Kalpana Devi et al /J. Pharm. Sci. & Res. Vol. 15(2), 2023, 1025-1032

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Comparative Study of Pyrimidine –Hydroxy and Thiol Derivatives as Antioxidant and Anti-inflammatory Activity

Kalpana Devi* and Monica Kachroo

Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Bangalore-27, India

Abstract:

In the present research work an attempt has been made to synthesize some novel pyrimidine derivatives by bioisosteric replacement of one heteroatom witrh another (replacing oxygen with sulphur) and comparing the anti-oxidant and anti-inflammatory activity. The target molecules were synthesized via chalcone as an intermediateand the pyrimidine –ol and thiol derivatives were obtained by cyclization using urea and thiourea respectively. Compared to thiol derivatives, the hydroxy derivatives showed greater denaturation of proteins and were also able to scavenge DPPH and NO radical

Keywords: Pyrimidine, Chalcone, Anti-oxidant, Anti-inflammatory

INTRODUCTION

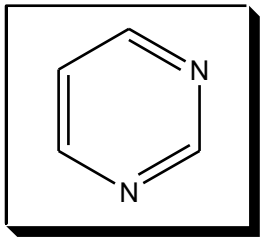
Many heterocyclic rings are found as key components in biological system. Significant numbers of compounds synthesized each year are heterocyclic in nature. The presence of heterocyclic structure in such compounds is strongly indicative due to its profound effects. Such structures exert on physiological activity and recognition of this is reflected in efforts to discover newer and newer synthetic drugs

Thus medicinal chemist need to be aware of the major new developments in this area.

Chalcone (1, 3-diaryl-2-propene-1-one) which possesses α, β – unsaturated carbonyl systems, is one of the most ubiquitously found secondary metabolites in the plant kingdom. This structure has always been considered as privileged pharmacophore because of its application in synthesis of various five and six membered heterocyclic compounds as well as its therapeutic activity against a wide spectrum of diseases

Nitrogen containing heterocyclic compounds is the most explored because of their occurrence in a numerous natural and biologically active compounds

One of the most popular nitrogen containing heterocyclic moiety is the six membered ring containing two nitrogen atom at 1 and 3 position of the benzene ring is pyrimidine.



This moiety has gained popularity due to wide spectrum of pharmacological applications such as antimalarial, anthelmintic, anticancer antimicrobial, anti-convulsion and anti-inflammatory activities. This moiety which is present as pyrimidine salts serve as the building blocks of both deoxyribonucleic acids (DNA) and ribonucleic acid (RNA) is one possible reason for their widespread therapeutic applications. The literature survey indicates a wide range of pharmacological activities exhibited by the pyrimidine nucleus [1-3].

Reactive oxygen species (ROS) are highly reactive chemical molecules formed due to the electron acceptability of O_2 which include peroxides, superoxide, hydroxyl radical, singlet oxygen,[4] and alpha-oxygen. ROS are formed as a natural byproduct of the normal aerobic metabolism of oxygen and have important roles in cell signaling and homeostasis[5]. ROS has a dual role, its effects as harmful, protective or signaling, depends on the balance between its production and disposal at the right time and place[6]. During times of environmental stress, ROS levels can increase dramatically which may result in significant damage to cell structures commonly known as oxidative stress which is an underlying cause of many diseases like inflammation, stroke, acute myocardial infarction, cancer etc

