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SYNTHESIS & BIOLOGICAL EVALUATION OF SOME 2-SUBSTITUTED AMINO-BENZOTHIAZOLE DERIVATIVES AS AN ANTI-INFLAMMATORY AGENT

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ABSTRACT

The pressing need for safer anti-inflammatory compounds has motivated us to investigate benzothiazole-derived molecules as promising candidates for replacing the conventional NSAIDs. Five novel 2-substituted aminobenzothiazoles (YG-1 to YG-5) were rationally designed and synthesized in a systematic three-step process with yields ranging from 46-77%. The compounds were fully characterized by FTIR and ^{1}H-NMR spectroscopy to validate their integrity. Molecular docking tests against cyclooxygenase-2 (COX-2) indicated favorable binding affinities between -7.632 and -8.412 kcal/mol, with the most active compound being YG-3. Interestingly, the compounds also satisfied Lipinski's Rule of Five, a drug-likeness requirement for potential oral drug delivery. In vivo anti-inflammatory activity in carrageenan- induced paw edema test led to identification of YG-1 as the most active compound, 80% edema inhibition at 100 mg/kg—equally effective as diclofenac sodium. Structure-activity relationship studies demonstrated that dual nitro substitution patterns significantly influence enhancing biological activity. These findings position 2-substituted aminobenzothiazoles as a valid lead structure for the identification of novel anti-inflammatory therapies. YG-1 is a lead worthy of further preclinical optimization, which could possess better safety profiles than existing NSAIDs without loss of therapeutic potency.

KEYWORDS: Benzothiazole, Virtual Screening, Organic Synthesis, ADMET, NSAID.

1. INTRODUCTION

Inflammation constitutes a critical physiological response to noxious stimuli, encompassing pathogens, cellular damage, or irritants, and serves as a cornerstone of the body's defense mechanism for homeostasis restoring.[1] This complex process, characterized by the classical signs of rubor (redness), calor (heat), tumor (swelling), dolor (pain), and functio laesa (loss of function), is orchestrated by a sophisticated network of mediators, including cytokines, prostaglandins, and eicosanoids, which regulate vascular responses and leukocyte recruitment.[1] While acute inflammation[2] facilitates tissue repair and pathogen clearance, dysregulated or chronic inflammation is implicated in pathogenesis of numerous debilitating conditions i.e. RA, IBD, psoriasis, atherosclerosis, and even carcinogenesis.[2] The global prevalence of inflammatory disorders, coupled with their significant morbidity, underscores the urgent need for novel therapeutic agents that offer enhanced efficacy and improved safety profiles over existing treatments.[3]

Conventional anti-inflammatory therapies, primarily nonsteroidal anti-inflammatory drugs (NSAIDs)[4] and corticosteroids[4], effectively mitigate inflammatory symptoms but are associated with substantial limitations.[4] Prolonged use of NSAIDs, which primarily inhibit cyclooxygenase (COX) enzymes[5] to reduce prostaglandin synthesis, predisposes patients to gastrointestinal toxicity, cardiovascular complications, and renal dysfunction.[5] Similarly, corticosteroids, despite their potent anti-inflammatory effects, are linked to adverse outcomes such as metabolic disturbances, immunosuppression, and osteoporosis.[6] These challenges have driven extensive research into alternative molecular scaffolds capable of selectively modulating inflammatory pathways while minimizing adverse effects.[7] Among these, heterocyclic compounds are promising as to versatility, synthetic accessibility, and favorable pharmacokinetic properties.[6]

Benzothiazole, a bicyclic heterocyclic framework comprising a benzene ring fused to a 1,3- thiazole moiety[8], has garnered significant attention in medicinal chemistry for its broad- spectrum pharmacological activities, including antimicrobial, anticancer, antidiabetic, and anti- inflammatory properties.[9] The benzothiazole nucleus possesses unique electronic characteristics, with nitrogen and sulfur atoms facilitating diverse non-covalent interactions, with

various biomacromolecular targets.[8] Notably, 2-substituted aminobenzothiazoles[10] have demonstrated considerable promise as anti-inflammatory agents,[11] exhibiting inhibitory effects on key inflammatory mediators, including COX-2, 5-lipoxygenase (5-LOX), and TNF-α and IL-6. The structural amenability of the 2-amino group to modifications,[11] such as acylation, alkylation, or condensation, enables the generation of diverse chemical libraries, facilitating systematic structure-activity relationship (SAR) studies to optimize pharmacological and pharmacokinetic profiles.[11]

This study aims to design, synthesize, and evaluate novel 2-substituted aminobenzothiazole derivatives as potential anti-inflammatory agents, with a focus on achieving selective COX-2 inhibition and improved safety profiles. Their anti-inflammatory potential was assessed through in vitro enzyme inhibition assays, in vivo carrageenan-induced paw edema models, and in silico molecular docking studies targeting COX-2 (PDB ID: 4COX). Additionally, in silico ADMET predictions were conducted to evaluate pharmacokinetic properties and toxicity risks, while SAR analyses elucidated the structural determinants of activity.

