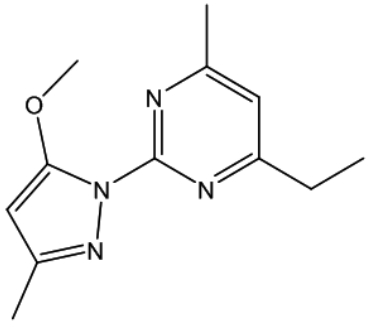

Fig. 2. Structure of Epirazole(Drawn by using ChemBio Draw Ultra 14.0)

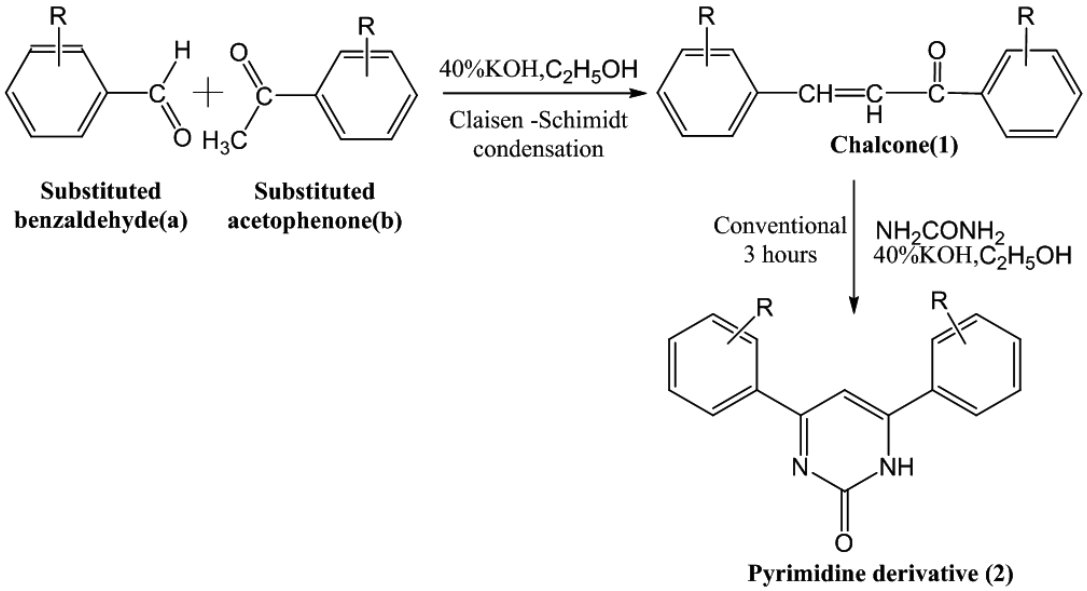

Fig. 3. General Reaction of pyrimidine derivative(Drawn by using ChemBio Draw Ultra 14.0)

mixture was being stirred. The mixture refluxed for four hours in a water bath, periodically checking with TLC for completion. After cooling, it was added to ice-cold water and neutralised with diluted HCl. The obtained precipitate, after filtration, water washing, and drying, was reconstituted using rectified spirit for recrystallization [30].

Method-2: Biginelli synthesis of pyrimidine derivatives

We mixed one mmol of benzaldehyde, ethyl acetoacetate, urea/thiourea, and a small amount of HCl/NH_4Cl in a round-bottom flask. The mixture was refluxed at 100°C for three hours, with TLC used to monitor the reaction's progress. After cooling to room temperature, the mixture was added to cold water, and the solid product was collected by filtration. Following drying and recrystallization with heated alcohol, the pure product was obtained, and its melting point was determined [31].

Biological activity evaluation

Anti-inflammatory activity

Egg albumin denaturation was used as a protein denaturation assay to measure the in vitro anti-inflammatory efficacy.

Procedure

50ml Control Solution

To make the control solution, 28ml of pH 6.4 phosphate buffer saline were added to 2ml of newly made egg albumin, and 20ml of distilled water were added as well [32].