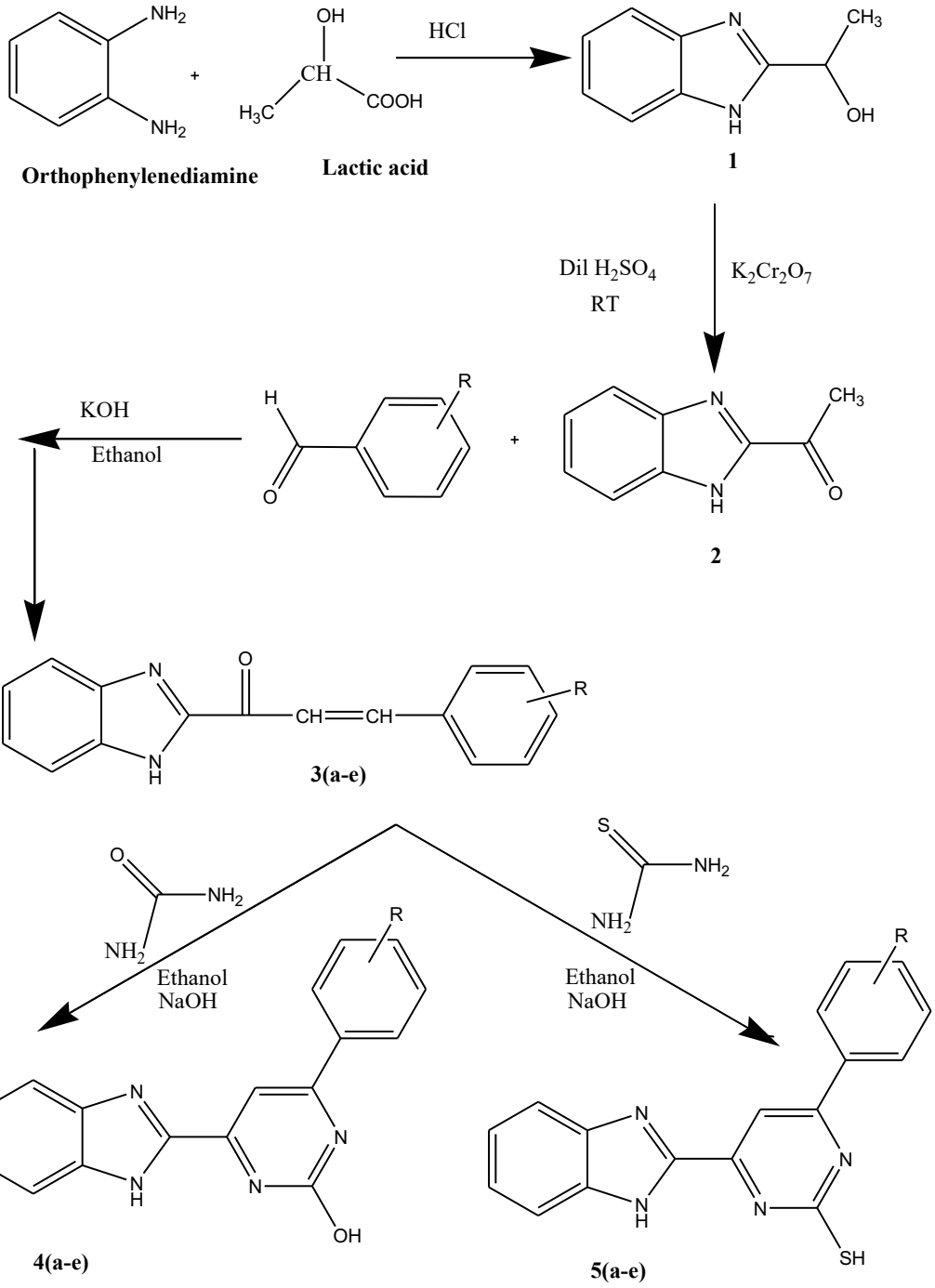
Non-steroidal anti-inflammatory drugs (NSAIDs) are the most prescribed therapeutic drugs for the treatment of pain and inflammation[7] .NSAIDs activity is due to the suppression of biosynthesis of prostaglandin from arachidonic acid by inhibiting the cyclooxygenase (COX) enzymes[8-9]. COX exists in two isoforms, COX- 1 and COX-2. COX-1 is constitutive and COX-2 is inducible. Substitution of isoleucine at position 523 in COX-1 with valine in COX-2 is the main difference between the two isoforms. The smaller Val_{523} residue in COX-2 allows access to a hydrophobic side-pocket in the enzyme (while Ile_{523} sterically hinders). Drug molecules, such as DuP-697 and the coxibs derived from it, bind to this alternative site and are considered to be selective inhibitors of COX-2[10]. The classical *COX* inhibitors like aspirin and ibuprofen are not selective and inhibit all types of *COX*. The most frequent adverse effect of NSAIDs is irritation of the gastric mucosa as prostaglandins normally have a protective role in the gastrointestinal tract[11]. Selective *COX-2* drugs like celecoxib, etoricoxib etc is usually specific to inflamed tissue, and there is much less gastric irritation associated with COX-2 inhibitors, with a decreased risk of peptic ulceration. But still even long term use of selective COX-2 inhibitors may have an effect on kidney, heart leading to thrombosis and stroke[12]

A bioisostere is a molecule resulting from the exchange of an atom or of a group of atoms with an alternative, broadly similar, atom or group of atoms[13]. The replacement may have an effect on the potency of the compounds exhibiting biological acticvity, reducing the toxicity, modifying the activity, altering the pharmacokinetics etc of the parent molecule without making significant changes in chemical structure

Based upon the above biological uses of pyrimidine and to improve the pharmacological activity it was thought to synthesize some pyrimidine derivatives and make biosiosters of it by replacing one atom with another and comparing the bioisosters synthesized molecules in terms of antioxidant and anti-inflammatory activities

MATERIALS AND METHODS

Scheme



Procedure

STEP 1: Procedure for the preparation of 1-(1H-Benzoimidazol-2-yl)-ethanol (1)

A mixture of orthophenylenediamine (0.012 mol), lactic acid (0.036 mol) and 4N HCl (40 ml) were taken in around bottom flask and refluxed for 4 hours. The reaction mixture was cooled and pH was adjusted to 7.2 using NaOH pellets. The resulting solution was filtered, washed with water, dried and recrystallized from methanol.