2. EXPERIMENTAL

Materials

Chemicals and Reagents

Reagents utilized were of analytical grade(12) (AR) or laboratory reagent (LR). Carrageenan and diclofenac sodium (reference standard) were obtained from HiMedia Laboratories and a certified pharmaceutical supplier, respectively. Solvents such as methanol, acetone, chloroform, and dimethyl sulfoxide (DMSO) were of high purity.

Instrumentation

Synthesis, purification, and characterization of compounds were performed using the following instruments: a Veego, Model: VMP-D for melting point determination, a Shimadzu IR Affinity-1 spectrophotometer for FTIR spectroscopy (KBr, 4000–400 cm^{-1}, 4 cm^{-1} resolution), and a Bruker 400 MHz spectrometer for ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy (DMSO-d_6 solvent, tetramethylsilane as internal standard). TLC was conducted using Merck silica gel 60, visualized under UV (254 nm and 366 nm). A plethysmometer was used for in vivo paw volume measurements. Molecular docking was performed using Schrödinger Glide software, with protein structures visualized via PyMOL and interactions analyzed using LigPlot+. In-silico ADMET using SwissADME (http://www.swissadme.ch).[13]

METHODOLOGY

In Silico ADMET Prediction

ADMET properties were predicted using SwissADME,[13] evaluating molecular weight, lipophilicity (LogP), hydrogen bond donors/acceptors, rotatable bonds, and drug-likeness (Lipinski rules).[14]

Molecular Docking Studies

Ligand structures were drawn using ChemSketch, optimized with MM2 force field PDB format. COX-2 (PDB ID: 4COX) retrieved from Protein Data Bank[15], prepared by removing water molecules, heteroatoms.[16] Polar hydrogens added, and a grid box was defined around the active site.[16] Docking was performed using Schrödinger Glide (exhaustiveness = 8, modes = 9, energy range = 3).(16) The best pose was selected based on lowest binding energy and visualized using PyMOL and LigPlot+.(16) The docking protocol was validated by root- mean-square deviation (RMSD) < 2.0 Å.[16]

Synthetic Procedures

A series of 2-substituted aminobenzothiazole derivatives (YG-1 to YG-5) were synthesized via a three-step protocol, optimized for yield and purity, as outlined in Fig. 1.

Step 1: Synthesis of 5- and 6-Substituted 2-Aminobenzothiazole Derivatives (1a–1c)

Equimolar quantities (0.05 mol) of substituted anilines (3-nitroaniline, 4-nitroaniline, or 4- bromoaniline) and ammonium thiocyanate were dissolved in ethanol containing concentrated hydrochloric acid and stirred for 30 minutes to form substituted 1-phenylthiourea. Conc. H_2SO_4 added, refluxed for 1.5 hours. Reaction completion monitored by TLC (silica gel, ethyl acetate:hexane). Precipitate washed with cold water to remove acid, dried, and recrystallized to 5-nitro-2-aminobenzothiazole (1a), 6-nitro-2-aminobenzothiazole (1b), and 6-bromo-2- aminobenzothiazole (1c).

Step 2: Synthesis of N-Substituted Chloroacetanilides (2a–2d)

Substituted anilines (0.1 mol; 3-nitroaniline, 4-nitroaniline, 4-bromoaniline, or 2-chloroaniline) were dissolved in glacial acetic acid by sodium acetate, warmed, & cooled with stirr. Chloroacetyl chloride (0.12 mol) was added dropwise, stirred to 30 minutes. Precipitated yeild filtered, washed, and recrystallized to afford 2-chloro-(3-nitrophenyl)-acetamide (2a), 2-chloro-(4-nitrophenyl)-acetamide (2b), 2-chloro-(4-bromophenyl)-acetamide (2c), and 2- chloro-(2-chlorophenyl)-acetamide (2d).

Step 3: Synthesis of 2-Substituted Aminobenzothiazole Derivatives (YG-1 to YG-5)

Equimolar quantities of substituted 2-aminobenzothiazole (1a–1c) and chloroacetanilide (2a–2d) were dissolved in 1,4-dioxane. Triethylamine added as a base, refluxed for 2 hours. The mixture was cooled, precipitate was filtered, dried, and recrystallized for target compounds: YG-1 (2-(3-nitroanilino)-N-(6-nitro-1,3-benzothiazol-2-yl)acetamide), YG-2 (2-(2-chloroanilino)-N-(4-nitro-1,3-benzothiazol-2-yl)acetamide), YG-3 (N-(5-bromo-1,3- benzothiazol-2-yl)-2-(4-nitroanilino)acetamide), YG-4 (2-(4-

nitroanilino)-N-(6-nitro-1,3- benzothiazol-2-yl)acetamide), and YG-5 (2-(4-bromoanilino)-N-(6-nitro-1,3-benzothiazol-2- yl)acetamide).

