In-silico Based Designing of Benzo[d]thiazol-2-amine Derivatives as Analgesic and Anti-Inflammatory Agents

Arun K. Mishra * , Kamal Y Thajudeen , Shahana Salam , Mhaveer Singh , Gulam Rasool , Arvind Kumar , Harpreet Singh , Kalicharan Sharma , Amrita Mishra

Posted Date: 19 June 2023

doi: 10.20944/preprints202306.1064.v1

Keywords: Benzothiazole; Docking; Analgesic; Anti-inflammatory

Article

In-silico Based Designing of Benzo[d]thiazol-2-amine Derivatives as Analgesic and Anti-Inflammatory Agents

Arun K. Mishra *, Kamal Y Thajudeen, Shahana Salam, Mhaveer Singh, Gulam Rasool, Arvind Kumar, Harpreet Singh, Kalicharan Sharma, Amrita Mishra

Posted Date: 19 June 2023

doi: 10.20944/preprints202306.1064.v1

Keywords: Benzothiazole; Docking; Analgesic; Anti-inflammatory

Article

In-silico based designing of benzo[d]thiazol-2-amine derivatives as analgesic and anti-inflammatory agents

Arun K. Mishra [1,*], Kamal Y. Thajudeen [2], Shahna Salam [3], Mhaveer Singh [1], Gulam Rasool [4], Arvind Kumar [4], Harpreet Singh [4], Kalicharan Sharma [5] and Amrita Mishra [5]

[1] Central Facility of Instrumentation, Pharmacy Academy, IFTM University, Moradabad, 244001, India
[2] Department of Pharmacognosy, College of Pharmacy, King Khalid University, Abha, 62529, Saudi Arabia
[3] Department of Pharmaceutical chemistry, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan 333001, India.
[4] Drug Design Laboratory, School of Pharmaceutical Sciences, IFTM University, Moradabad, 244001, India
[5] School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences & Research University, New Delhi 110017
* Correspondence: arun_azam@rediffmail.com

Abstract: The synthesis of the presented benzo[d]thiazol-2-amine derivatives was performed by condensing-(4-chlorobenzylidene) benzo[d]thiazol-2-amine with a number of substituted phenols in the presence of potassium iodide and anhydrous potassium carbonate in dry acetone. IR spectroscopy, ^{1}HNMRspectroscopy and spectroscopy methods were used to characterize the structural properties of newly synthesized derivatives. In order to observe the attributes of drug-like candidates of these derivatives, number of their molecular properties was estimated. Benzo[d]thiazol-2-amine derivatives were molecularly docked with selective enzymes COX-1 and COX-2. Findings suggested that Compounds G3, G4, G6, G8and G11 possess higher binding affinity than diclofenac sodium, when docking was performed with enzyme COX-1. Compounds G1, G3, G6, G8and G10 showed lower binding affinity than Indomethacin when docking performed with enzyme COX-2.*In vitro* evaluation of the COX-1 and COX-2 enzyme inhibitory activities were performed for synthesized compounds. Compounds 10 and 11 exhibited significant COX-1 and COX- enzyme inhibitory action with IC_{50} value of 5.0 and 10 μM, respectively. Using the hot plate method and the carrageenan-induced rat paw edema model, the synthesized compounds were screened for their biological activities, including analgesic and anti-inflammatory activities. Highest analgesic action was exhibited by derivative **G11**and the compound **G10** showed highest anti-inflammatory response.

Keywords: Benzothiazole; Docking; Analgesic; Anti-inflammatory

1. Introduction

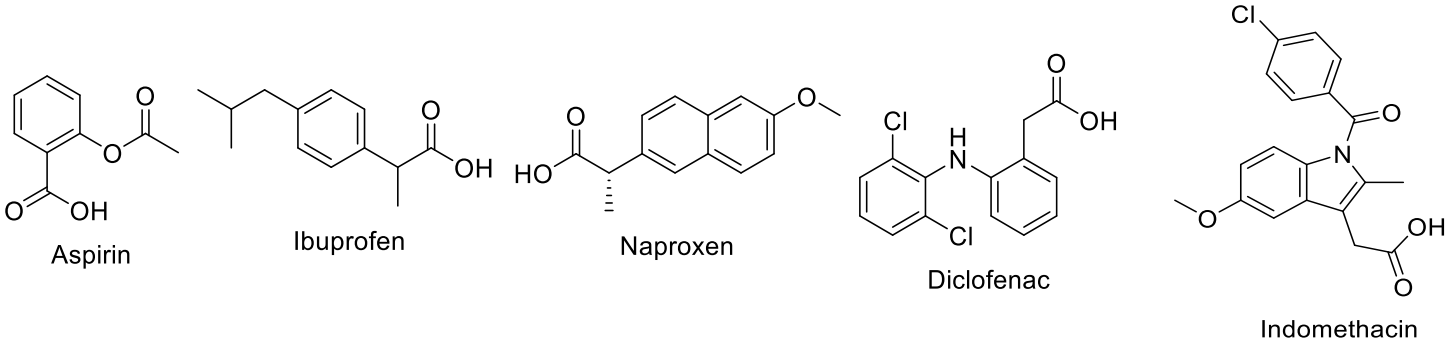
The availability of commercially important intermediate molecules, which are required for the synthesis of several classes of biologically active drugs, is greatly influenced by the benzo[d]thiazol-2-amine and its derivatives. Exploration of the synthesis of some new benzo[d]thiazol-2-amine, characterization, and their computational investigations, along with evaluation of their pharmacological activity, were taken into consideration as a crucial component of present research.

Trauma, foreign bodies, and pathogens cause biochemical inflammation [1,2]. In extreme cases of allergies, autoimmune illnesses, and organ rejection, inflammatory disorders can be catastrophic. Chronic inflammation is implicated to many diseases, notably cardiovascular, cancer, diabetes, arthritis, Alzheimer's, pulmonary, and many others [3]. Non-steroidal anti-inflammatory drugs (NSAIDs) are analgesic, antipyretic, and anti-inflammatory [4]. NSAIDs impede cyclooxygenase (COX) production. COX, commonly known as prostaglandin H-synthase or prostaglandin endoperoxide synthase, is the rate-limiting enzyme in prostanoid production. COX exists in two different isoforms,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

cyclooxygenase-1 (COX-1) andcyclooxygenase-2 COX-2 [5]. COX-1, performs normal physiological activities in all tissues [6].

COX-2 transforms arachidonic acid of biological tissues to prostaglandins, promoting cytokine inflammation [7,8]. In order to effectively treat pain and inflammation, new synthetic compounds are being sought. The present task has been carried out as a continuation of the previous work. The commonly used drugs of the said NSAIDs class are aspirin [8], ibuprofen [9], naproxen [10], diclofenac sodium [11,12] and indomethacin [13].



The majority of NSAIDs work by reducing the activity of COX-1 and COX-2 to inhibit the production of prostaglandins and thromboxane. Nevertheless, blocking the COX-2 enzyme delivers the expected anti-inflammatory, analgesic, and antipyretic effects [14,15]. Considering the side effects of current anti-inflammatory compounds, new and potent compounds should be developed. Researchers are interested in benzo[d]thiazol-2-amine, bicyclic heterocycles with fused benzene, thiazole rings and electron-rich heteroatoms including nitrogen and sulphur, due to their high pharmacological activity [16].

Particularly, benzo[d]thiazol-2-amine has a significant impact on medicinal chemistry and has already exhibited a wide spectrum of significant biological activities (Figure 1). As a result, the synthesis of benzo[d]thiazol-2-amines may be quite interesting because of their powerful and important biological activities as well as their high medicinal value.

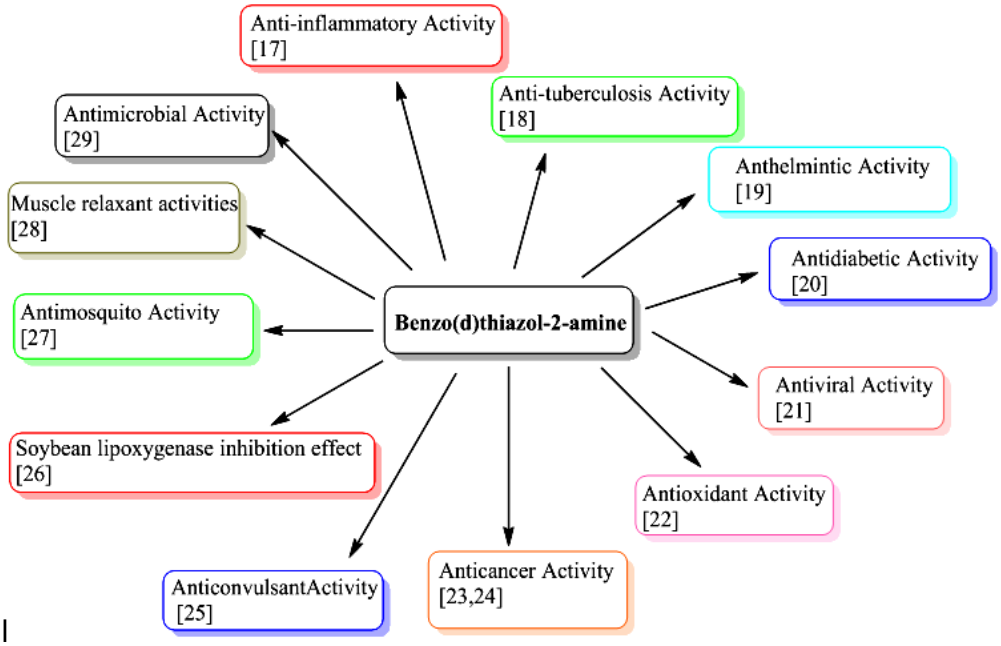


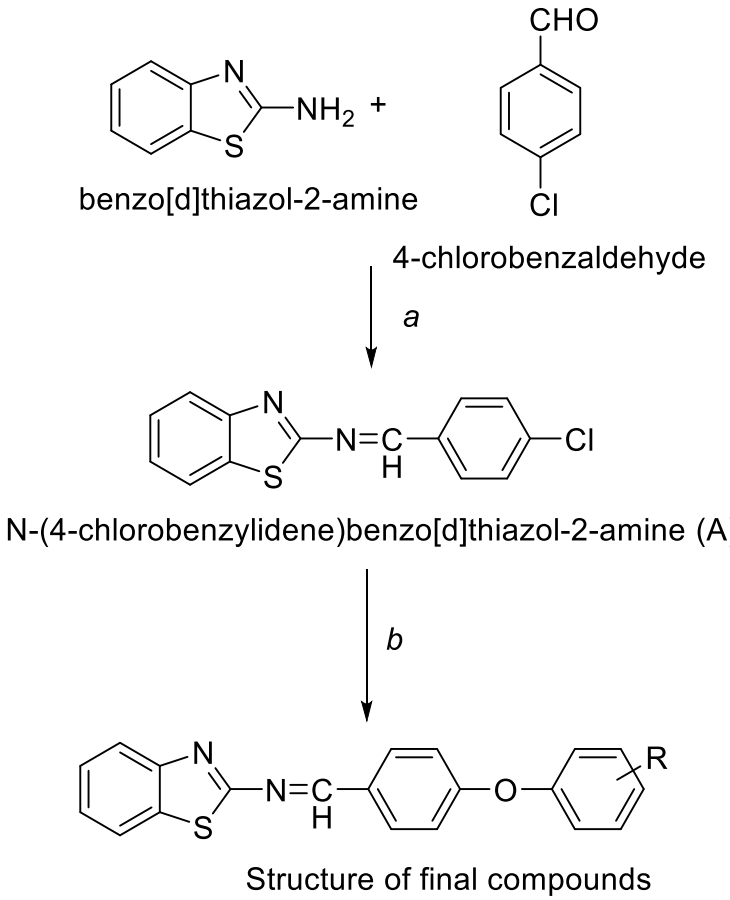
Figure 1. Schematic representation of biological activities ofbenzo[d]thiazol-2-amine.