M.W: 162, yield 90 %, M.P: 160-162 ºC, Rf value 0.62, solvent ratio: ethyl acetate: n-hexane (4:1).

IR (KBr υmax cm^{-1}): 3591 (b, OH str), 1588 (C=N str)

STEP 2: Procedure for the preparation of 1-(1H-Benzoimidazol-2-yl)-ethanone (2)

To a solution of **(1)** (0.05 mol) in dilute H_2SO_4 (5%, 40 ml) was added a solution of $K_2Cr_2O_7$ (0.05 mol) in water and conc H_2SO_4 drop wise with vigorous stirring. The separated solid was filtered and washed with water. The precipitate was resuspended in water and treated with aqueous NH_3 to a pH of 6.0-6.5. The suspension was stirred for 0.5 hrs and filtered. The residue was washed with water and dried to obtain the product

M.W: 160, yield 70 %, M.P: 200-202 ºC, Rf value 0.78, solvent ratio: ethyl acetate: n-hexane (2:1).

IR (KBr υmax cm^{-1}): 2931 (Al CH str), 1701 (C=O str).

STEP 3: Procedure for the preparation of 1-(1H-Benzoimidazol-2-yl)-3-(substituted phenyl)-propenone (3a-e)

(2) (0.01 mol) and substituted aromatic aldehyde derivatives (0.01 mol) were dissolved in ethanol and stirred. An aqueous solution of 40% KOH (10 ml) was added to this mixture. The stirring was continued for 7-8 hrs and the mixture was kept overnight. The mixture was then poured into crushed ice and acidified with HCl. The precipitate obtained was filtered, dried and recrstallized using ethanol

1-(1H-benzoimidazol-2-yl)-3-(4-hydroxy-3-methoxy-phenyl)-propenone 3a

M.W: 294, yield 65 %, M.P: 150-152ºC, Rf value 0.64, solvent ratio: ethyl acetate: n hexane (1:1)

IR (KBr υmax cm^{-1}): 3439 (b, OH, str), 2918 (Ar CH, str), 1696 (C=O str), 1595 (C=N str), 1491 (C=C str)

1-(1H-benzoimidazol-2-yl)-3-(3, 4-dimethoxy-phenyl)-propenone 3b

M.W: 308, yield 63 %, M.P: 148-150ºC, Rf value 0.54, solvent ratio: ethyl acetate: n hexane (1:1)

IR (KBr υmax cm^{-1}): 2921 (Ar CH, str), 1609 (C=O str), 1569 (C=N str), 1507 (C=C str)

1-(1H-benzoimidazol-2-yl)-3-(2-nitro-phenyl)-propenone 3c

M.W: 293, yield 50 %, M.P: 156-158ºC, Rf value 0.52, solvent ratio: ethyl acetate: n hexane (1:1)

IR (KBr υmax cm^{-1}): 2919 (Ar CH, str), 1691 (C=O str), 1556 (C=N str), 1507 (C=C str), 823 (Ar NO_2 str)

1-(1H-benzoimidazol-2-yl)-3-(4-hydroxy-phenyl)-propenone 3d

M.W: 264, yield 55 %, M.P: 140-142ºC, Rf value 0.58, solvent ratio: ethyl acetate: n hexane (1:1)

IR (KBr υmax cm^{-1}): 3459 (b, OH,str), 2922 (Ar CH, str), 1608 (C=O str), 1559 (C=N str), 1507 (C=C str),

1-(1H-benzoimidazol-2-yl)-3-(4-dimethylamino-phenyl)-propenone 3e

M.W: 291, yield 62 %, M.P: 154-156ºC, Rf value 0.53, solvent ratio: ethyl acetate: n hexane (1:1)

IR (KBr υmax cm^{-1}): 2922 (Al CH, str), 1685 (C=O str), 1608 (C=N str), 1456 (C=C str)

STEP 4: Procedure for the preparation of 4-(1H-benzoimidazole-2-yl)-6-(substituted –phenyl)-pyrimidine-2-ol 4(a-e) and 4-(1H-benzoimidazole-2-yl)-6-(substituted –phenyl)-pyrimidine-2-thiol 5(a-e).