Physicochemical Characterization

Synthesized compounds were characterized as follows

Physical Properties: Appearance (color, state), solubility (water, methanol, ethanol, acetone, chloroform, DMSO), and melting points (Veego VMP-D, uncorrected).

TLC: Performed on silica gel 60 with optimized mobile phases. Visualized under UV (254 nm, 366 nm), and Rf calculated.

FTIR Spectroscopy: Spectra were recorded using KBr pellets, identifying functional groups via characteristic absorption bands.

^{1}H-NMR Spectroscopy: Spectra acquired in DMSO-d_6, chemical shifts (δ, ppm) reported relative to tetramethylsilane and coupling constants (J, Hz) noted.

In Vivo Anti-Inflammatory Activity

The carrageenan-induced paw edema model was employed to evaluate anti-inflammatory activity.[17] Wistar rats (n=6 per group) were divided into: Group I (control, normal saline), Group II (reference, diclofenac sodium 10 mg/kg), and Groups III–VII (test compounds YG-1 to YG-5, 10 mg/kg), administered orally.[17] One hour post-treatment, 0.1 mL of 1% w/v carrageenan in saline was injected into the subplantar region of the right hind paw.[17] Paw volume was measured at 0, 1, 2, 3, and 4 hours using a plethysmometer.[17] Percentage edema inhibition was calculated as.

% Inhibition = [(Vt - V0)control - (Vt - V0)treated] / (Vt - V0)control × 100, where Vt is paw volume at time t, and V0 is initial paw volume.[17]

3. RESULTS

3.1. In-Silico Computational Studies

3.2. 3.2. Chemistry

Given in Table 1.

3.2.1. Spectral Data of Intermediates

5 and 6 Substituted 2-aminobenzothiazole (YG-A): Yield %: 85; m.p: 121-124^{0}C; **IR υ cm−1 (KBr):** N–H (amine) 3060.48; Aromatic/Alkyne C–H 3060.48; C≡C / C≡N (alkyne/nitrile) 2121.31; Aromatic C=C / C=N 1592.91, 1466.85; Aromatic C=C 1466.85; C–N / C–S (thiazole ring) 1309.43, 1175.41, 1130.59.

N-Substituted Chloroacetamide (YG-B): Yield %: 76; m.p: 156-161^{0}C; **IR υ cm−1 (KBr):** N–H (amine) 3275.5; Aromatic C–H 3121.22; C=N (imine/thiazole) 1617.98; Aromatic C=C 1514.89, 1444.89; C–N (amine/thiazole) 1184.08, 1097.7.

3.2.2. Spectral Data of the Targeted Compounds

2-(3-nitroanilino)-N-(6-nitro-1,3-benzothiazol-2-yl)-acetamide (YG-1): Brown; Yield %: 69; m.p: 201-206^{0}C; **IR υ cm−1 (KBr):** N–H stretching (amide) 3475.1, 3330.46, 3150.15; C=O stretching (amide) 1684.57; C–N stretching (amide) 1332.57; N–H bending (amide) 1503.24; S=O stretching (sulfonyl) 1332.57; NO_2 asymmetric stretch 1503.24; NO_2 symmetric stretch 1332.57; Aromatic C–H bending 849.49, 750.17**; ^{1}H NMR-DMSO D6:** 10.77 NH (amide); 10.407 Phenolic OH; 8.251, 8.272, 8.309 Aromatic (nitro ring); 8.143, 8.112, 8.125 Aromatic (near carbonyl/NO_2); 7.951, 7.935, 7.849 Aromatic (central ring); 7.880, 7.839 Aromatic (near amide); 7.608, 7.646 Aromatic (shielded); 6.773, 6.808 Aromatic (methoxy ring); 3.329, 3.344, 3.577 OCH_3; 2.986 CH_2; 2.036, 2.130 CH_3 (Ar–CH_3); 1.291, 1.205, 1.123 Aliphatic (ethyl/propyl).