2. Results and Discussion

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

2.1. Synthesis

The efficient synthetic route for the synthesis of benzo[d]thiazol-2-amine derivatives is presented (**Scheme 1)**. The key compound*N*-(4-chlorobenzylidene) benzo[d]thiazol-2-amine (**A**) was synthesized by substitution reaction of 2-aminobenzothiazole with 4-chlorobenzaldehyde in acetone. Finally,*N-(4-chlorobenzylidene) benzo[d]thiazol-2-amine* (**A**) was reacted with various substituted phenols in the presence of the anhydrous potassium carbonate and potassium iodide in dry acetone to obtain the synthetic derivatives, namely **G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12** and **G13**.



Where,R= H (G1), 2,5-CH_3 (G2), 2-NO_2 (G3), 3-OCH_3 (G4), 4-Br (G5), 2-Cl (G6), 4-OCH_3 (G7), 4-F (G8), 4-NO_2 (G9), 4-CHO (G10), 3-C_2H_5 (G11), 4-Cl (G12), 3-CH_3(G13)

Scheme 1. Synthetic routes for accessing the synthesis of different benzo[d]thiazol-2-amine derivatives. Reagents and conditions: *a) Ethanol, glacial acetic acid, reflux,10h b) Substituted phenols, acetone, anhydrous potassium carbonate, potassium iodide, reflux, 21h*.

The structures of all the synthesized benzo[d]thiazol-2-amine derivatives were identified by IR, ^{1}HNMR spectroscopy and Mass spectrometry. The physical characterization data of synthesized derivatives are presented in Table 1. Highest yield was obtained for G10. The structures of all synthesized compounds are presented in supplementary file.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

Table 1. Physical characterization data of benzo[d]thiazol-2-amine derivatives.

S. No.	Compound Code	R	M.P (^{0}C)	Molecular formula	Molecular weight	% Yield	R_f Value
1.	G1	H	140-142	$C_{20}H_{14}N_2OS$	330.40	57.89	0.75
2.	G2	2,5-CH_3	120-122	$C_{22}H_{18}N_2OS$	358.46	63.27	0.70
3.	G3	2-NO_2	132-134	$C_{20}H_{13}N_3O_3S$	375.40	59.58	0.65
4.	G4	3-OCH_3	128-130	$C_{21}H_{16}N_2O_2S$	360.43	55.35	0.80
5.	G5	4-Br	145-147	$C_{20}H_{13}BrN_2OS$	409.30	57.36	0.68
6.	G6	4-Cl	85-87	$C_{20}H_{13}ClN_2OS$	364.85	61.15	0.58
7.	G7	4-OCH_3	165-167	$C_{21}H_{16}N_2O_2S$	360.43	59.33	0.47
8.	G8	4-Fl	173-175	$C_{20}H_{13}FN_2OS$	348.39	52.61	0.53
9.	G9	4-NO_2	140-142	$C_{20}H_{13}N_3O_3S$	375.40	61.75	0.63
10.	G10	4-CHO	160-162	$C_{21}H_{14}N_2O_2S$	358.4	64.33	0.77
11.	G11	4-C_2H_5	110-112	$C_{22}H_{18}N_2OS$	358.46	51.84	0.51
12.	G12	2-Cl	138-140	$C_{20}H_{13}ClN_2OS$	364.85	56.71	0.59
13.	G13	3-CH_3	122-124	$C_{21}H_{16}ClN_2OS$	344.43	54.73	0.55

2.2. Computational Evaluation

To determine the drug-like candidate nature of synthesized compounds, the physicochemical features of the compounds were assessed to predict the pharmacokinetic properties of the compounds (Table 2).

Table 2. Physicochemical Properties of newly synthesized benzo[d]thiazol-2-amine compounds.

CC	MW[a]	log P	MR[b]	SAS[c]	MSA[d]	SEV[e]	TPSA[f]	MTI[g]	WI[h]	OV[i]	HBA[j]	HBD[k]	nRB[l]
G1	330.40	4.73	9.80	565.51	297.81	249.26	33.95	12531	1633	1.554	3	0	4
G2	358.45	5.39	10.73	613.91	329.76	283.30	33.95	15477	2012	1.580	3	0	4
G3	375.40	4.62	10.41	584.30	311.72	267.21	85.76	16057	2219	1.553	4	0	5
G4	360.43	4.63	10.42	605.88	321.82	271.15	43.18	15371	2050	1.588	4	0	5
G5	409.30	5.53	10.58	597.22	318.04	269.30	33.95	13546	1847	1.577	3	0	4
G6	364.84	5.36	10.29	589.47	312.66	263.55	33.95	13546	1847	1.572	3	0	4
G7	360.43	4.63	10.42	605.89	321.83	271.16	43.18	15611	2086	1.588	4	0	5
G8	348.39	4.89	9.82	571.57	301.47	252.47	33.85	13546	1847	1.560	4	0	4
G9	375.40	4.62	10.41	595.76	317.86	266.74	85.76	16705	2327	1.582	4	0	5
G10	358.41	4.45	10.30	591.45	314.33	264.18	51.02	15565	2086	1.578	4	0	5
G11	358.45	5.64	10.73	621.07	332.20	282.21	33.95	16045	2086	1.596	3	0	5
G12	364.84	5.36	10.29	589.47	312.66	263.56	33.95	13546	1847	1.572	3	0	4
G13	344.43	5.39	10.73	613.91	329.76	283.30	33.95	15477	2012	1.580	3	0	4
Ind.	357.8	4.17	11.26	547.16	298.94	266.94	66.84	9936	1424	1.4910	3	1	5

[a] Molecular Weight, [b] Molar refractivity, [c] Connolly solvent accessible surface area, [d] Connolly molecular surface area, [e] Connolly solvent excluded volume, [f] Topological polar surface area, [g] Molecular topological index, [h] Wienner index, [i] Ovality, j Hydrogen bond acceptor, [k] Hydrogen bond donor, [l] Number of rotable bonds.

All the synthesized compounds were found to have the log P values less than 5.6 and this shows a good solubility profile on the basis of log P, It be said that it can penetrate into lipid layer. All the compounds had nRB less than 10 signifying that these compounds may be suitable for oral bioavailability. It was observed that all synthesized compounds can permeate the cell because the calculated TPSA is less than 140 $Å^2$in each case. The percentage solubility of the synthesized derivatives was calculated from % Absorption (ABS) and it was observed that all synthesized compounds have excellent solubility at 85.94 to 97.28 %, there after these may exert excellent bioavailability.

2.3. Screening through in silicoPharmacokinetics, drug likeness and toxicity parameters

The findings of pharmacokinetics and toxicity study are presented in supplementary file. The risk of toxicity (*in silico*) measurable outcomes for the test derivatives (**G1-G13**) were evaluated and it was compared with reference drug. The findings suggested that the synthesized derivatives may be good at high doses or long-term therapeutic use. The outcome of predicted toxicity parameters was complying with standard drugs.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

2.4. Molecular Modeling Studies

The important parameter as a result of molecular docking is binding energy. It offers us an idea of the interaction's strength and affinities between the ligand and the receptor. The interaction is weaker if there is the higher the binding energy. Therefore, whenever we do a docking study, we aim to find the ligand that exhibits the lowest binding energy and best affinity among the test compounds. Docking of COX-1 with benzo[d]thiazol-2-amine derivatives was performed in comparison to diclofenac sodium as control. Control group showed binding affinity of -7.4 kcal/mol. Compounds G3, G4, G6, G8 and G11 showed higher binding affinity than control.

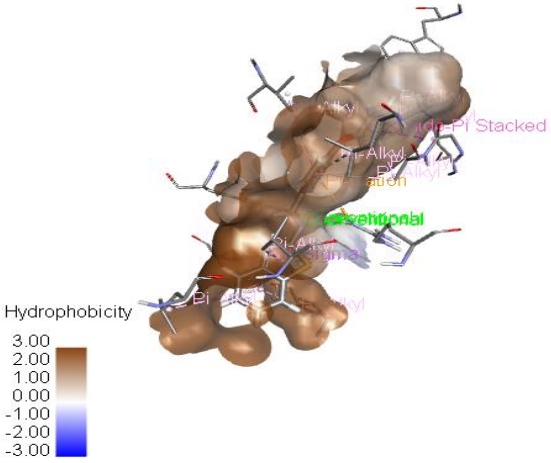
Docking of COX-2 with benzo[d]thiazol-2-amine compounds was done with indomethacin which served as the control. Control group showed binding affinity of -8.7 kcal/mol. Compounds G1, G3, G6 and G10 showed lower binding affinity than control. Binding affinity of newly synthesized benzo[d]thiazol-2 amine derivatives and reference standard compounds are presented in Table 3.

Table 3. Binding affinity of newly synthesized benzo[d]thiazol-2 amine derivatives and reference standard compounds.

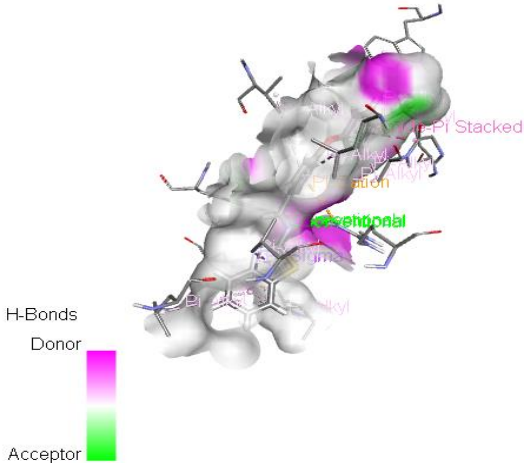
Compounds	Binding affinity (kcal/mol)	
	COX-1	COX-2
Indomethacin		-8.7
Diclofenac Sodium	-7.4	
G1	-7.1	-8.5
G2	-7.4	-8.1
G3	-7.7	-8.2
G4	-8.0	-8.2
G5	-5.7	-7.4
G6	-7.7	-8.3
G7	-6.7	-7.3
G8	-7.9	-8.4
G9	-6.9	-7.0
G10	-8.1	-8.6
G11	-8.4	-7.3
G12	-6.4	-7.9
G13	-6.9	-6.5

The hydrophobic interaction, hydrogen bond surface interactionand COX-1 complex of most significant synthesized compound (G11) benzo[d]thiazol-2 amine derivatives on COX-1 receptor are presented in Figure 2 a, 2 b and 2 c, respectively.