Respective chalcones **3(a-e)** (0.01 mol) and urea/thiourea (0.01 mol) were dissolved in ethanol (30 ml) containing few drops of NaOH. The reaction mixture was refluxed on heating mantle for about 8 hours for urea and 12 hours for thiourea. The hot mixture was then filtered and allowed to cool. The resulting solid so obtained was filtered, washed several times with water, dried and recrystallized from ethanol

4-(1H-benzoimidazol-2-yl)-6-(4-hydroxy-3-methoxy-phenyl)-pyrimidine-2-ol (4a)

M.W: 334, yield 52 %, M.P: 110-112ºC, Rf value 0.79, solvent ratio: benzene: chloroform 8:2

IR (KBr υmax cm^{-1}): 3333 (b, OH, str), 2933 (Al CH str), 1578 (C=N str)

^{1}H-NMR400 MHz, DMSO, δ-ppm: 2.43 (3H, OCH_3), 4.19 (1H, NH), 5.04 (2H, OH), 6.69-7.69 (m, 8H, ArH)

^{13}C-NMR, DMSO, δ- ppm**:** 53, 101, 112, 113, 119, 124, 128, 133, 136, 140, 143, 149, 156, 166 and 171

m/z: (M+1) 335

CHN**:** Found C=64.55 %, H=4.16 %, N=16.73 % Calculated C=64.66 %, H=4.22 %, N= 16.76%

4-(1H-benzoimidazol-2-yl)-6-(3, 4-dimethoxy-phenyl)-pyrimidine-2-ol (4b)

M.W: 348, yield 55 %, M.P: 98-100ºC, Rf value 0.73, solvent ratio: benzene: chloroform 8:2

IR (KBr υmax cm^{-1}): 3591 (b, OH, str), 1558 (C=N str), 1051 (C-O-C str)

^{1}H-NMR400 MHz, DMSO, δ-ppm: 3.43 (s, 6H, OCH_3), 4.33 (s, 1H, NH), 5.11 (s, 1H, OH), 6.44-7.78 (m, 8H, ArH)

^{13}C-NMR, DMSO, δ- ppm**:** 56, 102, 113, 114, 122, 125, 130, 137, 141, 149, 157, 162 and 172

m/z: (M+1) 349

CHN**:** Found C=65.72 %, H=4.56 %, N=16.17 % Calculated C=65.51 %, H=4.63 %, N= 16.08 %

4-(1H-benzoimidazol-2-yl)-6-(2-nitro-phenyl)-pyrimidine-2-ol (4c)

M.W: 333, yield 40 %, M.P: 92-94ºC, Rf value 0.61, solvent ratio: benzene: chloroform 8:2

IR (KBr υmax cm^{-1}): 3657 (b, OH, str), 1554 (C=N str), 936 (Ar NO_2 str)

4-(1H-benzoimidazol-2-yl)-6-(4-hydroxy-phenyl)-pyrimidine-2-ol (4d)
M.W: 304, yield 53 %, M.P: 112-114ºC, Rf value 0.80, solvent ratio: benzene: chloroform 8:2
IR (KBr υmax cm^{-1}): 3453 (b, OH, str), 2360 (Ar CH str), 1585 (C=N str)

4-(1H-benzoimidazol-2-yl)-6-(4-dimethylamino-phenyl)-pyrimidine-2-ol (4e)
M.W: 331, yield 59 %, M.P: 128-130ºC, Rf value 0.86, solvent ratio: benzene: chloroform 8:2
IR (KBr υmax cm^{-1}): 3337 (b, OH, str), 2922 (Ar CH str) 2850 (Al CH str), 1596 (C=N str)

4-[6-(1H-benzoimidazol-2-yl)-2-mercapto-pyrimidin-4-yl]-2-methoxy-phenol (5a)
M.W: 350, yield 45 %, M.P: 196-198ºC, Rf value 0.68, solvent ratio: benzene: chloroform 8:2
IR (KBr υmax cm^{-1}): 3444 (b, OH, str), 2906 (SH str), 1562 (C=N str), 1444 (C-O-C str)
^{1}H-NMR400 MHz, DMSO, *δ*-ppm: 2.33 (3H, OCH_3), 3.20 (1H, SH), 4.11 (1H, NH), 5.02 (1H, OH) 6.79-7.79 (m, 8H, ArH)
^{13}C-NMR, DMSO, *δ*- ppm**:** 54, 114, 116, 119, 123, 126, 130, 136, 140, 143, 148, 162, 165 and 180
m/z: (M+1) 351
CHN**:** Found C=61.82 %, H=4.73 %, N=15.42 % S=9.26 % .Calculated C=61.70 %, H=4.03 %, N= 15.99 %S=9.15 %

4-(1H-Benzoimidazol-yl)-6-(3,4-dimethoxy-phenyl)-pyrimidine-2-thiol (5b)
M.W: 364, yield 50 %, M.P: 168-170ºC, Rf value 0.70, solvent ratio: benzene: chloroform 8:2
IR (KBr υmax cm^{-1}): 2906 (Ar CH str), 2548 (Al CH str), 1564 (C=N str), 1100 (C-O-C str)
^{1}H-NMR400 MHz, DMSO, *δ*-ppm: 3.15 (s, 1H, SH), 3.73 (s, 6H, OCH_3), 5.03 (s, 1H, NH) 6.63-7.83 (m, 8H, ArH)
^{13}C-NMR, DMSO, *δ*- ppm**:** 56, 113, 115, 118, 121, 124, 129, 137, 141, 147, 166 and 181
m/z: (M+1) 365
CHN**:** Found C=62.86 %, H=4.76 %, N=15.48 % S=8.25 % .Calculated C=62.62 %, H=4.43 %, N= 15.37%S=8.80 %