2-(2-chloroanilino)-N-(4-nitro-1,3-benzothiazol-2-yl)-acetamide (YG-2): Brown; Yield %: 77; m.p: 210-215^{0}C; **IR υ cm−1 (KBr):** N–H stretching (amide) 3357.46, 3150.15; C=O stretching (amide) 1690.63; N–H bending (amide) 1520.6; NO_2 asymmetric stretch 1520.6; C– Cl stretch (aryl-Cl) 731.85, 593.96; Aromatic C–H bending 867.81, 731.85**; ^{1}H NMR-DMSO**

D6: 11.663, 11.605, 11.580 NH (amide/thioamide); 10.663, 10.661, 10.645 NH near triazole; 8.401, 8.370 Aromatic (ortho to NO_2); 8.179, 8.162 Aromatic (para/meta to NO_2); 7.934, 7.926 Triazole/phenyl (deshielded); 7.597, 7.570 Aromatic (chlorophenyl); 7.558, 7.550 Aromatic (overlapping); 7.465, 7.430 Aromatic (meta/para); 3.557, 3.573 CH_2 (CH_2–N/CH_2–S); 2.971, 2.943 Methyl/Aliphatic; 2.010, 2.007 CH_2 or CH_3 (slightly shielded).

N-(5-bromo-1,3-benzothiazol-2-yl)-2-(4-nitroanilino)-acetamide (YG-3): Brown; Yield %: 46; m.p: 199-203^{0}C; IR υ cm−1 (KBr): N–H stretching (amide) 3381.21, 3352.64, 3250.43; C–H stretching (aromatic) 2896.56; C=O stretching (amide) 1640.95; N–H bending (amide) 1505.28; NO_2 asymmetric stretch 1505.28; S=O stretching (sulfonyl) 1150.28; C–Br stretch (aryl–Br) 566.57; Aromatic C–H bending 845.70, 849.78, 954.50; ^{1}H NMR-DMSO.

D6: 10.684 NH (amide/thioamide); 9.231, 9.158 NH (adjacent to triazole or S); 8.273, 8.218, 8.243 Aromatic (ortho/para to NO_2); 7.931, 7.913, 7.894 Aromatic (meta/para to Br); 7.865, 7.858, 7.838 Triazole-adjacent (possibly doublets); 7.188, 7.208, 7.250 Aromatic (deshielded/overlapping); 6.208, 6.250, 6.297 Aromatic/heteroaromatic (fused ring); 4.290, 4.305 CH_2–N or CH_2–S; 3.984, 3.960, 3.918 CH_2 (aliphatic side chain); 3.540, 3.553, 3.589 OCH_3 or CH_2 (overlapping); 2.986, 2.903 CH_3 or CH_2 (near aromatic); 2.120, 2.063 CH_3/CH_2 (aliphatic); 1.221, 1.163 Terminal CH_3 or CH_2.

2-(4-nitroanilino)-N-(6-nitro-1,3-benzothiazol-2-yl)-acetamide (YG-4): Dark Brown, Yield %: 56; m.p: 232-

237^{0}C; **IR υ cm−1 (KBr):** N–H stretching (amide) 3475.1, 3376.75, 3222.45; C=O stretching (amide) 1694.15; N–H bending (amide) 1570.74; NO_2 asymmetric stretch 1570.74; NO_2 symmetric stretch 1360.43; S=O stretching (sulfonyl) ~1360.43 (overlapping); Aromatic C–H bending 856.25, 890.034; C–N stretching 1093.26**; ^{1}H NMR- DMSO D6:** 10.953 NH (amide or triazole-adjacent); 8.264–8.181 Aromatic (ortho to NO_2); 7.958–7.827 Aromatic (NO_2-phenyl/triazole ring); 7.817–7.603 Aromatic (para/ortho to OH); 6.493, 6.401 OH (phenolic, exchangeable); 4.352, 3.748 CH_2 (near heteroatoms); 2.558, 2.503 CH_2 (aliphatic, near electronegative atoms); 1.226, 1.008 CH_3 or CH_2 (aliphatic, upfield).

2-(4-bromoanilino)-N-(6-nitro-1,3-benzothiazol-2-yl)-acetamide (YG-5): Dark Brown; Yield %: 67; m.p: 234-239^{0}C; **IR υ cm−1 (KBr):** N–H stretching (amide) 3391.21, 3352.64, 3250.43; C–H stretching (aromatic) 2896.56; C=O stretching (amide) 1649.95; N–H bending (amide) 1505.28; NO_2 asymmetric stretch 1505.28; S=O stretching (thiazole) 1150.28; C–Br stretching (aryl–Br) 686.57, 566.57; Aromatic C–H bending 845.70, 954.50**; ^{1}H NMR-DMSO D6:** 10.943 NH (amide, strongly deshielded); 8.260–8.181 Aromatic (ortho to NO_2); 7.958– 7.827 Triazole/substituted phenyl; 7.817–7.603 Aromatic (bromo-phenyl); 6.939, 6.497 Possibly OH or NH (exchangeable/aromatic).