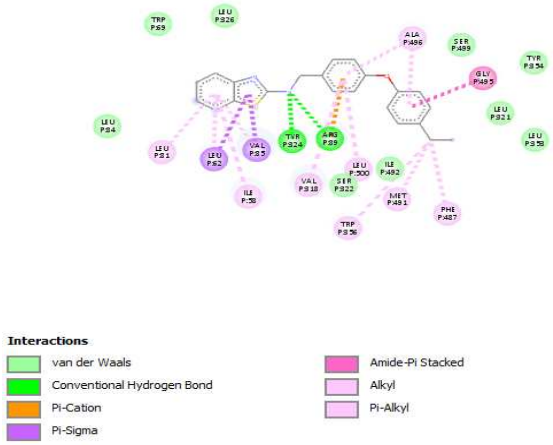
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1



2 (a)The hydrophobic interactions surface for molecule G11 with COX-1.



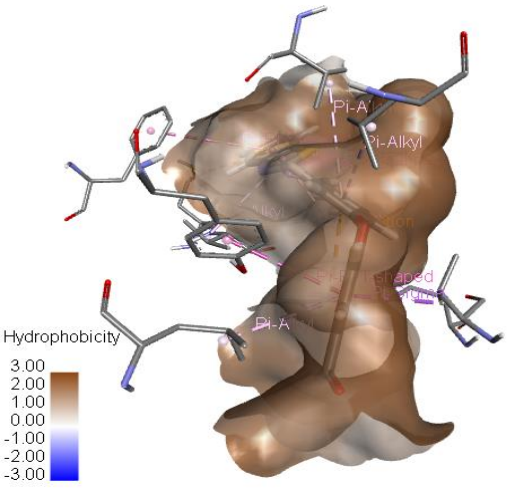
2(b)The hydrogen bond surfaces for molecule G11 with COX-1.

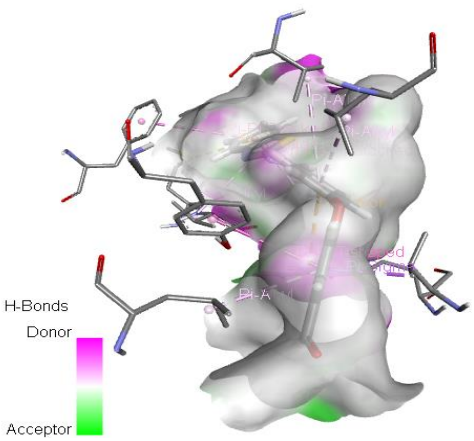


2(c)COX-1 complex with G11.

Figure 2. (a, b,c). The hydrophobic interaction, hydrogen bond surface interaction and COX-1 complex of most significant synthesized compound (G11) benzo[d]thiazol-2 amine derivatives.

The hydrophobic interaction, hydrogen bond surface interaction and COX-1 complex of most significant synthesized compound (G10) benzo[d]thiazol-2 amine derivatives on COX-2 receptor are presented in Figure 3 a, 3 b and 3 c,respectively.

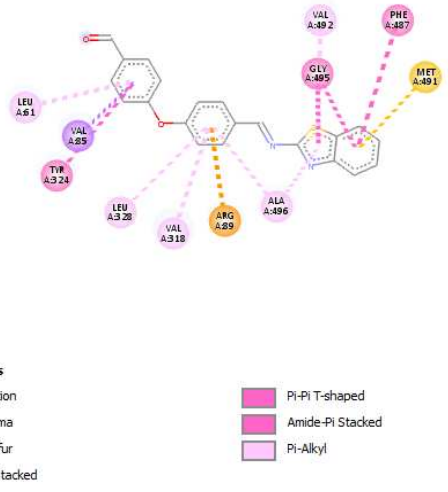




Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

3 (a)The hydrophobic interactions surface for molecule G10 with COX-2.

3 (b)The hydrogen bond surfaces for molecule G10 with COX-2.



3 (c) COX-2 complex with G10.

Figure 3. (a, b,c). The hydrophobic interaction, hydrogen bond surface interaction and COX-2 complex of most significant synthesized compound (G10) benzo[d]thiazol-2 amine derivatives.

In order to find out drug likeness properties of the newly synthesized benzo[d]thiazol-2-amine derivatives, bioavailability radar graph of benzo[d]thiazol-2-amine derivatives were drawn and the same is presented in supplementary file.

2.5. In vitro Cyclooxygenase enzyme inhibitory activity

Table 4 showed the results of *In vitro* Cyclooxygenase inhibitory activity. Diclofenac sodium and Indomethacin were used as a standard drug. Synthesized compounds G4, G10 and G11 demonstrated potent COX-1 inhibitory action with IC_{50} value of 13, 11 and 10 μM but standard drug diclofenac sodium and indomethacin demonstrated IC_{50} value 2.97 and 0.72 μM. Synthesized compounds G1, G8 and G10demonstrated potent COX-2 inhibitory action with IC_{50} value of 7.0, 8.0 and 5.0 μM but standard drug diclofenac sodium and indomethacin demonstrated IC_{50} value 0.79 and 0.98 μM.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

2.6. Acute toxicity

According to OECD recommendations, rats were used to test the preliminary toxicity of all the synthesized compounds G1 to G13. All the synthesized compounds were found to be safe up to 1000 mg/kg b.w. Thus, 1/10th of highest tolerated dose (100 mg/kg), was used as a therapeutic dose for biological activity. No significant changes were found in body weight and results are presented in Table 5. Acute toxicity results showed that none of the newly synthesized derivatives are harmful and toxic by nature and these compounds can be investigated further.

Table 4. *In vitro* COX-1 and COX-2 enzyme inhibition data for the synthesized compounds.

Compounds	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	SI COX-1/COX-2
Indomethacin	0.72 ±0.27	0.98±0.04	0.73
Diclofenac Sodium	2.97 ± 0.03	0.79 ± 0.07	3.75
G1	24±0.98	7.0±0.19	3.42
G2	21±0.79	15±0.26	1.40
G3	18±0.17	12±0.31	1.5
G4	13±0.21	11±0.29	1.18
G5	38±0.68	18±0.33	2.11
G6	19±0.32	10±0.20	1.90
G7	28±0.42	22±0.78	1.27
G8	16±0.36	8.0±0.11	2.00
G9	27±0.40	26±0.28	1.03
G10	11±0.14	5.0±0.12	2.20
G11	10±0.16	23±0.60	0.43
G12	30±0.32	16±0.24	1.87
G13	25±0.87	13±0.30	1.92

All values are expressed as a mean of three replicates ± SD. Selectivity index = (COX-1 IC_{50}/COX-2 IC_{50}).

Table 5. Effect of compounds on body weight.

Group	Doses	Body Weight		
		Initial day	7th day	14th day
Control	-	153.23 ± 3.02	158.02 ± 4.21	158.55 ± 5.61
G1	1000 mg/kg	154.20 ± 4.03 ^{ns}	157.19 ± 4.34 ^{ns}	156.33 ± 4.84 ^{ns}
G2	1000 mg/kg	155.21±5.17 ^{ns}	156.43±4.27 ^{ns}	157.12±5.04 ^{ns}
G3	1000 mg/kg	154.36±3.73 ^{ns}	156.68±4.95 ^{ns}	157.24±5.36 ^{ns}
G4	1000 mg/kg	153.21 ± 6.25 ^{ns}	155.25 ± 5.01 ^{ns}	156.41 ± 5.79 ^{ns}
G5	1000 mg/kg	152.23 ± 3.25 ^{ns}	154.04 ± 4.23 ^{ns}	155.15±5.02 ^{ns}
G6	1000 mg/kg	154.13 ± 3.51 ^{ns}	156.39 ± 4.72 ^{ns}	156.97±4.31 ^{ns}
G7	1000 mg/kg	155.41 ± 4.14 ^{ns}	157.14 ± 4.14 ^{ns}	156.74±5.32 ^{ns}
G8	1000 mg/kg	154.15 ± 3.68^{ns}	156.35 ± 4.81 ^{ns}	158.14±5.03 ^{ns}
G9	1000 mg/kg	155.84 ± 3.11 ^{ns}	157.15 ± 4.27 ^{ns}	159.11±4.72 ^{ns}
G10	1000 mg/kg	152.27 ± 6.03 ^{ns}	159.69± 6.24 ^{ns}	160.16±6.07^{ns}
G11	1000 mg/kg	153.63 ± 5.71 ^{ns}	160.05 ± 4.74 ^{ns}	161.12±5.12 ^{ns}
G12	1000 mg/kg	149.13 ± 2.03 ^{ns}	158.07 ± 4.31 ^{ns}	158.19±3.07 ^{ns}
G13	1000 mg/kg	154.21 ± 6.95 ^{ns}	160.08 ± 5.84 ^{ns}	161.14±7.11 ^{ns}

*ns- Not Significant,Body weight is presented as mean ± SD (standard deviation), n=6 each group, one-way Analysis of Variance (ANOVA) test was used,p value < 0.05 was considered statistically significant.

2.7. Biological activity

Hot plate method and carrageenan induced rat paw edema method were used to evaluate analgesic and anti-inflammatory activity at a dose of 100 mg/kg b.w. and Table 6 showed the results of analgesic activity. Table 7presents the results of anti-inflammatory activity. Diclofenac sodium and Indomethacin were used as a standard drug.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

Table 6. The response of analgesic activity by hot plate method.

Treatment	Dose(mg/kg) b.w.	Analgesic Response by hot plate method Reaction time in seconds (Mean ±SEM)				
		0 m	60 m	120 m	180 m	240m
Control (normal saline)	5 ml/kg	2.39±0.23	2.46±0.17	2.43±0.21	2.45±0.18	2. ±0.22
Diclofenac sodium	10	4.37±1.32**	7.52±0.74***	11.49±0.94***	13.35±1.01***	10.28±0.93***
G1	100	2.52±0.73	5.22±0.67***	8.32±0.75***	9.41±0.88***	8.29±0.67***
G2	100	2.55±0.81	5.28±0.69***	8.39±0.71***	9.36±0.85***	8.41±0.73***
G3	100	3.12±0.97*	6.21±0.59***	9.33±0.70***	10.29±0.91***	9.36±0.89***
G4	100	3.14±0.68	6.42±0.61***	10.11±0.72***	11.37±0.97***	8.39±0.91***
G5	100	2.55±0.83	3.58±0.65**	4.37±0.79	5.22±0.75	4.41±0.65**
G6	100	3.15±0.96*	6.19±0.61***	9.21±0.73***	10.11±0.93***	9.25±0.90***
G7	100	3.35±0.71*	4.48±0.79**	5.36±0.95**	6.10±0.83***	5.25±0.97**
G8	100	3.32±0.65*	6.28±0.60***	10.10±0.71***	11.14±0.95***	8.30±0.90***
G9	100	2.45±0.88	4.52±0.97**	5.49±0.97**	6.23±0.85***	5.48±0.98**
G10	100	3.23±0.71*	4.36±0.77**	5.21±0.93**	5.84±0.81***	5.19±0.68**
G11	100	2.50±0.81	7.34±0.69***	10.28±0.72***	12.46±1.02***	9.28±0.91***
G12	100	2.57±0.83	4.14±0.69**	5.12±0.91**	5.37±0.79***	4.63±0.65**
G13	100	2.58±0.84	3.63±0.63**	4.89±0.79	5.62±0.73	4.26±0.65**

Data are presented as mean ±SEM, n=6 each group, one-way Analysis of Variance (ANOVA) test was used,p value < 0.05 was considered statistically significant.