50ml Standard Solution

To freshly prepared egg albumin (2 ml), 28 milliliters of phosphate buffer saline (pH 6.4) was added. Next, to prepared the standard solution, a diclofenac sodium solution with different concentrations ranging from 100 to 500 ppm (20 ml) was added.

50ml Test Solution

After adding 20 ml of the drug solution with varying concentration ranges from 100-500 ppm to freshly manufactured egg albumin (2 ml), The prepared test solution was mixed with 28 milliliters of phosphate buffer saline (pH 6.4).

After being incubated for 15 minutes at 37 ± 2 °C, each solution was heated for 5 minutes at 70 °C on a water bath. Room temperature cooling was allowed for the solutions. As the after that, absorbance was measured at 660 nm using a vehicle and a UV-visible spectrophotometer [33-35].

RESULTS

The design molecules of pyrimidine derivatives with docking scores were considered for detailed discussion (Table 1), as was their interaction with selected proteins on the receptor. The molecule numbers 2c (Ace dock) and 2a (Arguslab), which is 4-(3-nitrophenyl), -6-phenylpyrimidin2(1H)-one,4-(2-hydroxyphenyl)-6-(4-hydroxyphenylpyrimidin)-2(1H)-one, and 4,6-diphenylpyrimidine-2(1H)-one showed the highest docking score (affinity) with the FAAH (4DO3) receptor, which is -30.76, -12.06, indicating a scope for these molecules for further in vitro studies. This molecule is also compared with the standard molecule Epirazole, which is taken for comparison purposes. The docking score

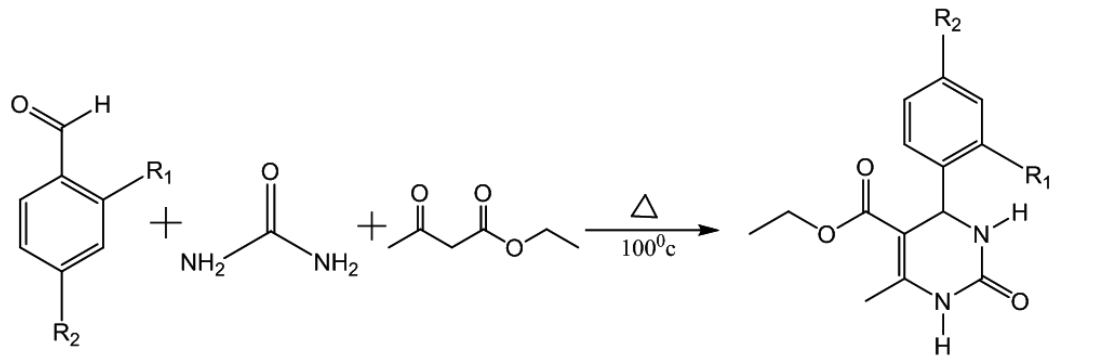

Fig. 4. General reaction of biginelli synthesis(Drawn by using ChemBio Draw Ultra 14.0)

(affinity) of this molecule is -13.93 (Ace dock) and -6.37 (Argus lab). Further, the remaining designed molecules showed docking scores (Affinity) in the -13.45 to -30.76 (Ace dock) and -9.15 to -12.06 (Arguslab) ranges, as did the ligand molecule for the FAAH protein, with an acceptable range of docking scores for standard Epirazole. These all-designed molecules, which have an acceptable