3.3. Biological Activity

Given in Table 2 and Fig. 2.

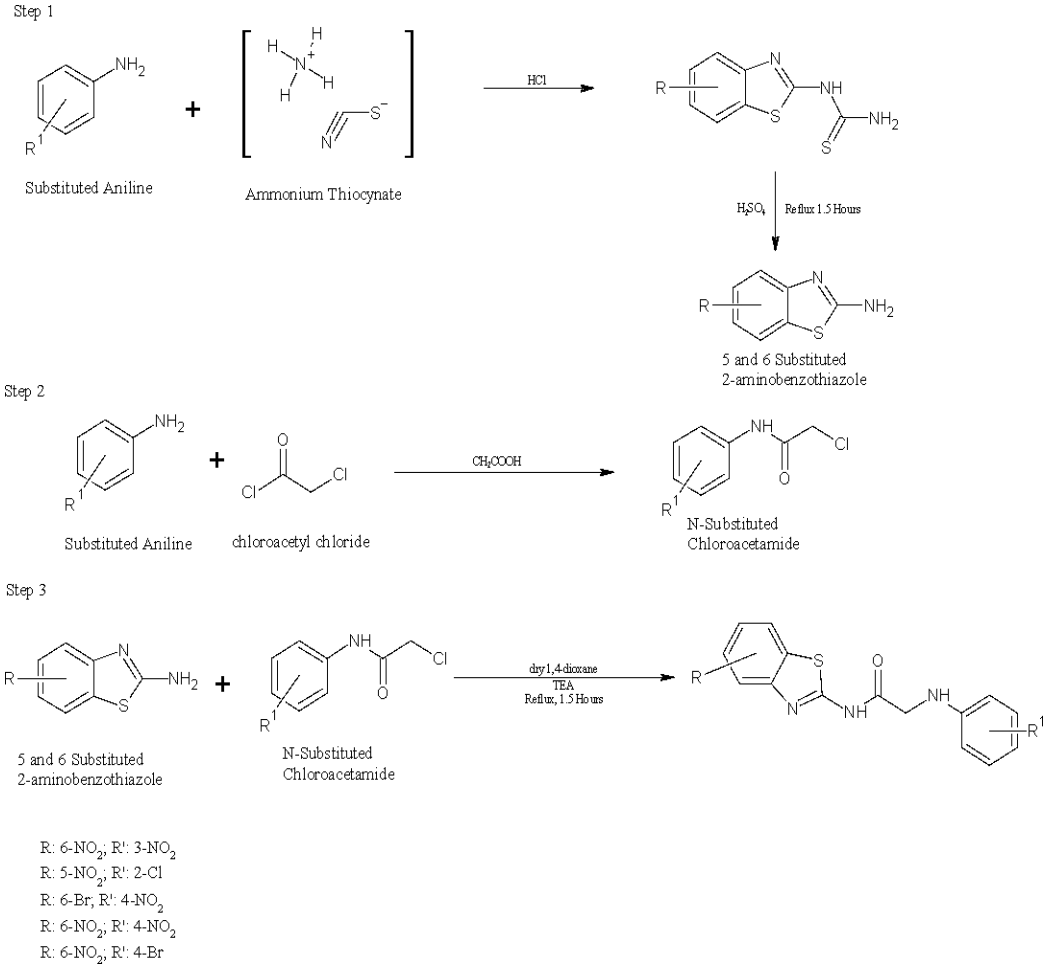

Figure 1. Synthetic Route to Target Compounds.

Table 1: In-silico analysis of Analogues with Rule of 5 (Ro5).

Title	docking score	glide energy	Ro5
YG3	-8.412	-51.956	0
YG2	-8.388	-43.089	0
YG4	-7.884	-51.489	0
YG1	-7.632	-43.840	0
diclofenac sodium	-7.582	-36.694	0

Table 2: Anti-inflammatory activity of analogues equivalent to Diclofenac Sodium.

Drug Group	Dose (mg/kg)	Initial Volume (ml)	Final Volume (ml)	% Inhibition of Oedema
YG-1	50	1.45 ± 2.12	1.90 ± 0.54	28
	75	1.10 ± 2.75	1.40 ± 0.21	52
	100	1.48 ± 1.23	1.60 ± 0.57**	80
YG-2	50	1.5 ± 0.31	2.1 ± 0.98	4
	75	1.3 ± 0.34	1.8 ± 0.23	20
	100	1.1 ± 0.21	1.5 ± 0.21	36
YG-3	50	1.74 ± 0.43	2.2 ± 1.35	26
	75	1.57 ± 1.63	1.9 ± 4.22	47
	100	1.34 ± 1.11	1.5 ± 0.15	74
YG-4	50	1.2 ± 0.12	1.58 ± 3.11	39
	75	1.53 ± 0.42	1.89 ± 4.22*	44
	100	1.48 ± 2.32	1.69 ± 5.43**	66
YG-5	50	1.00 ± 3.23	1.6 ± 2.32	4
	75	1.3 ± 8.56	1.8 ± 0.45*	52
	100	1.00 ± 0.32	1.3 ± 0.45*	52
Diclofenac Sodium (Std)	5	1.2 ± 2.43	1.5 ± 0.15**	52
	7.5	1.11 ± 1.21	1.3 ± 0.45**	69
	10	1.29 ± 0.12	1.33 ± 0.76***	93