Table 7. Anti-inflammatory activity by Carrageenan induced rat paw edema method.

Treatment	**Dose (mg/kg) b.w.**	**% inhibition (1-Vt/Vc)×100**				
		0 m	**60 m**	**120 m**	**180 m**	**240m**
Control	5 ml/kg	----	----	----	----	----
Indomethacin	10	0	68.81	72.63	74.36	69.27
G1	100	0	59.56	63.89	67.21	64.84
G2	100	0	58.11	62.29	65.42	63.11
G3	100	0	58.29	62.43	65.71	63.49
G4	100	0	57.16	61.49	63.76	62.81
G5	100	0	56.37	60.22	62.46	60.87
G6	100	0	58.89	62.77	66.10	63.82
G7	100	0	55.51	59.45	60.83	58.11
G8	100	0	59.23	63.11	66.77	64.29
G9	100	0	49.53	52.37	55.17	53.83
G10	100	0	63.71	64.51	67.88	65.29
G11	100	0	55.42	59.12	60.22	57.52
G12	100	0	57.10	61.32	63.41	62.33
G13	100	0	50.24.53	52.89	56.38	54.86

Anti-inflammatory response is presented as mean ± SD (standard deviation), n=6 each group, one-way Analysis of Variance (ANOVA) test was used,p value < 0.05 was considered statistically significant; m-Minutes.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

Highest analgesic response was observed by derivative **G11** having 4-C_2H_5 group in the phenyl ring and it showed reaction time ranging from 2.50±0.81-12.46±1.02whereas least analgesic response was demonstrated by derivative **G5** bearing 4-Br group in the phenyl ring and it demonstrated reaction time ranging from 2.55±0.83-5.22±0.75at 100 mg/kg b.w. Among the compounds tested for anti-inflammatory activity, compound **G10**was having 4-amino group demonstrated highest anti-inflammatory response which was 67.88% inhibition of edema but **G9** having 4-NO_2 group demonstrated lowest anti-inflammatory action around 54.79% inhibition of edema at 120 m.

The results suggested that the analgesic and anti-inflammatory activity reactions were because of good results in docking study. This shows that newly synthesized compounds are having potential to inhibit COX-1 and COX-2 enzymes resulting into inhibition of the synthesis of PGs and therefore results into analgesic and anti-inflammatory response.

2.7. Structure activity relationship (SAR)

The literature-based data and on the basis of observations of present research, some points has been concluded on structure activity relationship by comparing the analgesic as well as anti-inflammatory response of synthesized derivatives (G1-G13).Based upon the findings of present work, Structure Activity Relationship of benzo[d]thiazol-2-amine derivatives is presented in Figure 4.

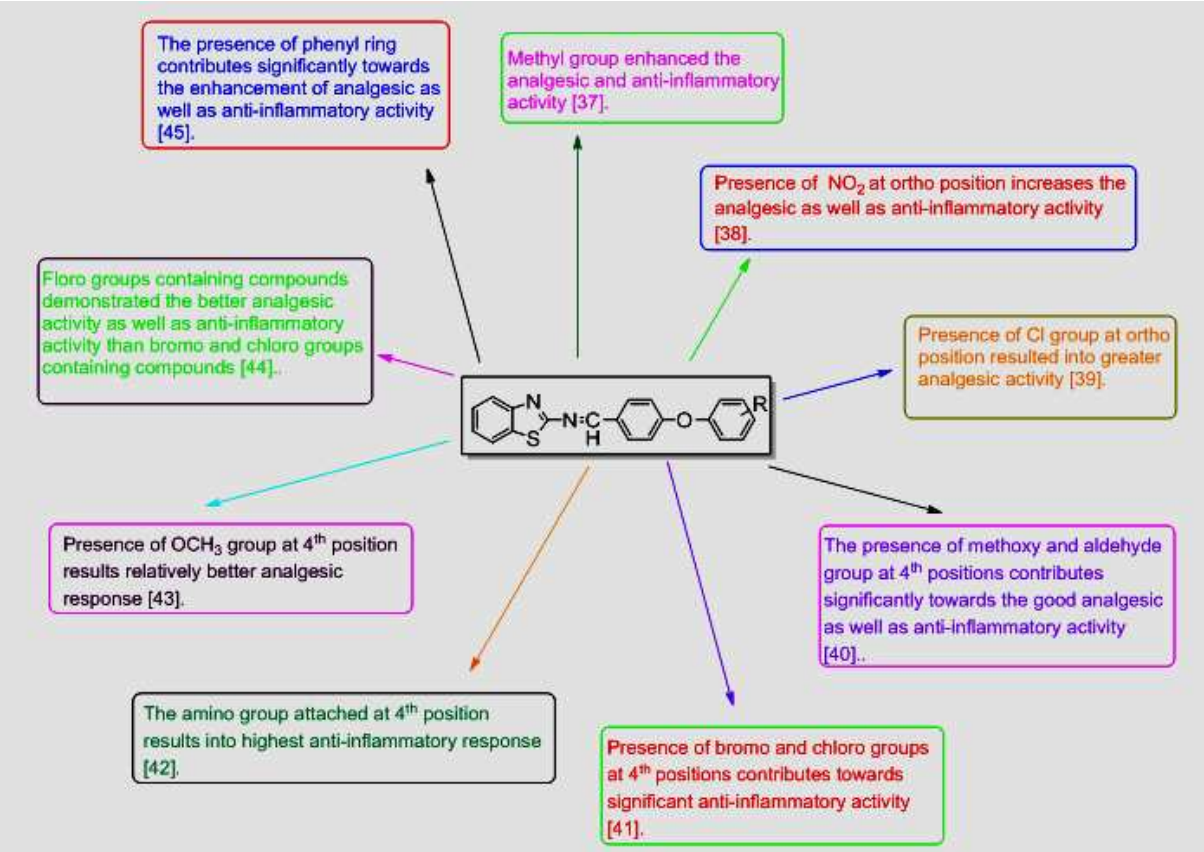


Figure 4. Graphical presentation of SAR of synthesized derivatives.

NSAIDshave been used to relieve pain by preventing the enzymes that make prostaglandins from being produced. However, it is clear that NSAIDs also have analgesic effects through a variety of additional peripheral and cerebral mechanisms in addition to peripheral reduction of prostaglandin synthesis. Analgesics are one of the most often used types of drugs for pain treatment.The existence of two structurally distinct cyclo-oxygenase enzyme variants is well established (COX-1 and COX-2). COX-1 is present in healthy

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

cells by nature;however, COX-2 is produced by inflammatory cells. The most likely mechanism of action for NSAID-mediated analgesia is COX-2 inhibition, while the ratio of COX-1 to COX-2 inhibition by NSAIDs should indicate the likelihood of side effects [46,47,48].

It has been suggested that a potential mechanism for efficient analgesic and anti-inflammatory actions is the inhibition of the COX-1 and COX-2 enzymes. Furthermore, docking investigations made the same clear.

According to the findings of the current investigation, the basic ring of the newly synthesised derivatives, benzo[d]thiazol-2-amine, has analgesic and anti-inflammatory properties.

In previous work as reported by other researchers, different benzothiazole derivatives were synthesized and tested for anti-inflammatory and analgesic activities.Structural activity data exhibited that substitution at the para position of the benzenesulphonamide and presence of electron rich NH or OH group enhanced the anti-inflammatory and analgesic properties.Further, it was observed that *N-(1,3-Benzothiazol-2-yl)-3-(1H-indol-2-yl)-2-[N-(4-nitrobenzenesulfonyl)-1 phenylformamido]propenamide* showed the highest anti-inflammatory and analgesic activitieswhen compared to indomethacin and celecoxib and also showed the highest binding energy against COX enzyme[49].

2-[[2-alkoxy-6-pentadecylphenyl)methyl]thio]-1H-benzimidazoles/benzothiazoles was synthesized by Paramashivappa*et al.* and in a human whole blood trial, it was tested for its ability to block the COX-2 enzyme, whereas the more potent compounds were tested for their capability to block the COX-1 enzyme. This compound was found to be 384-fold more selective than COX-1, and compound 19 was found to be more than 470-fold more active[50].

After observing the results of this study, we can say that these synthesized derivatives (G1-G13) could be developed and investigated as a novel class of analgesic and anti-inflammatory agents. However, further thorough biological evaluation at the molecular level would be necessary to identify the valuable pharmacologically active molecules with the fewest side effects.

3. Materials and Methods

3.1. Chemicals

2-aminobenzothiazole, 4-chlorobenzaldehyde, and other substituted phenols were procured from CDH Pvt. Ltd., New Delhi, and S.D. Fine, Mumbai, India. The synthetic strategy for the proposed research is presented in scheme 1.

All the synthesized compounds were recrystallized from ethanol. The Thin Layer Chromatography (TLC) was used to check the purity of newly synthesized compounds. Ethyl acetate: hexane (1:1) was used as solvent system for same. The conventional method (open capillary tube method) was used to observe the melting point of intermediates of newly synthesized compounds. The results were recorded as uncorrected. The Fourier Transform Infrared (FTIR) spectrum was captured using an FTIR spectrophotometer (8400SS Shimadzu, Japan) using potassium bromide pellets method. The ^{1}HNMR spectrum was recorded at 25 °C using a 500 MHz Bruker Avance III HD spectrometerin DMSO-d_6. As an internal standard, Tetramethylsilane (TMS) was used. The ^{1}HNMR chemical shifts were recorded as parts per million (ppm) downfield from TMS. Shimadzu GCMS-QP-2000 mass spectrometer was used to record the mass spectrum.

3.2. Synthesis

Synthesis of N-(4-chlorobenzylidene) benzo[d]thiazol-2-amine (A)

Equimolar of 2-aminobenzothiazole and 4-chlorobenzaldehyde was dissolved in ethanol (100ml) in 250ml round bottom flask (RBF) and 4-5 drops of glacial acetic acid was added in this. The reaction mixture was refluxed for 10 h. The reaction mixture was cooled to room temperature and set aside overnight for precipitation. The precipitated compounds were filtered, washed with water and recrystallized using ethanol. The reaction

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

mixture was concentrated and filtered. Yellow colored product was obtained which was dried at room temperature. Then, melting point and TLC were observed.