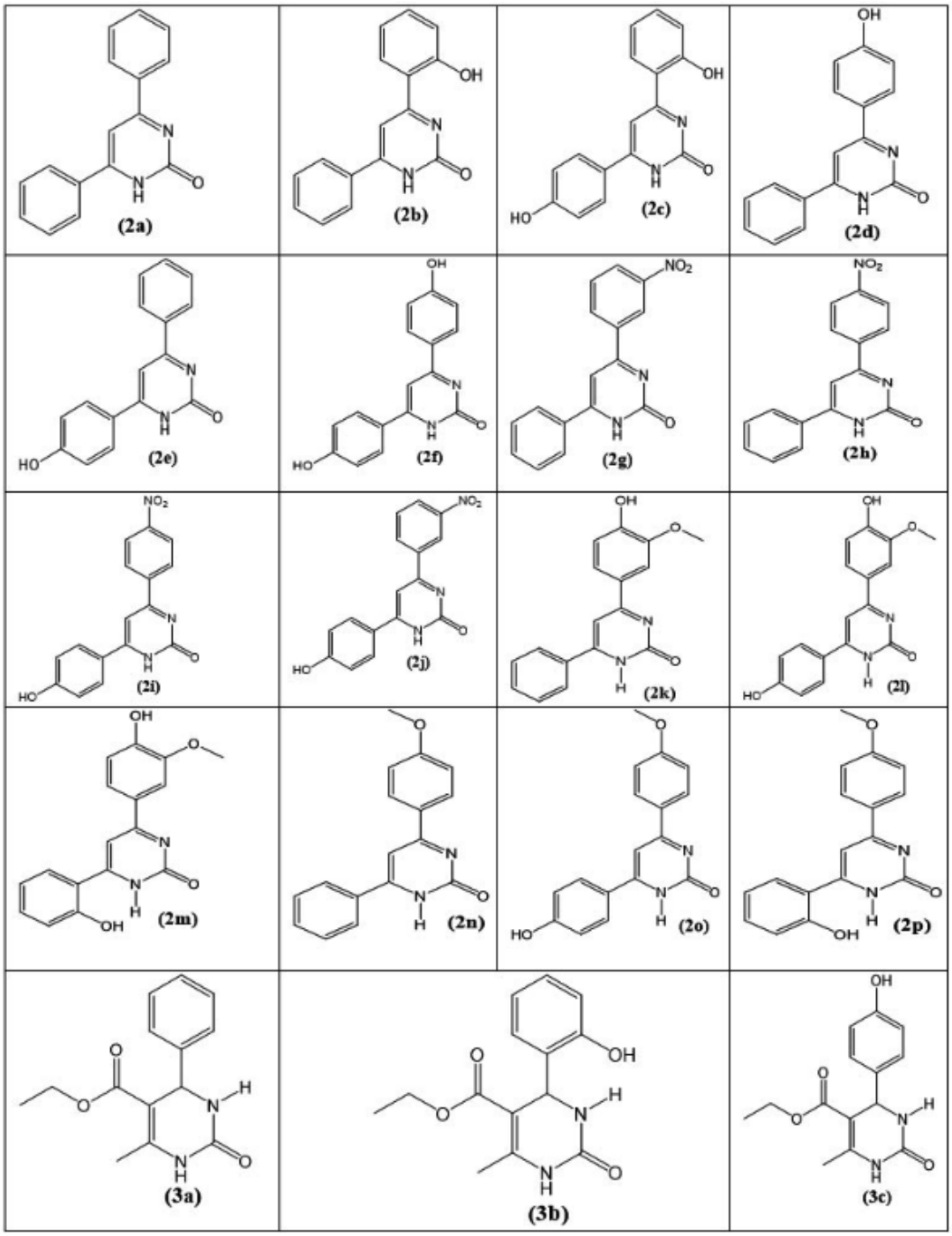

Fig. 5. Design molecules for molecular docking (Drawn by using ChemBio Draw Ultra 14.0)

docking score, may have anti-inflammatory activity.

The molecular weight of epirazole is 234.25, and the molecular weight of designed molecules is in the range of 248.28 to 310.3. The log P value of epirazole is 1 to 3, and that of designed molecules is 2.52 to 3.54. The hydrogen bond donor and acceptor of Epirazole are 0 and 19, respectively, and those of the designed molecules are also in an acceptable range. According to all these calculated properties, all molecules will obey the Lipinski rules of five, which is one of the most important rules for the design of novel molecules.

Estimating compound toxicities is a crucial aspect of the drug development process. It was established which compounds were hepatotoxic, carcinogenic, immunotoxic, mutagenic, and cytotoxic. With the exception of compounds 1e, 3b, and 3c, all of the given compounds were found to exhibit hepatotoxicity. A number of compounds were found to be carcinogenic: 2c, 2g, 2h, 2i, 2j, 2m, 2n, 2o, 2p, and 3a. Additionally, compounds 2l, 2m, and 2p showed increased immunotoxicity, indicated by a dark red colour (values exceeding 7 or 70%). Furthermore, compounds 2g, 2h, 2i, and 2j were found to have heightened mutagenicity, also represented by the red color. The ligand's acute toxicity was assessed using the PROTOX-II server.