Data analyzed by one way ANOVA followed by Dunnett's test (n=5). ∗P<0.05 and ∗∗P<0.01.

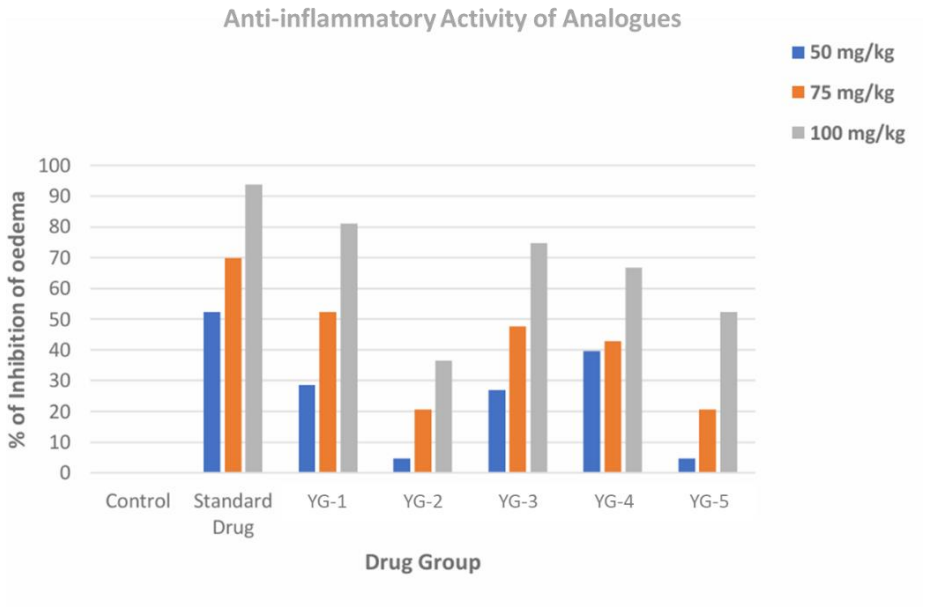

Figure 2: Anti-inflammatory activity of Analogues.

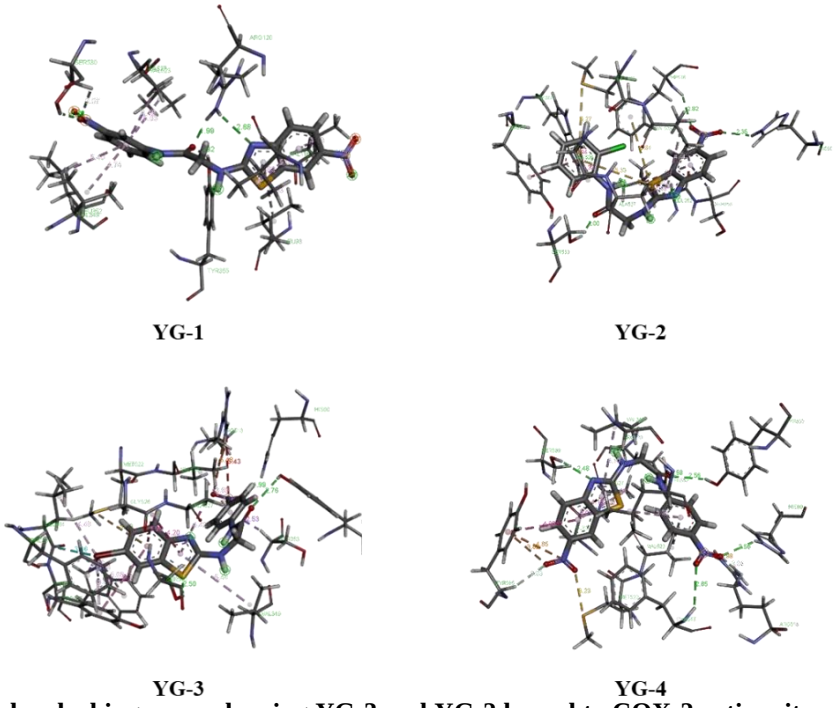

Figure 3: 3D molecular docking poses showing YG-3 and YG-2 bound to COX-2 active site with key interactions highlighted.