4-(1H-Benzoimidazol-yl)-6-(2-nitro-phenyl)-pyrimidine-2-thiol (5c)
M.W: 349, yield 40 %, M.P: 188-190ºC, Rf value 0.73, solvent ratio: benzene: chloroform 8:2
IR (KBr υmax cm^{-1}): 2954 (Ar CH str), 1585 (C=N str), 785 (Ar NO_2 str)

4-(1H-Benzoimidazol-yl)-6-(4-hydroxy-phenyl)-pyrimidine-2-thiol (5d)
M.W: 320, yield 50 %, M.P: 218-220ºC, Rf value 0.86, solvent ratio: benzene: chloroform 8:2
IR (KBr υmax cm^{-1}): 3419 (b, OH, str), 2978 (Ar CH str), 1510 (C=N str)

4-(1H-Benzoimidazol-yl)-6-(4-dimethylamino -phenyl)-pyrimidine-2-thiol (5e)
M.W: 347, yield 50 %, M.P: 186-188ºC, Rf value 0.81, solvent ratio: benzene: chloroform 8:2
IR (KBr υmax cm^{-1}): 2918 (Ar CH str), 1521 (C=N str)

DOCKING STUDIES

The synthesized molecules **4(a-e)** and **5(a-e)** were subjected to molecular docking studies using AutoVina and PyRx. The interactions between ligands and the respective proteins were visualized using Discovery Studio Visualizer.

Antiinflammatory

The synthesized compounds were docked into the active site of COX-2 (PDB: 1CX2)[14]

DRUG LIKELINESS PROPERTIES

It is an integrated approach for preliminary screening of the drug compounds. The famous Lipinski Rule of 5 i.e the number of rotatable bonds, polar surface area, molecular weight, the number of hydrogen bond acceptor and donar and Log P are determined using online MOLINSPIRATION tool. All the properties should not violate the Lipinski rules. Druglikeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs[15].

Table 1: 4-(1H-benzoimidazole-2-yl)-6-(substituted –phenyl)-pyrimidine-2-ol/thiol with their binding energy scores (Kcal/mol) and H-bonds interactions against COX-2 (PDB: 1CX2)

Ligand Code	Binding energy score (Kcal/mol)	H-bond Interacting Residues
4a	-8.8	Asn 382, Tyr 385
4b	-8.7	------
4c	-9.3	-----
4d	-9	-------
4e	-8.9	--------
5a	-8.5	His 388
5b	-8.5	His 388
5c	-9.1	------
5d	-8.6	His 388
5e	-8.7	His 388
Reference Ligand SC-558(Selective COX-2 Inhibitor)	-8	Try 387, Ala 202

Table 2: Calculation of molecular properties of 4-(1H-benzoimidazole-2-yl)-6-(substituted –phenyl)-pyrimidine-2-ol/thiol Pyr-OH 4(a-e) & Pyr-SH 5(a-e).

Comp Code	miLog P	TPSA	Natoms	MW	nON	nOHNH	nviolations	nrotb	volume
4a	2.83	104.16	25	334	7	3	0	3	284.95
4b	3.14	93.16	26	348	7	2	0	4	302.48
4c	3.40	120.52	25	333	8	2	0	3	274.72
4d	3.01	94.92	23	304	6	3	0	2	259.40
4e	3.59	77.93	25	331	6	2	0	3	297.29
5a	3.44	83.93	25	350	6	2	0	3	294.59
5b	3.75	72.94	26	364	6	1	0	4	312.12
5c	4.01	100.29	25	349	7	1	0	3	284.36
5d	3.62	74.69	23	320	5	2	0	2	269.05
5e	4.20	57.70	25	347	5	1	0	3	306.94

Table 3: Online toxicity screening of 4-(1H-benzoimidazole-2-yl)-6-(substituted –phenyl)-pyrimidine-2-ol/thiol Pyr-OH 4(a-e) & Pyr-SH 5(a-e).