Synthesis

Method 1:

Greener synthesis of pyrimidine derivatives:

Method 2:

One pot synthesis of pyrimidine derivatives [PietrioBeginelli]:

Biological evaluation

Anti-inflammatory activity

The chemical's ability to prevent protein denaturation was assessed in order to learn more about the mechanism behind the anti-inflammatory effect. The results of this study showed that the inhibition of protein denaturation ranged from 100 to 500 ppm for both synthesized compounds, and diclofenac sodium (reference) is in a concentration-dependent manner. Notably, compound 2d demonstrated the highest inhibition at 100 ppm, reaching 68.59%, while diclofenac sodium displayed the maximum inhibition of 80.58% at the

Table 1. ADME Properties and Affinity (Docking Score) of pyrimidine molecules

Sr. No	Molecule	Molecular weight	Lipophilicity	Hydrogen bond donar	Hydrogen bond acceptor	ACE-DOCK	ARGUS-LAB
	Standard						
1.	Epirazole	234.25	1.3	0	19	-13.93	-6.37
	Pyrimidine analogue						
1.	2a	248.28	3.1	1	14	-26.80	-12.06
2.	2b	264.28	2.81	2	15	-21.23	-11.64
3.	2c	280.28	2.52	3	16	-30.76	-10.89
4.	2d	264.32	2.94	2	19	-28.04	-11.43
5.	2e	264.24	2.81	2	15	-28.11	-11.57
6.	2f	280.28	2.52	3	16	-25.74	-11.72
7.	2g	293.28	3.54	1	15	-18.23	-10.89
8.	2h	293.28	3.54	1	15	-18.16	-10.12
9.	2i	309.28	3.24	2	16	-17.85	-10.60
10.	2j	309.28	3.24	2	16	-21.32	-11.07
11.	2k	294.31	2.82	2	18	-21.12	-10.48
12.	2l	310.3	2.52	3	19	-22.14	-10.61
13.	2m	310.3	2.52	3	19	-19.06	-10.72
14.	2n	278.31	3.11	1	17	-19.45	-11.34
15.	2o	294.31	2.82	2	18	-22.11	-10.62
16.	2p	294.31	2.82	2	18	-17.83	-10.73
17.	3a	260.29	2.54	2	21	-19.16	-9.94
18.	3b	276,29	2.24	3	22	-13.45	-9.15
19.	3c	276.29	2.24	3	22	-20.22	-9.85

same concentration. It looked like these compounds kept the protein membrane from breaking down, which suggests that the presence of pyrimidine-based compounds might be what made them anti-denaturation. By blocking tyrosine kinase, neutrophil degranulation, and cycloxygenase-2 (COX2), they have anti-inflammatory properties.

DISCUSSION

Inflammation is a globally common condition that affects a significant portion of the population. We are currently working on developing promising anti-inflammatory compounds. We have selected the pyrimidine moiety as the fundamental

