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AN INVESTIGATION TO THE IN SILICO DESIGN AND SYNTHESIS OF N-SUBSTITUTED AMINO THIOPHENES AS NOVEL CYCLOOXYGENASE-2 INHIBITORS

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ABSTRACT: 2-Amino thiophene act as synthons for biologically active organic molecules. Computational screening performed on a series of 2-amino thiophene scaffolds and evaluated their potential to be a lead for development of novel drugs for the inhibition of Cyclooxygenase-2. Top ranked compounds were synthesized via modified Gewald's reaction and were characterized by physical and spectral methods.

KEY WORDS: In silico, Synthesis, 2-amino thiophene, COX-2, Anti inflammatory

INTRODUCTION

The design and production of drugs is a field in which pharmaceutical chemistry has had incomparable impact on longevity and excellence over the past century. It is widely known that the design and development of a new drug costs more than 1 billion dollars in total and takes a minimum of 10 years. While even-with all these efforts, only a very limited number of drug discovery proposes lead to the official release of a new drug. Several technologies have been developed to excuse the process by shortening longanimity, expense. One of which is computer-aided drug design (CADD). CADD uses computing resources, algorithms, and 3D-visualization to generate new ideas about how to plan or modify molecules, and to make resolution in the fulfilling of the drug discovery process [I]. Docking studies are based on the available 3D structure and prior knowledge about the binding site of the target protein. This is suitable for a library of a lesser number of small molecules [II].

2-Aminothiophenes are important five membered heterocyclic building blocks in organic synthesis, and the chemistry of these small molecules is still developing based on the discovery of cyclization synthetic method by Gewald[III]. Another attractive feature of 2-aminothiophene scaffolds is their ability to act as synthons for the synthesis of biological active thiophene-containing heterocycles, conjugates and hybrids. Currently, the biological actions of 2-aminothiophenes or their 2-*N*-substituted analogues are still being investigated because of their various mechanisms of action (*e.g.*, pharmacophoric and pharmacokinetic properties). Likewise, the 2-aminothiophene family is used as diverse promising selective inhibitors,

receptors, and modulators in medicinal chemistry, and these compounds even exhibit effective pharmacological properties in the various clinical phases of appropriate diseases[IV].
Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. It can be classified as either acute or chronic, and involves a cascade of biochemical events comprising the local vascular system, the immune system, and different cell types found in the injured tissue. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. Chronic inflammation concerns a progressive change in the type of cells present at the site of the inflammatory reaction and is characterized by simultaneous destruction and healing of the injured tissue[V]. Regardless of the triggering factor, the mechanisms involved in the inflammatory process are common to all. The title compounds(AT9-AT11) were screened from a molecular library and synthesized via Scheme-01 followed by the in-vitro estimation of anti-inflammatory activity.

EXPERIMENTAL SECTION

Table 01: Molecular library of 2-amino thiophene scaffolds

CODE	MOLECULAR FORMULA	MOLECULAR WEIGHT	CODE	MOLECULAR FORMULA	MOLECULAR WEIGHT
AT1	C9H11NO4S	229.5	AT7	C9H12N2OS	196.27
AT2	C9H13N3OS	227.28	AT8	C10H12N2O4S	288.34
AT3	C7H7N3OS	181.21	AT9	C18H20N2O3S	344.428
AT4	C7H10N4OS	198.25	AT10	C18H21NO4S2	379.49
AT5	C15H17N3O3S	319.38	AT11	C18H19NO3S	329.41
AT6	C9H13N3O3S	243.28			

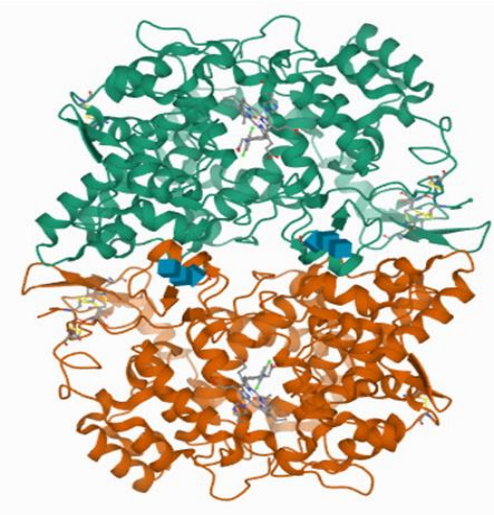
Evaluation of pharmacokinetic properties[VI,VII]:

Designed molecules were evaluated for their pharmacokinetic parameters using Swiss ADME, an online free tool for the evaluation of pharmacokinetic properties of organic compounds. For the evaluation of pharmacokinetic properties, number of hydrogen bond acceptors(HBA), number of hydrogen bond donors (HBD), consensus log P. Lipinski violation, GI absorption were selected. In spite of these parameters, bioavailability prediction and permeability prediction by boiled egg method was carried out using the same tool.

Toxicity Studies[VIII]:
Evaluation of toxicity of the proposed derivatives carried out using PreADMET, an online web tool. Ames test, Carcino mouse, Carcino rat, hERG inhibition models were used to evaluate the toxicity profile of all the eleven derivatives.

Docking studies:
All the structures of the ligands were drawn using ChemDraw Professional 15.0 and the 3D structures were generated using online CORINA software. Crystal structure of protein COX 2 was downloaded from PDB (PDB ID: 1PXX). The downloaded protein was cleaned using PyMol2. Latter the docking was done using Autodock vina in PyRx15.0. The results were visualized using Biovia Drug Discovery Studio 2021.