Comp	LD_{50} mg/kg	Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
4a	800	IV	Active	Inactive	Inactive	Active	Inactive
4b	568	IV	Active	Inactive	Inactive	Active	Inactive
4c	1000	IV	Active	Active	Inactive	Active	Inactive
4d	715	IV	Active	Inactive	Inactive	Inactive	Inactive
4e	200	III	Active	Inactive	Inactive	Active	Inactive
5a	800	IV	Active	Active	Inactive	Active	Inactive
5b	800	IV	Active	Active	Inactive	Active	Inactive
5c	1000	IV	Active	Active	Inactive	Active	Inactive
5d	1000	IV	Active	Inactive	Inactive	Active	Inactive
5e	1000	IV	Active	Inactive	Inactive	Active	Inactive

TOXICITY SCREENING

Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound. Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals. LD50 values are given in [mg/kg]:

- Class I: fatal if swallowed (LD50 ≤ 5)
- Class II: fatal if swallowed (5 < LD50 ≤ 50)
- Class III: toxic if swallowed (50 < LD50 ≤ 300)
- Class IV: harmful if swallowed (300 < LD50 ≤ 2000)
- Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000)
- Class VI: non-toxic (LD50 > 5000)

The online toxicity prediction was performed using **ProTox-II software tool**[16]

IN VITRO BIOLOGICAL ACTIVITIES

***In vitro* antiinflammatory activity By Bovine Serum Albumin Denaturation Method:**

A solution of 0.2% w/v of BSA was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 1000µg/ml of all test samples were prepared by using methanol as a solvent, from these stock solutions two different concentrations of 100µg/ml and 200µg/ml were prepared by using methanol as a solvent. 100µl (0.1ml) of each test sample was transferred to volumetric flask (10 ml) using 1ml micropipette; 5ml of 0.2% BSA was added to all the above flasks. The control consists of 5ml 0.2%w/v BSA solution with 0.1ml methanol. The 0.1ml standard consist 100цg/ml of indomethacin in methanol with 5ml 0.2%w/v BSA solution. The volumetric flasks were heated at 72˚C for five minutes and then cooled for 10 min. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm. The % inhibition of precipitation (denaturation of the protein) was determined on a percentage basis relative to the control using the following formula[17]:-

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Table 4: In vitro anti- inflammatory activity*:*

Comp Code	% Inhibition	
	100 µg/ml	200µg/ml
4a	76	78
4b	74	79
4c	72	76
4d	67	77
4e	69	74
5a	57	69
5b	61	66
5c	55	59
5d	60	64
5e	68	70
Indomethacin	90	94

***In vitro* antioxidant activity**

(A) Screening of antioxidant activity by DPPH method[18]

Preparation of Control (DPPH) Solution:

10 mg of DPPH was dissolved in 10 ml of methanol. From this stock solution dilutions were made to obtain concentrations of 10 to 40 µg/ ml. The absorbance was recorded for these dilutions at 516 nm.

Preparation of standard solution (Ascorbic acid):

10 g of ascorbic acid was dissolved in 10 ml of methanol. From this stock solution dilutions were made to obtain concentrations of 10, 50 and 100 µg/ ml. 1 ml from each of these solutions was taken in different volumetric flasks to which 1 ml of DPPH solution (300 µg/ ml concentration) was added and volume was made up to 10 ml. The absorbance was recorded for these dilutions at 516 nm after duration of 30 min.

Preparation of test or sample solutions:

The test solution were prepared in similar manner as that of standard ascorbic acid and the absorbance were recorded at 516 nm after duration of 30 min.

Table 5: Screening of antioxidant activity by DPPH method

Compound code	% Inhibition		
	10 µg/ml	50 µg/ml	100 µg/ml
4a	43	44	47
4b	39	41	45
4c	40	42	43
4d	70	74	80
4e	53	56	59
5a	57	58	60
5b	42	43	48
5c	46	47	50
5d	57	59	63
5e	64	64	69
Ascorbic acid	97	98	99

(B) Nitric oxide free radical scavenging activity[19]

Sodium nitroprusside solution

Weighed accurately 0.2998 g of sodium nitroprusside and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask (10 mM)

Naphthyl ethylene diaminedihydrochloride (NEDD, 0.1%)

Weighed accurately 0.1 g of NEDD and dissolved in 60 ml of 50% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask with distilled water

Preparation of test or sample solutions:

The reaction mixture (6 ml) containing 10 mM sodium nitroprusside in phosphate buffer solution and the test or reference compound (ascorbic acid) at different concentrations (10, 50 and 100 µg/ml) were incubated at 25^0 C for 150 min. About 0.5 ml aliquot of the incubated sample was removed at 30 min interval and 0.5 ml Griess reagent was added. The absorbance of the chromophore formed was measured at 546 nm. Inhibition of the nitric oxide generated was measured by comparing the absorbance values of control, test samples and ascorbic acid.