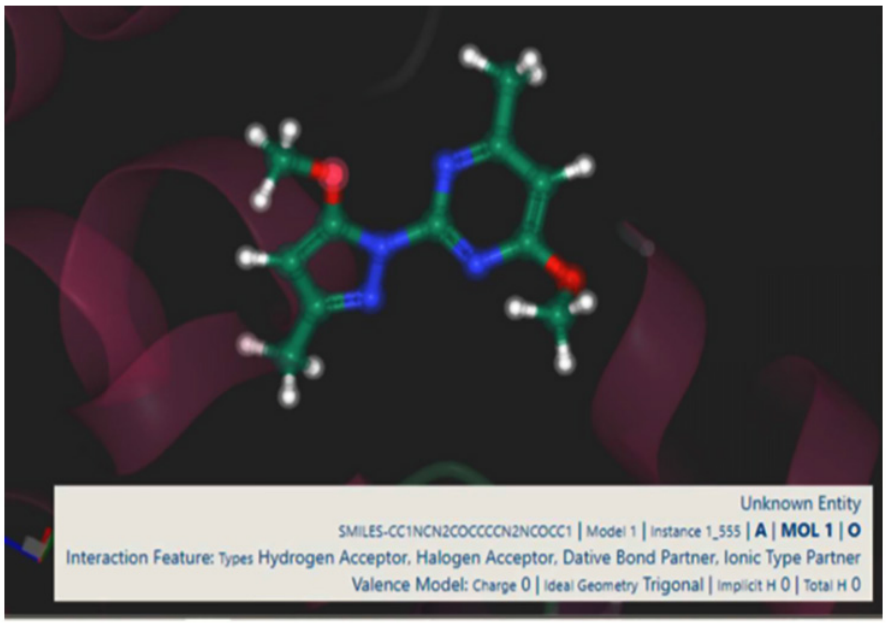

Fig. 6a. 3D Representation of drug receptor interaction of Epirazolewith fatty acid amide hydrolase (4DO3) protein (Ace dock)(Generated from Acedock software)

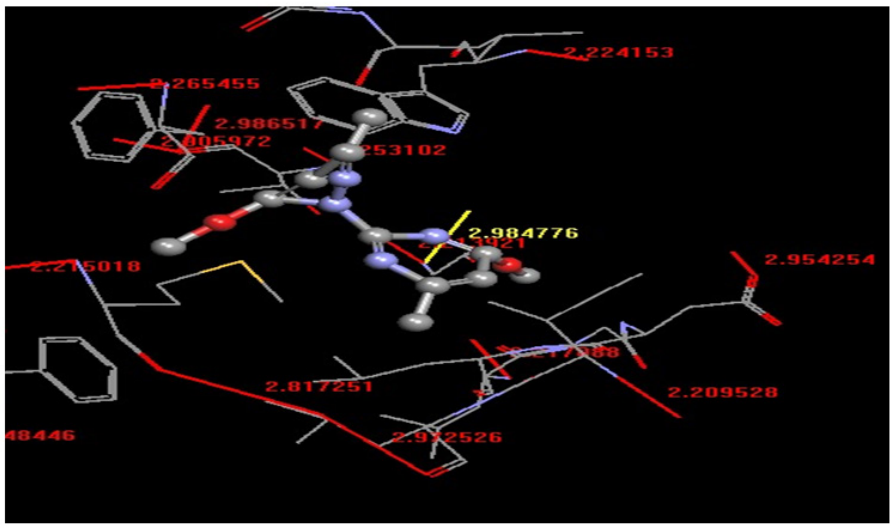

Fig. 6b. 3D Representation of drug receptor interaction of Epirazolewith fatty acid amide hydrolase (4DO3) protein (Arguslab)(Generated from Arguslab software)

structure for synthesizing the desired compounds. The in silico analysis was conducted using Ace Dock and Argus Lab software. We compared the anti-inflammatory activity of all the developed compounds for this investigation. We selected the pyrimidine derivatives for further analysis (Table 1), focusing on their interaction with specific proteins on the receptor, based on their docking scores. The molecules 2c (Ace dock) and 2a (Arguslab), had the best docking scores (affinities) with the FAAH (4DO3) receptor, with -30.76 and -12.06. This suggests that these molecules have the potential for further in vitro studies. This molecule is also compared to the reference molecule, Epirazole, which serves as a benchmark for comparison.

The molecule's docking score (affinity) is -13.93 in Ace Dock and -6.37 in Argus Lab.