M.P: 90-92^{0}C; R_f. Value: 0.74; IR (KBr) cm-^{1}: 1488 (C-C Str. Aromatic), 1596 (C=C Str. Aromatic), 3054 (C-H Str. Aromatic), 1728 (C=N Str.), 1342 (C-N Str.), 718 (C-Cl Str.); ^{1}HNMR (500 MHz) (ppm): 8.56 (s, 1H, CH), 8.17 (d, *J* = 10 Hz, 1H, benzothiazole), 7.99 (d, *J* = 5.14 Hz, 1H, benzothiazole), 7.75 (d, *J* = 4 Hz, 2H, Ar-H), 7.53 (t, J = 6.73 Hz, 2H, benzothiazole), 7.51 (d, *J* = 9.0 Hz, 2H, Ar-H); 13CNMR (500MHz) 121.3, 121.5, 124.3, 125.1, 125.5, 148.42, 174.3 (C, Benzothiazole), 159.83 (CH), 128.70, 128.73, 129.85, 130.10, 133.31, 135.32 (C, Ar-C.); MS (FAB) $[M+1]^+$:(m/z) 273.01

General procedure for synthesis of Benzo[d]thiazol-2-amine derivatives

Equimolar of **A** and various substituted phenols were dissolved in 125 ml dry acetone in 250ml RBF. Thenequimolarof anhydrous potassium carbonate was added in reaction mixture and potassium iodide was added. The reaction mixture was refluxed for 21 h. After the completion of reaction, the reaction mixture was cooled and subjected to freeze overnight for precipitation. The reaction mixture was concentrated, filtered, washed with water and dried. Then, melting point and TLC was performed. In the reaction procedure, different phenols were used which resulted into newbenzo[d]thiazol-2-amine derivatives namely G1-G13.

(E)-N-(4-Phenoxybenzylidene) benzo[d]thiazol-2-amine (G1)

IR (KBr) cm-^{1}:3115 (C-H Aromatic), 1651 (C=N), 1613 (C-N), 1505 (C-C Str. Aromatic), 1482 (C=C Str. Aromatic), 1261 (C-S), 1128 (C-O str.); ^{1}HNMR (500 MHz) (ppm): 8.53 (s, 1H, CH), 8.15 (d, *J* = 9.96 Hz,1H, benzothiazole), 7.98 (d, *J* = 5.37 Hz, 1H, benzothiazole), 7.87 (d, *J* = 4.12 Hz, 2H,.), 7.52 (t, *J* = 6.49 Hz, 2H, benzothiazole), 7.39 (d, *J = 7.0 Hz, 2H*,Ar-H), 7.21 (d, *J* = 7.5 Hz,2H, Ar-H), 7.18 (d,*J* = 8.27 Hz, 2H, Ar-H), 7.15 (t, *J* = 9.0 Hz,1H, Ar-H), ^{13}CNMR (500MHz) 121.42, 121.60, 124.33, 125.21, 125.49, 148.53, 174.50 (C, Benzothiazole), 159.98 (CH), 117.12, 117.57, 118.52, 118.63, 120.83, 128.65, 128.75, 129.80, 129.92, 130.15, 156.49, 158.37 (C, Ar-C.);MS (FAB) $[M+1]^+$:(m/z) 331.08

(E)-N-(4-(2,5-Dimethylphenoxy) benzylidene) benzo[d]thiazol-2-amine (G2)

IR (KBr) cm-^{1}:3112 (C-H Aromatic), 1649 (C=N), 1614 (C-N), 1507 (C-C Str. Aromatic), 1485 (C=C Str. Aromatic), 1264 (C-S), 1130 (C-O str.); ^{1}HNMR(500 MHz) (ppm): 8.53 (s, 1H, CH), 8.16 (d, *J* = 9.98 Hz,1H, benzothiazole), 7.96 (d, *J = 5.71 Hz*,1H, benzothiazole), 7.87 (d, *J* = 4.15 Hz,2H, Ar-H.), 7.51 (t, *J* = 6.43 Hz,2H, benzothiazole), 7.89 (d, *J* = 5.0 Hz,2H, Ar-H), 6.84 (d, *J* = 8.0 Hz,1H, Ar-H), 2.34 (s, 6H, CH_3); ^{13}CNMR (500MHz) 121.39, 121.55, 124.37, 125.29, 125.53, 148.46, 174.57 (C, Benzothiazole), 159.95 (CH), 117.09, 117.51, 118.49, 118.57, 120.78, 128.67, 128.71, 129.83, 129.95, 130.16, 156.51, 158.32(C, Ar-C.), 15.21 (CH_3), 21.22 (CH_3); MS (FAB) $[M+1]^+$:(m/z) 359.11

(E)-N-(4-(2-Nitrophenoxy) benzylidene) benzo[d]thiazol-2-amine (G3)

IR (KBr) cm-^{1}:3115 (C-H Aromatic), 1651 (C=N), 1617 (C-N), 1501 (C-C Str. Aromatic), 1479 (C=C Str. Aromatic), 1303 (N-O Str.), 1259 (C-S), 1128 (C-O str.); ^{1}HNMR(500 MHz) (ppm): 8.58 (s, 1H, CH), 8.21 (d,*J* = 7.50 Hz, 1H, Ar-H.), 8.17 (d, *J* = 9.50 Hz, 1H, benzothiazole), 8.10 (d, *J* = 5.50 Hz, 1H, benzothiazole), 7.89 (d, *J* = 4.35 Hz, 2H, Ar-H), 7.76 (t, *J* = 6.50 Hz, 2H, Ar-H), 7.72 (d, *J* = 1.50 Hz, 1H, Ar-H), 7.50 (t, *J* = 4.98 Hz, 2H, benzothiazole), 7.20 (d, *J* = 5.0 Hz, 2H, Ar-H); ^{13}CNMR (500MHz) 121.33, 121.54, 124.31, 125.22, 125.49, 148.43, 174.52 (C, Benzothiazole), 159.83 (CH), 117.31, 117.53, 118.43, 118.52, 120.65, 128.71, 128.73, 129.79, 129.98, 130.19, 149.63, 159.28 (C, Ar-C.); MS (FAB) $[M+1]^+$:(m/z) 376.05

(E)-N-(4-(3-Methoxyphenoxy) benzylidene) benzo[d]thiazol-2-amine (G4)

IR (KBr) cm-^{1}:3119 (C-H Aromatic), 1655 (C=N), 1619 (C-N), 1509 (C-C Str. Aromatic), 1481 (C=C Str. Aromatic), 1262 (C-S), 1130 (C-O str.); ^{1}HNMR(500 MHz) (ppm): 8.56 (s, 1H, CH), 8.16 (d, *J* = 9.0 Hz, 1H, benzothiazole), 8.11 (d, *J* = 5.25 Hz, 1H, benzothiazole), 7.90 (d, *J = 4.45 Hz*, 2H, Ar-H), 7.52 (t, *J = 6.0 Hz*, 2H, benzothiazole), 7.18 (d, *J = 5.50 Hz*, 2H, Ar-H); 7.14 (t, *J* = 7.50 Hz, 1H, Ar-H), 6.35 (t, *J = 4.58 Hz*, 1H, Ar-H), 6.31 (d, *J* = 5.0Hz, 1H, Ar-H), 6.21 (d, *J* = 1.5 Hz, 1H, Ar-H), 3.81 (s, 3H, OCH_3), ^{13}CNMR (500MHz) 121.28, 121.49, 124.28, 125.19, 125.46, 148.39, 174.55 (C, Benzothiazole), 160.31 (CH), 117.30, 117.52,

 doi:10.20944/preprints202306.1064.v1

118.40, 118.57, 120.59, 128.67, 128.75, 129.81, 129.92, 130.17, 157.65, 159.28 (C, Ar-C.), 55.31 (C, OCH_3); MS (FAB) $[M+1]^+$:(m/z) 361.08

(E)-N-(4-(4-Bromophenoxy) benzylidene) benzo[d]thiazol-2-amine (G5)

IR (KBr) cm^{-1}:3114 (C-H Aromatic), 1653 (C=N), 1616 (C-N), 1510 (C-C Str. Aromatic), 1475 (C=C Str. Aromatic), 1260 (C-S), 1132 (C-O str.), 705 (C-Br Str.); ^{1}HNMR(500 MHz) (ppm): 8.50 (s, 1H, CH), 8.13 (d,*J* = 9.25 Hz, 1H, benzothiazole), 8.02 (d, *J* = 5.34 Hz, 1H, benzothiazole), 7.88 (d, *J* = 4.36 Hz, 2H, Ar-H), 7.55 (d, *J* = 6.5 Hz, 2H, Ar-H), 7.50 (t, *J* = 7.0 Hz,2H, benzothiazole), 7.46 (d, *J* = 6.0 Hz, 2H, Ar-H), 7.20 (d, *J* = 7.05 Hz, 2H, Ar-H), ^{13}CNMR (500MHz) 121.29, 121.50, 124.31, 125.24, 125.51, 148.47, 174.61 (C, Benzothiazole), 159.88 (CH), 117.29, 117.49, 118.43, 118.54, 120.48, 128.63, 128.71, 129.84, 129.96, 130.13, 157.62, 159.30 (C, Ar-C.); MS (FAB) $[M+1]^+$:(m/z) 410.99

(E)-N-(4-(4-Chlorophenoxy)benzylidene)benzo[d]thiazol-2-amine (G6)

IR (KBr) cm^{-1}:3116 (C-H Aromatic), 1655 (C=N), 1618 (C-N), 1512 (C-C Str. Aromatic), 1478 (C=C Str. Aromatic), 1259 (C-S), 1129 (C-O str.), 705 (C-Cl Str.); ^{1}HNMR (500 MHz) (ppm): 8.58 (s, 1H, CH), 8.14 (d, *J* = 9.33 Hz, 1H, benzothiazole), 7.95 (d, *J* = 4.98 Hz, 1H, benzothiazole), 7.90 (d, *J* = 4.45 Hz, 2H, Ar-H), 7.55 (d, *J* = 6.0 Hz, 2H, Ar-H), 7.50 (t, *J* = 7.09 Hz, 2H, benzothiazole), 7.41 (d, *J* = 6.12 Hz, 2H, Ar-H), 7.20 (d, *J* = 7.10 Hz, 2H, Ar-H), ^{13}CNMR (500MHz) 121.27, 121.55, 124.34, 125.29, 125.54, 148.43, 174.57 (C, Benzothiazole), 159.79 (CH), 117.26, 117.45, 118.38, 118.50, 120.46, 128.57, 128.73, 129.88, 129.94, 130.21, 157.58, 159.42 (C, Ar-C.); MS (FAB) $[M+1]^+$:(m/z) 365.05