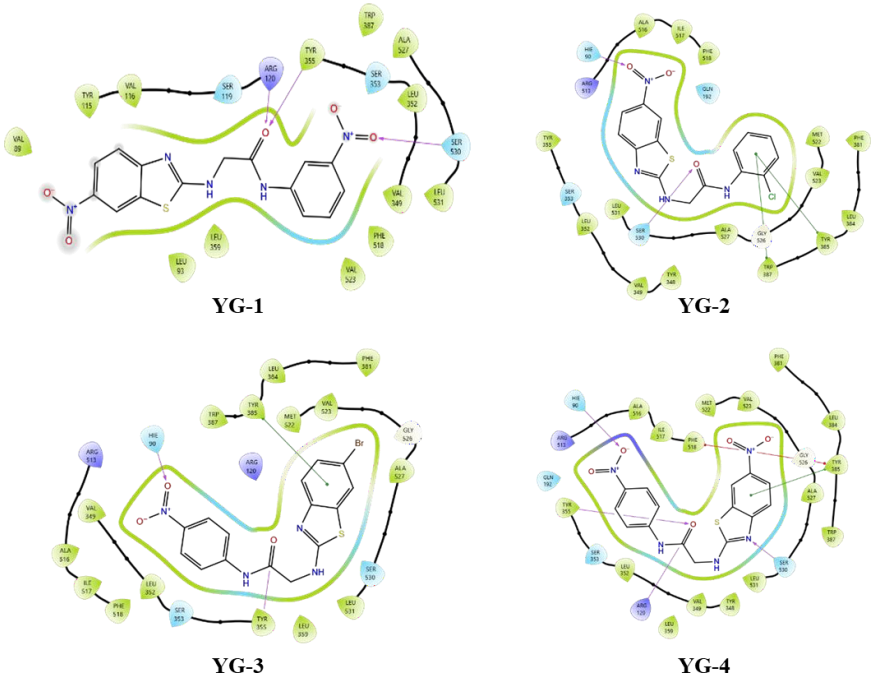

Figure 4: 2D interaction diagrams showing hydrogen bonds, hydrophobic contacts, and halogen bonds for top-performing compounds.

4. DISCUSSION

The comprehensive approach integrating synthetic chemistry, in silico molecular modeling, and in vivo pharmacological assessment has yielded valuable insights into the structure-activity relationships governing the anti-inflammatory efficacy of this class of compounds.

The three-step synthetic protocol employed for the preparation of target compounds YG-1 through YG-5 proved to be efficient and reproducible, with overall yields ranging from 46% to 77%.[18] The optimized reaction conditions, particularly the use of 1,4-dioxane as solvent and triethylamine as base in the final coupling step[19], facilitated clean product formation with minimal side reactions.[19] The moderate to good yields obtained are consistent with the inherent reactivity patterns of aminobenzothiazole nucleophiles toward chloroacetanilide electrophiles[20], where electron-withdrawing substituents on the benzothiazole ring enhance nucleophilicity while simultaneously reducing

overall reactivity through resonance effects.[21]

Spectroscopic characterization confirmed the successful formation of the desired amide linkages, with FTIR spectroscopy revealing characteristic carbonyl stretching frequencies in the range of 1640-1694 cm^{-1}, consistent with secondary amide functionality.[22] The presence of multiple N-H stretching vibrations (3150-3475 cm^{-1}) corroborated the formation of the acetamide bridge connecting the benzothiazole and substituted aniline moieties.[22] ^{1}H-NMR analysis provided definitive structural confirmation, with the amide NH protons appearing as characteristic downfield signals (δ 10.6-11.7 ppm).[22]

The molecular docking studies against COX-2 (PDB ID: 4COX) revealed significant binding affinities for all synthesized compounds, with docking scores ranging from -7.632 to -8.412 kcal/mol, favorably comparing to the reference standard diclofenac sodium (-7.582 kcal/mol).[23] Compound YG-3 demonstrated the highest binding affinity (-8.412 kcal/mol), followed closely by YG-2 (-8.388 kcal/mol), suggesting these derivatives possess enhanced complementarity to the COX-2 active site architecture.[23]

Docking performance of YG-3 due to 5-bromo substituent on the benzothiazole ring, which likely engages in favorable halogen bonding interactions with COX-2 binding pocket.[23] Bromine atom's electron-deficient sigma-hole facilitates directional non-covalent interactions with electron-rich regions of the protein, potentially including backbone carbonyls or aromatic π-systems of phenylalanine or tyrosine residues.[23] Similarly, the enhanced binding of YG-2 may result from the strategic positioning of the 2-chloro substituent on the aniline ring, which could optimize hydrophobic contacts within the enzyme's active site Fig. 3 and Fig. 4.[23]