Fig. 7a. 3D Representation of drug interaction of 4-(2-hydroxyphenyl)-6-(4-hydroxyphenylpyrimidin)-2(1H)-one with fatty acid amide hydrolase (4DO3) protein (Ace dock)(Generated from Acedock software)

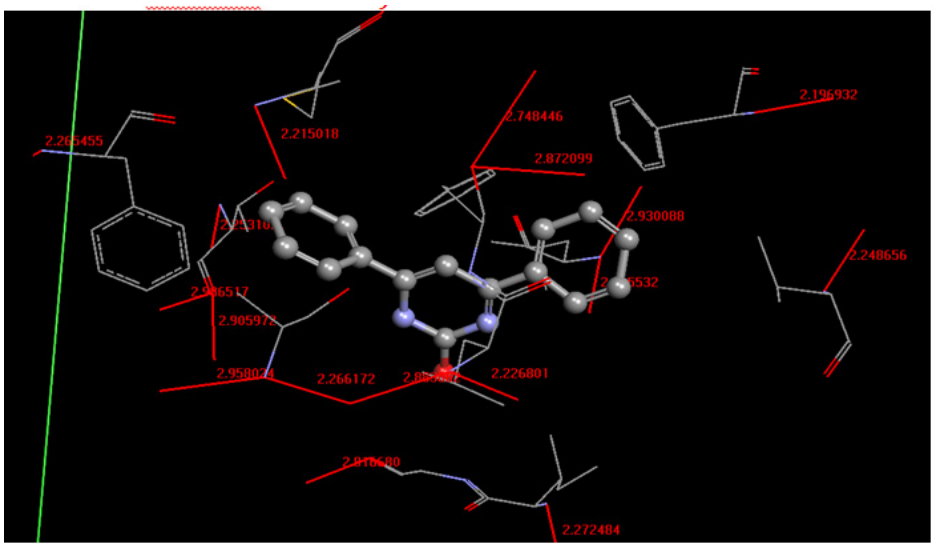

Fig. 7b. 3D Representation of drug interaction of 4,6- diphenylpyrimidine-2(1H)-one with fatty acid amide hydrolase (4DO3) protein (Argus lab)(Generated from Arguslab software)

In addition, the remaining compounds that were developed exhibited docking scores (affinity) ranging from -13.45 to -30.76 (Ace dock) and -9.15 to -12.06 (Arguslab), similar to the ligand molecule for the FAAH protein. These docking scores are within the allowed range for the standard Epirazole. These compounds, which have a satisfactory docking score, may possess anti-inflammatory properties.

Epirazole has a molecular weight of 234.25, while the molecular weights of the proposed molecules range from 248.28 to 310.3. The log P value of epirazole ranges from 1 to 3, while the log P values of the proposed compounds range from 2.52 to 3.54. Epirazole has a hydrogen bond donor value of 0 and a hydrogen bond acceptor value of 19. The proposed molecules also fall within an acceptable range for hydrogen bond donor and acceptor values. Based on the predicted properties, all molecules will adhere to the Lipinski rules of five, which is a crucial guideline for designing new compounds. Assessing the combined harmful effects is a vital component of the process of developing drugs. We identified the chemicals that were hepatotoxic, carcinogenic, immunotoxic, mutagenic, and cytotoxic. We observed hepatotoxicity in all the provided compounds, with the exception of compounds 1e, 3b, and 3c. We discovered carcinogenic properties in several chemicals, specifically 2c, 2g, 2h, 2i, 2j,