(E)-N-(4-(4-Methoxyphenoxy) benzylidene) benzo[d]thiazol-2-amine (G7)

IR (KBr) cm^{-1}:3120 (C-H Aromatic), 1650 (C=N), 1615 (C-N), 1514(C-C Str. Aromatic), 1469 (C=C Str. Aromatic), 1250 (C-S), 1125 (C-O str.); ^{1}HNMR (500 MHz) (ppm): 8.55 (s, 1H, CH), 8.12 (d, *J* = 9.12 Hz, 1H, benzothiazole), 7.90 (d, *J* = 4.50 Hz, 1H, benzothiazole), 7.88 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.53 (d, *J* = 6.25 Hz, 2H, Ar-H), 7.49 (t, *J* = 7.05 Hz, 2H, benzothiazole), 6.97 (d, *J* = 7.42 Hz,2H, Ar-H), 3.85 (s, 3H, OCH_3); ^{13}CNMR (500 MHz) 121.39, 121.48, 124.45, 125.19, 125.73, 148.62, 174.46 (C, Benzothiazole), 159.83 (CH), 113.85, 114.32, 117.11, 117.39, 119.46, 128.32, 128.69, 129.83, 129.91, 131.21, 154.32, 158.76 (C, Ar-C.), 54.82 (C, OCH_3); MS (FAB) $[M+1]^+$:(m/z) 361.08

(E)-N-(4-(4-Fluorophenoxy) benzylidene) benzo[d]thiazol-2-amine (G8)

IR (KBr) cm^{-1}:3119 (C-H Aromatic), 1658 (C=N), 1621 (C-N), 1515 (C-C Str. Aromatic), 1477 (C=C Str. Aromatic), 1255 (C-S), 1131 (C-O str.), 687 (C-F Str.); ^{1}HNMR(500 MHz) (ppm): 8.57 (s, 1H, CH), 8.11 (d, *J* = 10 Hz, 1H, benzothiazole), 8.01 (d, *J = 5.0 Hz*, 1H, benzothiazole), 7.90 (d, *J* = 7.0 Hz,2H, Ar-H), 7.52 (t, *J = 6.50 Hz*,2H, benzothiazole), 7.43 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.20 (d, *J* = 7.45 Hz, 4H, Ar-H); ^{13}CNMR (500 MHz) 121.40, 121.51, 124.39, 125.23, 125.78, 148.65, 174.51 (C, Benzothiazole), 159.76 (CH), 113.71, 114.29, 117.19, 117.42, 119.53, 128.35, 128.62, 129.78, 129.95, 131.24, 154.36, 158.51 (C, Ar-C.);MS (FAB) $[M+1]^+$:(m/z) 349.08

(E)-N-(4-(4-Nitrophenoxy)benzylidene)benzo[d]thiazol-2-amine (G9)

IR (KBr) cm^{-1}:3116 (C-H Aromatic), 1653 (C=N), 1612 (C-N), 1507 (C-C Str. Aromatic), 1472 (C=C Str. Aromatic), 1305 (N-O Str.), 1251 (C-S), 1129 (C-O str.); ^{1}HNMR (500 MHz) (ppm): 8.57 (s, 1H, CH), 8.21 (d, *J* = 9.98 Hz, 2H, Ar-H), 8.17 (d, *J* = 7.0 Hz, 1H, benzothiazole), 8.02 (d, *J* = 5.12 Hz, 1H, benzothiazole), 7.89 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.49 (t, *J* = 6.25 Hz, 2H, benzothiazole), 7.21 (d, *J = 7.25 Hz*,4H, Ar-H); ^{13}CNMR (500 MHz) 121.38, 121.54, 124.37, 125.26, 125.69, 148.77, 174.56 (C, Benzothiazole), 159.68 (CH), 113.66, 114.31, 117.23, 117.45, 119.48, 128.39, 128.59, 129.72, 129.89, 131.27, 154.39, 158.48 (C, Ar-C.);MS (FAB) $[M+1]^+$:(m/z) 376.05

(E)-4-(4-((Benzo[d]thiazol-2-ylimino) methyl) phenoxy) benzaldehyde(G10)

IR (KBr) cm^{-1}:3118 (C-H Aromatic), 1656 (C=N), 1618 (C-N), 1510 (C-C Str. Aromatic), 1475 (C=C Str. Aromatic), 1257 (C-S), 1125 (C-O str.); ^{1}HNMR (500 MHz) (ppm): 9.85 (s, 1H, CHO), 8.52 (s, 1H, CH), 8.17 (d, *J* = 9.75 Hz, 1H, benzothiazole), 7.99 (d, *J = 5.25 Hz*,1H, benzothiazole), 7.89 (d, *J* = 7.75 Hz, 2H, Ar-H), 7.46 (t, *J* = 6.50 Hz, 2H, benzothiazole), 7.21 (d, *J* = 7.0 Hz, 2H, Ar-H), 6.79 (d, *J* = 5.50 Hz, 2H, Ar-H), 6.71 (d, *J* = 7.0 Hz, 2H, Ar-H); ^{13}CNMR (500 MHz) 121.29, 121.59, 124.28, 125.21, 125.65, 148.69, 174.48 (C, Benzotriazole),

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

159.65 (CH), 113.71, 114.42, 117.31, 117.48, 119.46, 128.41, 128.53, 129.69, 129.85, 131.23, 154.33, 158.51 (C, Ar-C.); MS (FAB) $[M+1]^+$:(m/z) 359.06

(E)-N-(4-(4-Ethylphenoxy)benzylidene)benzo[d]thiazol-2-amine (G11)

IR (KBr) cm^{-1}:3114 (C-H Aromatic), 1652 (C=N), 1614 (C-N), 1509 (C-C Str. Aromatic), 1481 (C=C Str. Aromatic), 1260 (C-S), 1129 (C-O str.); ^{1}HNMR (500 MHz) (ppm): 8.58 (s, 1H, CH), 8.18 (d, *J* = 9.83 Hz, 1H, benzothiazole), 8.01 (d, *J* = 5.50 Hz, 1H, benzothiazole), 7.91 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.50 (t, *J* = 6.75 Hz, 2H, benzothiazole), 7.35 (d, *J* = 4.50 Hz, 2H, Ar-H), 7.25 (d, *J* = 7.25 Hz,2H, Ar-H), 6.98 (d, *J* = 6.0 Hz, 2H, Ar-H); 2.55 (s, 2H, CH_2), 121 (s, 3H, CH_3); ^{13}CNMR (500 MHz) 121.31, 121.60, 124.31, 125.23, 125.68, 148.65, 174.41 (C, Benzothiazole), 159.52 (CH), 113.51, 114.46, 117.38, 117.51, 119.62, 128.43, 128.55, 129.63, 129.80, 131.28, 154.29, 158.48 (C, Ar-C.), 28.11 (C, CH_2), 14.23 (C, CH_3); MS (FAB) $[M+1]^+$:(m/z) 359.10

(E)-N-(4-(4-Chlorophenoxy) benzylidene) benzo[d]thiazol-2-amine (G12)

IR (KBr) cm^{-1}:3119 (C-H Aromatic), 1655 (C=N), 1618 (C-N), 1514 (C-C Str. Aromatic), 1471 (C=C Str. Aromatic), 1264 (C-S), 1135 (C-O str.), 708 (C-Cl Str.); ^{1}HNMR(500 MHz) (ppm): 8.57 (s, 1H, CH), 8.15 (d, *J* = 10 Hz, 1H, benzothiazole), 7.93 (d, *J = 7.95 Hz*, 1H, benzothiazole), 7.88 (d, *J* = 8.25 Hz, 2H, Ar-H), 7.53 (d, *J* = 6.50 Hz,2H, Ar-H), 7.48 (t, *J* = 7.0 Hz,2H, benzothiazole), 7.38 (d, *J = 7.50 Hz*, 2H, Ar-H), 7.18 (d, *J = 7.25 Hz*, 2H, Ar-H), ^{13}CNMR (500 MHz) 121.27, 121.63, 124.38, 125.29, 125.75, 148.59, 174.39 (C, Benzothiazole), 159.61 (CH), 113.42, 114.53, 117.42, 117.55, 119.58, 128.46, 128.52, 129.66, 129.83, 131.32, 154.26, 158.46 (C, Ar-C.); MS (FAB) $[M+1]^+$:(m/z) 365.03

(E)-N-(Benzo[d]thiazol-2-yl)-1-(4-(m-tolyloxy) phenyl) methanimine (G13)

IR (KBr) cm^{-1}:3111 (C-H Aromatic), 1643 (C=N), 1617 (C-N), 1501 (C-C Str. Aromatic), 1480 (C=C Str. Aromatic), 1265 (C-S), 1132 (C-O str.); ^{1}HNMR(500 MHz) (ppm): 8.55 (s, 1H, CH), 8.18 (d, *J* = 9.50 Hz, 1H, benzothiazole), 7.93 (d, *J* = 7.83 Hz, 1H, benzothiazole), 7.83 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.52 (t, *J = 6.25 Hz*, 2H, benzothiazole), 7.05 (d, *J = 4.50 Hz*, 1H, Ar-H), 7.20 (d, *J* = 7.25 Hz, 2H, Ar-H), 6.89 (d, *J* = 5.0 Hz, 1H, Ar-H), 6.75 (d, *J = 4.0 Hz*,1H, Ar-H); 1.23 (3H, CH_3); ^{13}CNMR (500 MHz) 121.19, 121.58, 124.41, 125.32, 125.68, 148.54, 174.35 (C, Benzothiazole), 159.58 (CH), 113.45, 114.48, 117.36, 117.58, 119.53, 128.39, 128.55, 129.61, 129.78, 131.30, 154.32, 158.53 (C, Ar-C.), 21.33 (C, CH_3); MS (FAB) $[M+1]^+$:(m/z) 359.11

3.3. Computational Study

The computational evaluation of all the synthesized compounds and reference standard compounds (Indomethacin) was done using the softwares Chem3D Ultra versions 12.0 and 16.0. Some of the important molecular metrics that were calculated includes e.g., log P, molecular weight, hydrogen bond acceptor, hydrogen bond donor, total polar surface area, number of rotatable bonds, molar refractivity,connolly molecular surface and connolly solvent accessible surface area.Findings of physicochemical properties and molecular metrics of all the newly synthesized benzo[d]thiazol-2-amine derivatives are presented in Table 2.

3.4. Screening through in silicoPharmacokinetics, drug likeness and toxicity parameters

Other than physicochemical properties, some features including parameters of pharmacokinetics (*Insilco* method) drug likeness and toxicity were determined also. The pharmacokinetic parameters were determined through *in-silico* method to determine Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET), which provided an idea of the therapeutic efficacy of the newly synthesized compound. Since a long time, pharmacokinetic properties of newly synthesized derivatives have been predicted using their physicochemical properties [30]. Compound percentage solubility was obtained using percent ABS=109–0.345TPSA [31]. In the present research work, pharmacokinetics and toxicity parameters were determined using SwissADME and OSIRIS property explorer (SwissADME; Osiris Property Explorer). Carcinogenicity, mutagenicity,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

reproductive/developmental toxicity, and skin irritating effects of synthesized compounds were screened to evaluate the toxicity.