The structure-activity relationship analysis reveals several critical structural determinants of anti-inflammatory activity.[23] The nitro group substituents, present in compounds YG-1, YG- 2, and YG-4, contribute to binding affinity through potential hydrogen bonding with polar residues, while simultaneously modulating the electronic properties of the aromatic systems.[16] The positioning of these electron-withdrawing groups influences the overall molecular dipole moment and may affect membrane permeability and protein-ligand recognition.[16]

The carrageenan-induced paw edema model provided robust evidence for the anti- inflammatory potential of the synthesized compounds. YG-1 emerged as the most potent derivative, achieving 80% edema inhibition at 100 mg/kg, which compares favorably to diclofenac sodium's 93% inhibition at 10 mg/kg.[24] This dose-dependent activity profile suggests a specific mechanism of action rather than non-specific cytotoxic effects.[24]

The anti-inflammatory efficacy order (YG-1 > YG-3 > YG-4 > YG-5 > YG-2) interestingly does not directly correlate with the docking scores, highlighting the complexity of translating in silico binding predictions to in vivo biological activity.[24]

YG-1's superior in vivo performance, despite moderate docking scores, suggests favorable pharmacokinetic properties that may include enhanced bioavailability, prolonged half-life, or improved tissue penetration.[25] The compound's dual nitro substitution pattern (6-nitro on benzothiazole and 3-nitro on aniline) may contribute to optimal physicochemical properties for oral absorption and systemic distribution.[25]

The observed anti-inflammatory activity likely involves selective COX-2 inhibition, as suggested by the molecular docking studies.[26] The benzothiazole scaffold's ability to occupy the COX-2 active site while avoiding the more constrained COX-1 binding pocket may contribute to the improved selectivity profile compared to traditional NSAIDs.[26] The acetamide linker provides the necessary flexibility for optimal positioning within the enzyme's active site while maintaining sufficient rigidity to preserve binding affinity.[27]

EWG (nitro and halogen) may enhance compounds' ability to engage in π-π stacking interactions, particularly with Phe518 and Tyr385, which are critical for substrate binding and catalytic activity.[28] Additionally, the thiazole nitrogen and sulfur atoms provide multiple hydrogen bond acceptor sites, potentially forming stabilizing interactions with Arg120 and Tyr355 residues.[28]

The in silico ADMET predictions indicate that all synthesized compounds comply with Lipinski's Rule of Five (Ro5 = 0), suggesting favorable drug-like properties and potential for oral bioavailability.[29] The calculated molecular weights (ranging from 389-434 Da) fall within the optimal range for small molecule drugs, while the predicted LogP values suggest appropriate lipophilicity for membrane permeation without excessive protein binding.[29]

The synthesized benzothiazole derivatives demonstrate several advantages over conventional NSAIDs. Structural features of these analogues suggest potential for enhanced COX-2 selectivity, which could translate to reduced gastrointestinal and cardiovascular adverse effects. The incorporation of the benzothiazole scaffold provides a distinct pharmacophore that may offer improved safety profiles compared to diclofenac or ibuprofen.

While current study gives compelling evidence for anti-inflammatory potential for 2- substituted aminobenzothiazole derivatives, several limitations warrant consideration. The in vivo evaluation was limited to acute inflammation models, and chronic

inflammatory conditions may require different mechanistic approaches. Additionally, the selectivity profile between COX-1 and COX-2 requires direct enzymatic assessment to confirm the proposed mechanism of action.

Future investigations should focus on expanding the structure-activity relationship studies to include additional substitution patterns, particularly exploring the effects of electron-donating groups and varied linker chemistries. Comprehensive toxicological evaluation, including hepatotoxicity, nephrotoxicity, and cardiovascular safety assessments, will be essential for advancing lead compounds toward clinical development.

5. CONCLUSION

This study successfully synthesized five novel 2-substituted aminobenzothiazole derivatives (YG-1 to YG-5) as anti-inflammatory agents. YG-1 demonstrated superior efficacy with 80% edema inhibition, while YG-3 showed highest COX-2 binding affinity (-8.412 kcal/mol). All compounds satisfied Lipinski's Rule of Five, indicating favorable drug-like properties. The structure-activity relationships revealed dual nitro substitution enhances in vivo activity. YG- 1 emerges as a promising lead compound for anti-inflammatory drug development.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENT

This should be brief and should acknowledge funding agencies (with grant/project details) and other special assistance. Trivial formalities like permission to carry out the study, etc., should be avoided.

Ethical issues

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