Table 2. Toxicity prediction of pyrimidine molecule

Sr. No	Molecule	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
			Standard			
1	Epirazole	Inactive0.56	Active0.56	Inactive0.98	Inactive0.60	Inactive0.85
Pyrimidine derivatives						
1	2a	Active(0.59)	Inactive(0.56)	Inactive(0.97)	Inactive(0.77)	Inactive(0.86)
3	2b	Active(0.59)	Inactive(0.50)	Inactive(0.89)	Inactive(0.76)	Inactive(0.94)
4	2c	Active(0.59)	Active(0.50)	Inactive(0.84)	Inactive(0.78)	Inactive(0.94)
5	2d	Inactive(0.54)	Inactive(0.58)	Inactive(0.99)	Inactive(0.56)	Inactive(0.69)
6	2e	Active(0.62)	Inactive(0.55)	Inactive(0.68)	Inactive(0.75)	Inactive(0.84)
7	2f	Active(0.62)	Inactive(0.52)	Inactive(0.60)	Inactive(0.76)	Inactive(0.85)
8	2g	Active(0.58)	Active(0.69)	Inactive(0.88)	Active(0.82)	Inactive(0.61)
9	2h	Active(0.58)	Active(0.59)	Inactive(0.90)	Active(0.82)	Inactive(0.61)
10	2i	Active(0.57)	Active(0.58)	Inactive(0.76)	Active(0.82)	Inactive(0.62)
11	2j	Active(0.57)	Active(0.58)	Inactive(0.76)	Active(0.82)	Inactive(0.62)
12	2k	Active(0.58)	Inactive(0.5)	Inactive(0.55)	Inactive(0.61)	Inactive(0.92)
13	2l	Active(0.56)	Inactive(0.52)	Active(0.58)	Inactive(0.62)	Inactive(0.92)
14	2m	Active(0.55)	Active(0.50)	Active(0.87)	Inactive(0.66)	Inactive(0.95)
15	2n	Active(0.55)	Active(0.51)	Inactive(0.78)	Inactive(0.61)	Inactive(0.83)
16	2o	Active(0.59)	Active(0.50)	Inactive(0.55)	Inactive(0.67)	Inactive(0.83)
17	2p	Active(0.55)	Active(0.52)	Active(0.86)	Inactive(0.69)	Inactive(0.95)
18	3a	Inactive(0.60)	Active(0.56)	Inactive(0.99)	Inactive(0.65)	Inactive(0.76)
19	3b	Inactive(0.56)	Inactive(0.50)	Inactive(0.98)	Inactive(0.72)	Inactive(0.88)
20	3c	Inactive(0.56)	Active(0.50)	Inactive(0.99)	Inactive(0.72)	Inactive(0.84)

Table 3. Synthesized compounds and their yields

Sr. No.	Name of Compound	Percent yield (%)	M.P (^{0}C)
1.	2a	55.52%	92
2.	2b	67.87	170
3.	2c	68.70	198
4.	2d	65.54	185

Table 4. Synthesized compounds and their yield

Sr. No.	Name of Compound	%Yield	M.P(^{0}C)
1	3a	85.45%	205
2	3b	87.67%	226
3	3c	75.65%	160

Table 5. Effect of different synthesized compounds on heat induced protein denaturation

Compound	Absorbance value (Mean)			
	Control	Standard	2a	2d
100ppm	1.468	2.651	1.442	2.475
200ppm	1.468	2.586	1.749	1.695
300ppm	1.468	2.274	1.381	2.067
400ppm	1.468	2.481	1.147	1.923
500ppm	1.468	1.456	1.384	2.283

Table 6. %Inhibition activity of control, standard and test compounds

Concentration	% Inhibition			
	Control	Standard	2a	2d
100ppm	0	80.58	1.74	68.59
200ppm	0	76.19	48.93	2.69
300ppm	0	52.90	5.92	40.80
400ppm	0	69.02	21.86	30.99
500ppm	0	0.81	5.67	55.56