3.5. Molecular Modeling Studies

The synthesizedbenzo[d]thiazol-2 amine derivatives (G1-G13) were molecularly docked using AutoDockVina software into the human COX-1 and COX-2 enzyme and the findings were compared to the results of diclofenac sodium and Indomethacin, which have already been known to have potentCOX-1 and COX-2 inhibitory activity. Each synthesized derivatives were subjected to docking. By observing the inhibition values towards COX-1 and COX-2 enzymes, the anti-inflammatory and analgesic effects of these newly synthesized benzo[d]thiazol-2 amine derivatives were evaluated.

COX-1 and COX-2 multi-chain protein crystal structures with PDB IDs 2OYU and 1PXX were obtained from the protein data bank (PDB). The docking method involved the use of a single chain-A. Molecules with two-dimensional (2D) structures were drawn using the ChemDraw/ChemSketch editor. The structural geometry of the 2D (.mol file format) structures was further tuned for the lowest energy state before being translated to a 3D structure. Finally, pdb files for stable 3D structures were saved. Docking was done following the creation of "pdb" files for both the protein structures and ligand molecules. For docking, Chain-A of the complete protein structure was taken into account. Multiple poses of each ligand with negative binding affinity values in kilocalories per mole (kcal/mol) unit wererecorded by each docking experiment. The optimal docked position at the binding site of individual target was determined by accessing best fitting docking pose with the highest negative binding affinity value.The results were determined by using Discovery studio (BIOVIA, Discovery Studio Modeling Environment, 2017[32].

Diclofenac sodium has been reported for inhibiting COX-1 and COX-2 both whereas literature suggests that Indomethacin has preferential COX-2 inhibition activity. This was the rationale behind selecting Indomethacin and diclofenac sodium as standard drug for present research work.

3.6. In vitro Cyclooxygenase enzyme inhibitory activity

The potential of the synthetic compounds G1 to G13, as well as diclofenac sodium and indomethacin as standard drug, to inhibit human COX-1 and COX-2 enzymes in terms of IC_{50}(μM) was screened. The human COX-1 and COX-2 inhibitor screening kit was used for the said purpose. All the synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and a mixture of COX-1 or COX-2 enzyme (10 μl), heme (10 μl) and samples (20 μl) were added to the supplied reaction buffer solution (160 μl, 0.1 M Tris–HCl), pH 8 containing 5 mMEthyleneDiamine Tetra Acetate (EDTA) and 2 mM phenol and were incubated for 10 m at 37 °C. This was followed by the addition of arachidonic acid (10 μl, final concentration in reaction mixture 100 μM) to initiate the reaction, after 2 m, The COX reactions were stopped using stannous chloride (30 μl) followed by incubation for 5 m at room temperature. This was followed by quantification of $PGF_{2\alpha}$formed in the samples by COX reactions by Enzyme-Linked ImmunoSorbent Assay (ELISA). Following itstransfer to a 96-well plate, the plate was incubated with samples for 18 h at room temperature. After incubation, the plate was washed to remove any unbound reagent and then Ellman's reagent (200 μl), containing acetyl cholinesterase, was added and incubated at room temperature for 60–90 m until the absorbance of *Bo* (maximum binding) well is in the range 0.3–0.8 A.U. at 410 nm. The plate was then read by an ELISA plate reader. By comparing sample treatment incubations to control incubations, the IC_{50} values of inhibition against both COX-1 and COX-2 enzymes were determined [33].

3.7. Biological Evaluation

3.7.1. Experimental Animals

For analgesic and anti-inflammatory activities, mature albino rats (150-180 g) of both sexes were procured from the Institutional Animal House, IFTM University, Lodhipur-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 June 2023 doi:10.20944/preprints202306.1064.v1

Rajput, Moradabad, India. Rats were kept in polypropylene cages at a temperature of 25±2 °C and a relative humidity of 45±55%. Prior to beginning the experiment, all of the rats spent a week in becoming used to lab conditions. These rats were given free access to water and conventional animal diet. The Committee of IAEC as per guidelines from for Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India, New Delhi reviewed, examined and approved the experimental protocols with Reg. No. 837/PO/ReBiBt/S/04/CPSCEA/38.

3.7.2. Preparation of Test Compounds

All the newly synthesized benzo[d]thiazol-2-amine derivatives and the standard drugs (diclofenac sodium and indomethacin) were used to prepare a suspension in 1% Tween 80. Animals of control group received 1 ml of tween 80 suspension orally.

3.7.3. Acute Toxicity Study

The acute toxicity study was conducted in accordance with recommendations made by the Organization for Economic Co-operation and Development (OECD) (OECD 2000). There were 90 animals, which were divided into 15 groups of 6 animals each (n=6). Animals were dosed with 10, 20, 100, 200, and 1000 mg/kg body weight (b.w) test compounds orally on the first day of the toxicity estimation. For three hours, the animals were separated and observed to check for any typical behavioral, neurological, or autonomic responses. With a difference of every 30 m, the assessment was continued for 24 hours or until the animals died. Relative body weight was also calculated to find out any adverse effect due to dosing of synthesized compounds.

3.8.1. Evaluation of Analgesic Activity

3.8.1.1. Eddy's Hot Plate Method

The Eddy's hot plate method was used to assess the analgesic activity of all freshly synthesizedbenzo[d]thiazol-2-amine derivatives [34,35].Total fifteengroups; each group with 6 animals (n=6) was selected. The hot plate that was used in this research work contained a shell that was heated by electricity and kept at a constant 55°C±1°C. Separate animals were placed on the hot plate, and a stopwatch was used to record the duration it took for any paw jumping or licking to occur. To prevent paw harm, the maximum limit of analgesic response of 15 s was used. As a standard drug, diclofenac sodium (10 mg/kg) was given. After the drug administration, the reaction time for each animal was noted at 0, 60, 120, 180 and 240 m.

3.9. Anti-inflammatory Activity

3.9.1.1. Carrageenan induced rat paw edema method

By using the carrageenan-induced rat paw edema method, all the newly synthesized benzo[d]thiazol-2-amine derivatives were assessed for their ability to reduce inflammation in experimental rats [36]. There were fifteen groups of six animals each (n=6). For comparison, Indomethacin (10 mg/kg b.w.) was used as the reference drug especially. Acute inflammation was induced by injecting the aqueous suspension of carrageenan (1%, w/v, 0.1 ml) in the right hind paw in the sub plantar region of each rat after 1 h after oral administration of the benzo[d]thiazol-2-amine derivatives and Indomethacin to the test groups and standard drug treatment group, respectively. To make the readings easier, a marking for identification was put on the leg.

The paw volume was calculated plethysmometrically at 0, 60, 120, 180 and 240 m after the injection of carrageenan. The formula used to calculate the % Inhibition was as follows-

$\%Inhibition = 100(1-V_t/V_c)$

Where V_t= mean change in paw volume of treated rats

V_c= mean change in paw volume of control rats.

4. Conclusions

Through this present work, we attempted for the synthesis, pharmacological assessment, computational studies and *in–silico*study of synthesized benzo(d)thiazole-2-amine derivatives as analgesic and anti-inflammatory agents. The designed and synthesized derivatives were effectively and well characterized by spectral data such as IR Spectroscopy, ^{1}HNMR Spectroscopy and Mass Spectrometry. Docking study concluded good of newly synthesized compounds interaction with COX-1 and COX-2 enzyme. In *silico* studies concluded all the synthesized derivatives with assuring results, mainly**G11***[(E)-N-(4-(4-ethylphenoxy) benzylidene) benzo[d]thiazol-2-amine]*as analgesic compound and the compound **G10***[(E)-N-(4-(4-aminophenoxy) benzylidene) benzo[d]thiazol-2-amine]*as anti-inflammatory agent, however, further clinical studies require to be doing to reconfirm and proved the obtained results. *In vitro* evaluation of theCOX-1 and COX-2 enzyme inhibitory activities was performed and finding suggested that compounds G10 and G11 arepossessing effective COX-1 and COX- inhibitory action with IC_{50}value of 5.0 and 10 μM, respectively.These findings concluded that the synthesized derivatives G10 and G11 showed promising analgesic and anti-inflammatory effect and these may be subjected to further clinical investigation for establishing this as a promising compound for humankind.

Acknowledgments: The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through short Research Project under Grant No: RGP/374/44.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The paper and supplementary material contain data.

Conflicts of Interest: The authors declare no conflict of interest, financial and otherwise.

References

1. Waliza, A.; Ghosh, S. Inflammation and Inflammatory Diseases, Markers, and Mediators: Role of CRP in Some Inflammatory Diseases. *Biol. C React. Protein Health Dis.* **2016**, 67-107.

2. Arshia, Jabeen, A.; Faheem, A.; Khan, K.M.; Shah, S.; Perveen, S. Benzophenone Esters and Sulfonates: Synthesis and their Potential as Antiinflammatory Agents. *Med. Chem.* **2019**, 15, 162-174.

3. Miller, R.G.; Mitchell, J.D.; Moore, D.H. Riluzole for amyotropic lateral sclerosis/motor neuron disease. *Cochrane Database Syst. Rev.* **2012**, 2, 1-5.

4. Wongrakpanich, W.; Wongrakpanich, A.; Melhado, K.; Rangaswami, J. A Comprehensive Review of Non-Steroidal AntiInflammatory Drug Use in The Elderly. *Aging Dis.* **2018**, 9, 143-150.

5. Simmons, D.L.; Botting, R.M.; Hla, T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacol. Rev.* **2004**, 56, 387-437.

6. Peskar, B.M. Role of cyclooxygenase isoforms in gastric mucosal defence. *J. Physiol. Paris.* **2001**, 95, 3-9.

7. Zidar, N.; Odar, K.; Glavač, D.; Jerše, M.; Zupanc, T.; Štajer, D. Cyclooxygenase in normal human tissues – is COX-1 really a constitutive isoform and COX-2 an inducible isoform. *J. Cell Mol. Med.* **2009**, 13, 3753-3763.

8. Wang, B.; Wu, L.; Chen, J.; Dong, L.; Chen, C.; Wen, Z.; Hu, J.; Fleming, I.; Wang, D.W. Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. *Signal Transduct. Target Ther.* **2021**, 6, 94.

9. Bushra, R.; Aslam, N. An Overview of Clinical Pharmacology of Ibuprofen. *Oman Med. J.* **2010**, 25, 155-166.

10. Steven, M.; Weisman, S.B. Efficacy and Safety of Naproxen for Acute Pain. *J. Fam. Pract.* **2020**, 69, S33-S85.

11. Costa, B.R.; Reichenbach, S.; Keller, N. Effectiveness of non-steroidal anti-inflammatory drugs for the treatment of pain inknee and hip osteoarthritis: a network meta-analysis. *Lancet.* **2017**, 390, e21-e33.