For both the methods % inhibition was calculated by following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Table 6: Nitric oxide free radical scavenging activity

Compound code	% Inhibition		
	10 µg/ml	50 µg/ml	100 µg/ml
4a	43	44	45
4b	35	36	39
4c	38	40	44
4d	48	50	51
4e	49	51	53
5a	33	34	37
5b	32	35	38
5c	38	42	46
5d	44	47	49
5e	42	43	47
Ascorbic acid	60	65	72

RESULTS AND DISCUSSIONS

The present research work involves the synthesis, characterization, in-silico studies comparison of invitro antioxidant and anti-inflammatory activities of pyrimidine ol and thiol bioisosters. In the first step orthophenylenediamine reacted with lactic acid in presence of HCl to form benzoimidazole-ethanol wherein the presence of OH group is exhibited by a broad peak at 3591cm^{-1}.The obtained intermediate was oxidized in presence of H_2SO_4 and $K_2Cr_2O_7$ at RT to form its corresponding enone. The disappearance of broad OH peak and the appearance of a peak at 1701cm^{-1} confirmed the formation of the enone. The obtained benzoimidazole enone underwent Claisen Schimdt condensation reactions with various substituted aromatic aldehydes in presence of a base and ethanol to form the corresponding chalcones .The presence of unsaturated carbonyl group is observed at a lower range of 1608-1696cm^{-1} .These chalcones were used for synthesizing two types of derivatives. In one set these chalcones were reacted with urea in presence of ethanol and NaOH to give pyrimidine 2-ol and in the other set the same chalcones reacted with thiourea in presence of ethanol and NaOH to give pyrimidine 2-thiol. The disappearance of the IR absorption peak of the carbonyl group proved that the cyclization had taken place. The compounds were further characterized through NMR spectra. The presence of phenolic OH is observed at δ value of 5.04-5.11 ppm whereas the mercapto proton is observed at δ value of 3.15-3.20 ppm. Also the phenolic carbon peak was seen at δ value of 172 ppm and the mercapto carbon is observed at δ value of 180 ppm. The mass values are in agreement with the molecular weight of the compound. The calculated and the obtained CHN values do not differ by ±0.5. Additionally the percentage of S element in mercapto series further confirms the formation of the derivatives.

Docking studies

Although the docking scores were more than the co-crystallized ligand (SC558-Selective COX-1 inhibitor in 1CX2) but most of them failed to form hydrogen bonds with the receptors. However the thiol pyrimidines proved to be a better antiinflammatory candidate than hydroxy pyrimidine as per docking scores and tendency to form multiple hydrogen bonds.

Drug Likeliness Profile

All the synthesized compounds were subjected to drug likeliness profile using MOLINSPIRATION online tool. All the derivatives fell within the limit criteria of Lipinski Rule of 5 (as shown in Table-1) where there were 0 deviations.

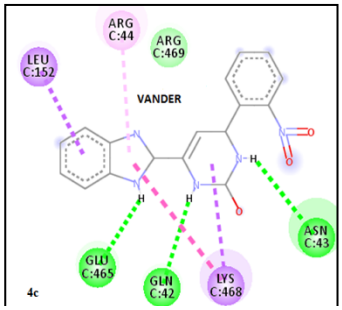
Online Toxicity Prediction

All the synthesized derivatives were subjected to online toxicity prediction using Pro Tox-II.. Most of the synthesized drugs fell in Class IV as the LD50 value is quite high indicating its safety. Although most of the drugs affected the liver but none of them were found to be immunotoxic and cytotoxic. Out of all the derivatives MKPy-OH-04 (4-hydroxy substitutent) may be considered as the safest drug as it was predicted to be neither carcinogenic, immunotoxic, mutagenic and cytotoxic with LD50 value of 715 mg/kg. It was observed from the table 3 that the thiol derivatives had LD50 value comparatively higher than the hydroxy derivatives indicating its broad range of safety

In Vitro biological activities

Antiinflammatory

Generally the mechanism of action of pyrimidine based antiinflammatory agents is associated with inhibition of PGE_2 generated by COX enzymes. They may bind to plasma albumin and prevent the thermal denaturation of albumin. Out of 10 compounds screened, pyrimidine –ol derivatives exhibited greater percentage inhibition of the denaturation of proteins compared to thiol derivatives. It was observed that all the derivatives exhibited concentration dependent inhibition of denaturation of proteins, where the % inhibition increased with the increase in the concentration of the synthesized derivatives. But none of the compounds had shown activity more than the standard



Antioxidant

The ability of newly synthesized compounds to act as hydrogen donars or free radical scavengers was tested by invitro antioxidant assays involving DPPH and NO radical and the results were compared with that of standard. In case of antioxidant activity the thiol derivatives showed moderate to good activity as compared to the hydroxy pyrimidines. Bioisosteric replacement of O with S at the pyrimidine ring did not have much significant contribution to the overall activity.