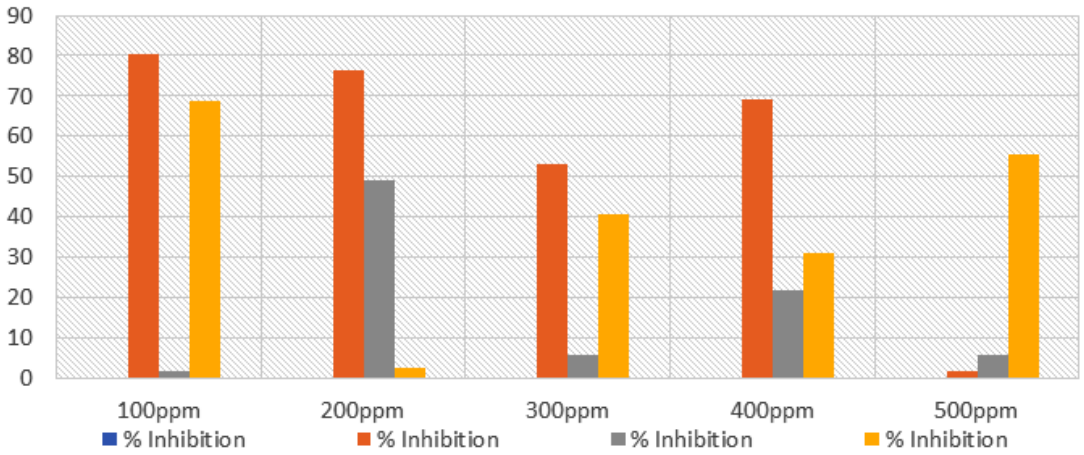

Fig. 8. Effects ofcompounds on heat-induced denaturation of proteins(Generated from Microsoft Excel)

2m, 2n, 2o, 2p, and 3a. Furthermore, chemicals 2l, 2m, and 2p exhibited heightened immunotoxicity, as indicated by a deep red hue (values above 7 or 70%). In addition, compounds 2g, 2h, 2i, and 2j exhibited increased mutagenicity, as shown by their red hue. We evaluated the acute toxicity of the ligand using the PROTOX-II server. Additionally, the pyrimidine-based compounds produced and characterized by spectroscopic techniques were screened for in silico drug design and chosen for in vitro activity.

In order to understand the mechanism of the anti-inflammatory activity, the produced compounds were examined for their capacity to inhibit the denaturation of egg albumin protein. This method is a practical and straightforward approach to assessing anti-inflammatory activity. Upon concluding the investigation, it was determined that concentrations ranging from 100 to 500 ppm of both synthetic compounds and the traditional medication diclofenac sodium effectively prevented protein denaturation, with the degree of effectiveness being dependent on the concentration. Compound 2d exhibited a peak inhibition of 68.59% when administered at a dosage of 100 parts per million (ppm). At a concentration of 100 ppm, diclofenac sodium demonstrated the most significant level of inhibition, measuring 80.58%. Therefore, it may be inferred that the produced compounds have the ability to safeguard the protein membrane against denaturation, and the anti-denaturation capabilities of these compounds may be attributed to their pyrimidine-based composition.

CONCLUSION

It may be concluded that, when compared to the reference molecule epirazole, the developed compounds (pyrimidine derivatives) for the in silico investigation against inflammatory enzymes like FAAH exhibit good interaction with these receptors. Using Protox-II software, the drug

likeness (Lipinski's rule of 5) is verified. The estimated properties of the designed derivatives show that all of the molecules properties fall within an acceptable range, indicating that the designed molecules abide by Lipinski's rule of five. If the designed molecules have a good docking score within the range of -13.45 to -30.76 (Ace dock) and -9.15 to -12.06 (Arguslab) with acceptable pharmacokinetic properties, then these molecules may have anti-inflammatory activity. Using the PROTOX-II server, the ligands' acute toxicity was examined. It may be concluded that pyrimidine derivatives were effectively synthesised with good yield and purity using the green method of synthesis. The compounds that were synthesized and the reference drug, diclofenac sodium, both showed concentration-dependent inhibition of protein denaturation at levels between 100 and 500 ppm. Maximum inhibition of 68.59% was observed at the concentration of 100 ppm of compound 2d. Diclofenac sodium showed the maximum inhibition (80.58%) at a concentration of 100 ppm. The results of this study can aid in understanding the molecular mechanism of these molecules as a potential lead for an anti-inflammatory drug. Studies conducted in vivo and in vitro can further validate the present findings.

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Conflict of Interest

The author declare that I have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' Contribution

Research work carried out: Mr. Sanket N. Aher, Miss Sanjana N. Sonawane, Mr. Pawan R. Sonawane, Dr. Khemchand R. Surana; Data collection, analysis and Interpretation of Results and Manuscript Draft Preparation: Dr. Khemchand R. Surana; Reviewed the results and approved the final version of the manuscript: Dr. Khemchand R. Surana, Dr. Sunil K. Mahajan, Dr. Dhananjay M. Patil, Dr. Pramod N. Katkade.

Availability of data and material

All required data is available.

Ethics approval Statement

Not applicable.

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