12. Shah, S.; Arshia, Kazmi, N.S.; Jabeen, A.; Faheem, A.; Dastagir, N.; Ahmed, T.; Khan, K.M.; Ahmed, S.; Raza, A.; Perveen, S. Diclofenac 1,3,4-Oxadiazole Derivatives; Biology-Oriented Drug Synthesis (BIODS) in Search of Better Non-Steroidal, Non-Acid Antiinflammatory Agents. *Med. Chem.* **2018**, 14, 674-687.
13. Nalamachu, S. Role of Indomethacin in Acute Pain and Inflammation Management: A Review of the Literature. *Postgrad. Med.* **2014**, 126, 92-97.
14. Goldstein, J.L.; Cryer, B. . Gastrointestinal injury associated with NSAID use: a case study and review of risk factors and preventative strategies. *Drug Heal Patient Saf.* **2015**, 7, 31-41.
15. Hörl, W.H. Nonsteroidal Anti-Inflammatory Drugs and the Kidney. *Pharmaceut.* **2010**, 3, 2291–2321.
16. Zhilitskaya, L.V.; Shainyan, B.A.; Yarosh, N.O. Modern Approaches to the Synthesis and Transformations of Practically Valuable Benzothiazole Derivatives. *Molecules.* **2021**, 26, 2190.
17. Ikpa, C.B.C.; Onoja, S.O.; Okwaraji, A.O. Synthesis And Antibacterial Activities Of Benzothiazole Derivatives Of Sulphonamides. *Acta Chem. Malays*. **2004**, 4, 55-57.
18. Kumar, G.; Singh, N.P. Synthesis, anti-inflammatory and analgesic evaluation of thiazole/oxazole substituted benzothiazole derivatives. *Bioorg. Chem.* **2021**, 107, 104608.
19. Venugopala, K.N.; Chandrashekharappa, S.; Pillay, M.; Bhandary, S.; Kandeel, K.; Mahomoodally, F.M.; Morsy, M.A.; Chopra, D.; Odhav, B. Synthesis and Structural Elucidation of Novel Benzothiazole Derivatives as Anti-tubercular Agents: In-silico Screening for Possible Target Identification. *Med. Chem.* **2019**, 15, 311.
20. Rajareddy, A.; Srinivas, M.. Synthesis, characterization, and anthelmintic activity of novel benzothiazole derivatives containing indole moieties. *Asian J. Pharm Clin. Res.* **2019**, 12, 321-325.
21. Mariappan, G.; Prabhat, P.; Sutharson, L.; Banerjee, J.; Patangia, U.; Nath, S. Synthesis and Antidiabetic Evaluation of Benzothiazole Derivatives. *J. Korean Chem. Soc.* **2012**, 56, 251-256.
22. Azzam, R.A.; Elboshi, H.A.; Elgemeie, G.H. Novel Synthesis and Antiviral Evaluation of New Benzothiazole-Bearing N-Sulfonamide 2-Pyridone Derivatives as USP7 Enzyme Inhibitors. *ACS Omega.* **2015**, 46, 30023.
23. Irfan, A.; Batool, F.; Andlee, S.; Naqvi, Z.; Islam, A.; Osman, S.M.; Nocentini, A.; Alissa, S.A.; Supurand, C.T. Benzothiazole derivatives as anticancer agents. *J. Enzyme. Inhib. Med. Chem.* **2020**, 35, 265–279.
24. Cabrea-Perez, L.C.; Padila-Martinez, I.I.; Cruz, A.; Mendieta-Wejebe, J.E.; Tamay-Cach, F.; Rosales-Hernández, M. C. Evaluation of a new benzothiazole derivative with antioxidant activity in the initial phase of acetaminophen toxicity. *Arabian J. Chem.* **2019**, 12, 3871-3882.
25. Gagoria, J.; Verma, P.K.; Khatkar, A. Anticonvulsant and neurological profile of benzothiazoles: a mini-review. *Cent Nerv Syst Agents Med Chem*. **2015**, 15, 11-16.
26 Haroun, M.; Chobe, S.S.; Alavala, R.R.; Mathure, S.M.; Jamullamudi, R.N. 1,5-Benzothiazepine Derivatives: Green Synthesis, In Silico and In Vitro Evaluation as Anticancer Agents. *Molecules* **2022**, 27, 3757.
27. Venugopala, K.N.; Krishnappa, M.; Nayak, S.K.; Subrahmanya, B.K.; Vaderapura, J.P.; Chalannavar, R.K.; Gleiser, R.M.; Odhav, B. Synthesis and antimosquito properties of 2,6-substituted benzo[d]thiazole and 2,4-substituted benzo[d]thiazole analogues against *Anopheles arabiensis*. *Eur. J. Med. Chem.* **2013**, 65, 295-303.
28. Khokra, S.L.; Arora, K.; Khan, S.A.; Kaushik, P.; Saini, R.; Husain, A. Synthesis, Computational Studies and Anticonvulsant Activity of Novel Benzothiazole Coupled Sulfonamide Derivatives. *Iran J. Pharm. Res.* **2019**, 18, 1-15.
29. Haroun, M.; Tratrat, C.; Petrou, A.; Geronikaki, A.; Ivanov, M.; Ćirić, A.; Soković, M.; Nagaraja, S.; Venugopala, K.N.; Nair, A.B.; Elsewedy, H.S.; Kochkar, H. Exploration of the Antimicrobial Effects of Benzothiazolylthiazolidin-4-One and In Silico Mechanistic Investigation. *Molecules.* **2021**, 26, 4061.
30. Karlgren, M.; Bergstrom, CA.S. How physicochemical properties of drug affect their metabolism and clearance. In: Wilson AGE, editor. New horizons in predictive drug metabolism and pharmacokinetic. **2016**, 49, 1.
31. Zhao, Y.; Abraham, M.H.; Lee, J. Rate-limited steps of human oral absorption and QSAR studies. *Pharm. Res.* **2002**, 19, 1446-1457.

32. Systemes Dassault, "BIOVIA, Discovery Studio Modeling Environment", Dassault SystemesBiovia, San Diego, CA, USA, **2017**.
33. Gedawy, E.M.; Kassab, A.E.; Kerdawy, A.M.F. Design, synthesis and biological evaluation of novel pyrazole sulfonamide derivatives as dual COX-2/5-LOX inhibitors. *Eur. J. Med. Chem.* **2020**, 189, 112066.
34. Eddy, N.B.; Leimbach, D.J. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J. Pharmacol. Exp. Ther.* **1953**, 107, 38593.
35. Yasir, A.; Isthiaq, S.; Jahangir, M.; Ajaib, M.; Salar, U.; Khan, K.M. Biology-Oriented Synthesis (BIOS) of Piperine Derivatives and their Comparative Analgesic and Antiinflammatory Activities. *Med Chem.* **2018**, 14, 269-280.
36. Winter, C.A.; Fisley, E.A.; Nuss, G.W. Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Anti-Inflammatory Drug. *Proc. Soc. Exp. Biol. Med.* **1962**, 111, 544-547.
37. Venkatesh, P.; Pandeya, S.N. Synthesis, characterisation and anti-inflammatory activity of some 2-amino benzothiazole derivatives. *Int. J. ChemTech Res.* **2009**, 1, 1354-1358.
38. Zhilitskaya, L.V.; Yarosh, N.O.. Synthesis of biologically active derivatives of 2-aminobenzothiazole. *Chem. Hetero. Comp.* **2021**, 57, 369-373.
39. Kumar, S.; Kumar, A.; Verma, A.; Mishra, A.; Mishra, A.K. Synthesis and Docking Study of Some Bioactive N-(benzo[d]thiazol-2-yl)- 2-(4-((substituted)phenoxy)acetamide on Cyclo-oxygenase-2 Enzyme and In vivo Analgesic Activity Evaluation. *Lett. Drug Design Discov.* **2021**, 18, 396-405.
40. Deodhar, M.N.; Dongre, A.C.; Kudale, S.D. Analgesic and Antiinflammatory Activity of Derivatives of 2-Aminobenzothiazole. *Asian J. Chem.* **2012**, 24, 2747-2752.
41. Gupta, P.; Yadav, H.L.; Garg, G.; Pawar, R.S.; Patil, U.K.; Singour, U.K. Synthesis and Biological Evaluation of Some Novel 2-Aminobenzothiazole Derivatives as Potential Analgesic Agents. *Asian J. Res. Chem.* **2010**, 3, 47-50.
42. Gill, R.K.; Rawal, R.K.; Bariwal, J. Recent Advances in the Chemistry and Biology of Benzothiazoles. *Archiv. Der. Pharmazie.* **2015**, 348, 42.
43. Kumar, K.R.; Karthik, K.N.; Begum, P.R.; Rao, C.M. Synthesis, characteristics and biological evaluation of benzothiazole derivatives as potential antimicrobial and analgesic agents. *Asian J. Res. Pharm. Sci.* **2017**, 28, 115-119.
44. Siddiqui, N.; Rana, A.; Khan, S.A.; Ahsan, W.; Alam, M.S.; Ahmed, S. Analgesic and Antidepressant Activities of Benzothiazole-Benzamides. *Biomed. Pharmacol. J.* **2008**, 1, 297-300.
45. Ugwu, D.I.; Okoro, U.C.; Ukoha, P.O.; Gupta, A.; Okafora, S.N. Novel anti-inflammatory and analgesic agents: synthesis, molecular docking and in vivo studies. J *Enzyme Inhib Med Chem.* **2018**, 33, 405-415.
46. Cashman, J.N. The mechanisms of action of NSAIDs in analgesia. *Drugs.* **1996**, 52, 13-23.
47. Habeeb, A.G.; Rao, P.N.; Knaus, E.E. Design and synthesis of 4,5-diphenyl-4-isoxazolines: novel inhibitors of cyclooxygenase-2 with analgesic and anti-inflammatory activity. *J. Med. Chem.* **2001**, 44, 2921-2927.
48. Saify, Z.S.; Khan, K.M.; Haider, S.M.; Zeeshan, S.T.A.; Shah, M.; Saeed, M.S.; Shekhani, W. Voelter. Syntheses and Evaluation of the Analgesic Activity of Some 4-Acetyl- 4-phenylpiperidine and 4-Hydroxy-4-phenylpiperidine Derivatives. *Zeitschrift für Naturforschung* B. **1999**, 54b,1327.
49. Ugwu, D.I.; Okoro, U.C.; Ukoha, P.O.; Gupta, A.; Okafor, S.N. Novel anti-inflammatory and analgesic agents: synthesis, molecular docking and in vivo studies. *J. Enz. Inh. Med. Chem.* **2018**, 33, 405-415.
50. Paramashivappa, R.; Kumar, P.P.; Rao, S. Design, synthesis and biological evaluation of benzimidazole/benzothiazole and benzoxazole derivatives as cyclooxygenase inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, 13, 657-660.