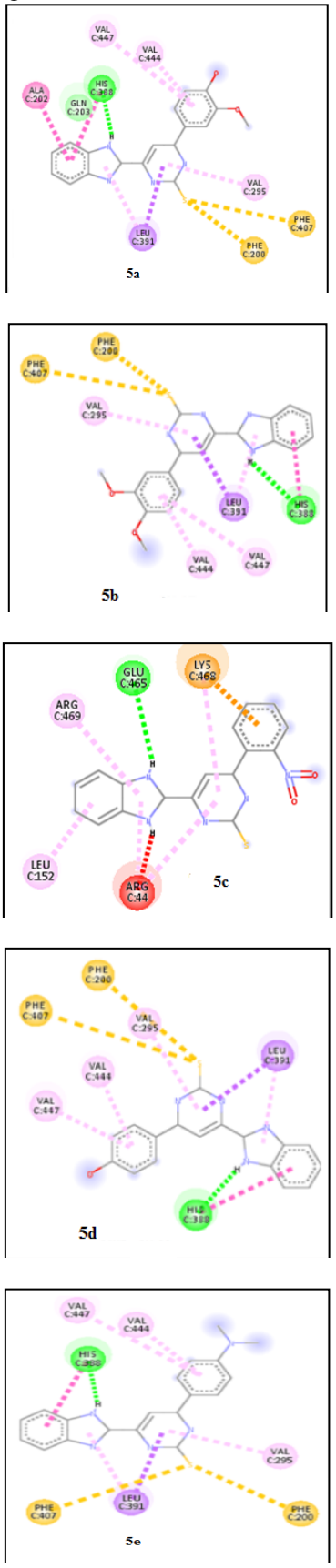


Fig: 1 Hydrogen bond interaction of the reference ligand (SC558), 4a, 4c, 5a, 5b, 5c, 5d and 5e with 1CX2

REFERENCES

1. Verma A, Sahu L, Chaudhary N, Dutta T, Dewangan D, etal. A Review: pyrimidine their chemistry and pharmacological potentials. Asi J Biochem & Pharma Res., 2012; 1(2):1-15.
2. Arikkatt S D, Baldwin M V, Joseph J, Chandran M, Bhat A R, etal. Pyrimidine derivatives and its biological potential-A review. Int J Org & Bio Org Chem. 2014; 4(1):1-5.
3. Dansena H, Dhongade H J, Chandrakar K. Pharmacological potentials of pyrimidine derivative: A Review. Asi J Pharm Clin Res., 2015; 8(4):171-7.
4. Hayyan M, Hashim M A, AlNashef I M. Superoxide Ion: Generation and chemical implications. Chem Rev., 2016; 116 (5): 3029–85.
5. Devasagayam T P, Tilak J C, Boloor K K, Sane K S, Ghaskadbi S S, etal. Free radicals and antioxidants in human health: current status and future prospects. The J Asso Phys Ind., 2004; 52: 794–804.
6. Edreva, Aglika. Generation and scavenging of reactive oxygen species in chloroplasts: a submolecular approach. Agri ecosys & envir.2005; 106 (2): 119–133.
7. https://en.wikipedia.org/wiki/Reactive_oxygen_species accessed on 18-02-2021.
8. Costa BRDA, Reichenbach S, Keller N, Nartey L, Wandel S, et al. Effectiveness of non-steroidal anti-inflammatory drugs for the treatment of pain in knee and hip osteoarthritis: a network meta-analysis. Lancet. 2017; 390: 21–33.
9. Smith C J, Zhang Y, Koboldt C M. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc Natl Acad Sci USA. 1998; 95:13313–8.
10. Warner T D, Giuliano F, Vaynovie I, Bukasa A, Mitchell J A, etal. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full *in vitro* analysis. Proc Natl Acad Sci USA. 1999; 96:563–8.
11. Kurumbail R G, Stevens A M, Gierse J K, McDonald J J, Stegeman R A, etal. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*. 1996; 384 (6610): 644–8.
12. https://en.wikipedia.org/wiki/Cyclooxygenase#cite_note-pmid8967954-2 accessed on 10-02-2021.
13. https://www.cambridgemedchemconsulting.com/resources/bioisoteres/accessed on 04-01-2023.
14. Ashish K T, Ved P S , Pratima Y , Garima G , Amit S and etal. Synthesis, biological evaluation and molecular modeling study of pyrazole derivatives as selective COX-2 inhibitors and anti-inflammatory agents. Bioorg Chem. 2014; 56:8–15.
15. https://molinspiration.com/docu/miscreen/druglikeness.html accessed on 31-03-2021.
16. http://tox.charite.de/protox_II/index.php?site=compound_input accessed on 31-03-2021.
17. Oyedapo AJ. Antiprotease and membrane stabilizing activities of extracts of fagara zanthoxylozdes, olax subscorpzozdes and tetrapleurteat raptera. Int J Pcog: 1995 (33), 65-69.
18. Vadivukkarasi S, Pavithra K. Evaluation of free radical scavenging activity of various leaf extracts from Kedrostis foetidissima. Biochem & Analyt Biochemistry. 2014; 3(2)