Figure 01: Crystal structure of target protein (PDB ID:1PXX) COX-2



RESULT AND DISCUSSIONS

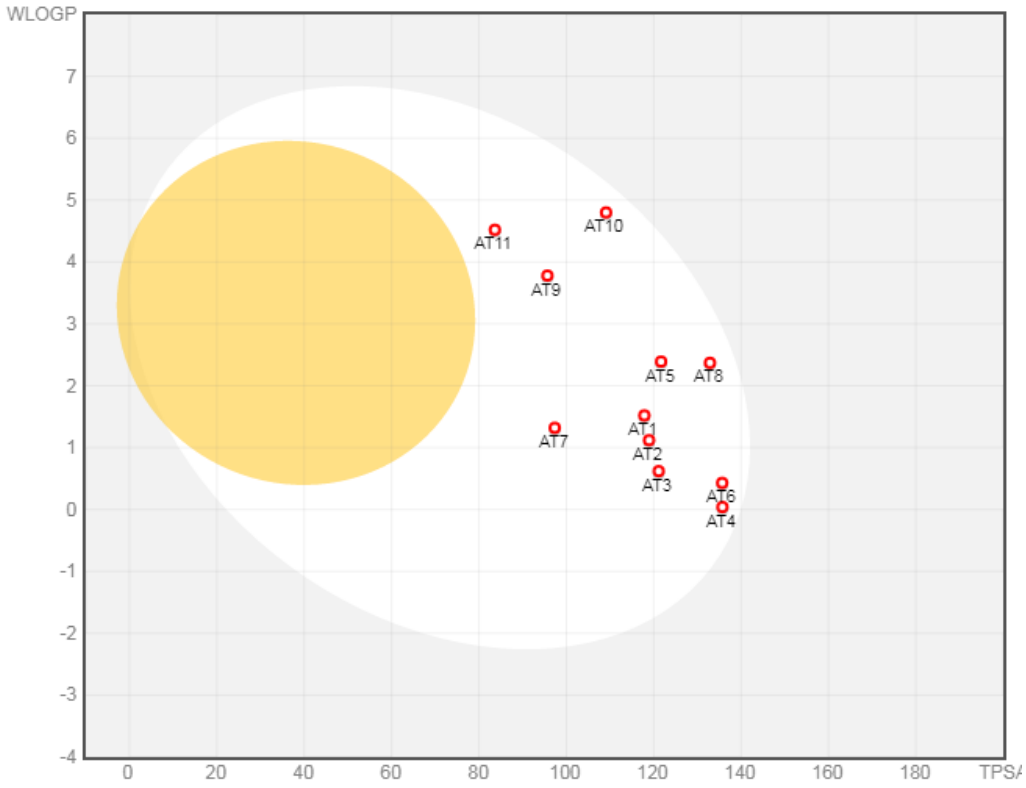
Evaluation of pharmacokinetic properties:
Swiss ADME studies shed light on to the pharmacokinetic properties of the proposed scaffolds. Out of the eleven derivatives, none of them are violating Lipinki rule. Consensus log P was found to be less than 5, which is optimal for all the derivatives. Among the scaffolds AT1, AT5, AT6, AT7, AT8 showing PAINS alert. Boiled egg experiment shows all compounds are having necessary systemic absorption and they do not show any CNS toxicities.

Table 02: Pharmacokinetic properties predicted using SwissADME

COMPOUND	AT1	AT2	AT3	AT4	AT5	AT6	AT7	AT8	AT9	AT 10	AT 11
HBA	4	3	2	2	3	4	1	5	3	4	3
HBD	2	2	2	3	3	3	2	1	2	1	1

Consensus log P	1.59	1.35	0.70	0.23	2.68	0.83	1.60	1.63	3.80	3.90	3.97
Lipinski's Violation	0	0	0	0	0	0	0	0	0	0	0
GI absorption	High	High	High	High	High	High	High	High	High	High	High
PAIN ALERT	1	0	0	0	1	1	1	0	0	0	0

Figure 02: Boiled egg experiment



Toxicity Studies:

In PreADMET study all the derivatives were found to be mutagenic in Ames test. But as it is a prokaryotic model, the result may not be applicable to eukaryotes. Carcinogenic models both in rat and mouse showed the scaffolds are not carcinogenic. Estimation of cardiac toxicity using hERG inhibition model showed AT5, AT6, AT9, AT10, AT11 are medium risk and rest are low risk scaffolds.

Table 03: PreADMET data showing the toxicity profile

Compound	Ames_test	Carcino_Mouse	Carcino_Rat	hERG_ inhibition

AT1	Mutagen	Negative	Negative	Low risk
AT2	Mutagen	Negative	Negative	Low risk
AT3	Mutagen	Negative	Negative	Low risk
AT4	Mutagen	Negative	Negative	Low risk
AT5	Mutagen	Negative	Negative	Medium risk
AT6	Mutagen	Negative	Negative	Medium risk
AT7	Mutagen	Negative	Negative	Low risk
AT8	Mutagen	Negative	Negative	Low risk
AT9	Mutagen	Negative	Negative	Medium risk
AT10	Mutagen	Negative	Negative	Medium risk
AT11	Mutagen	Negative	Negative	Medium risk

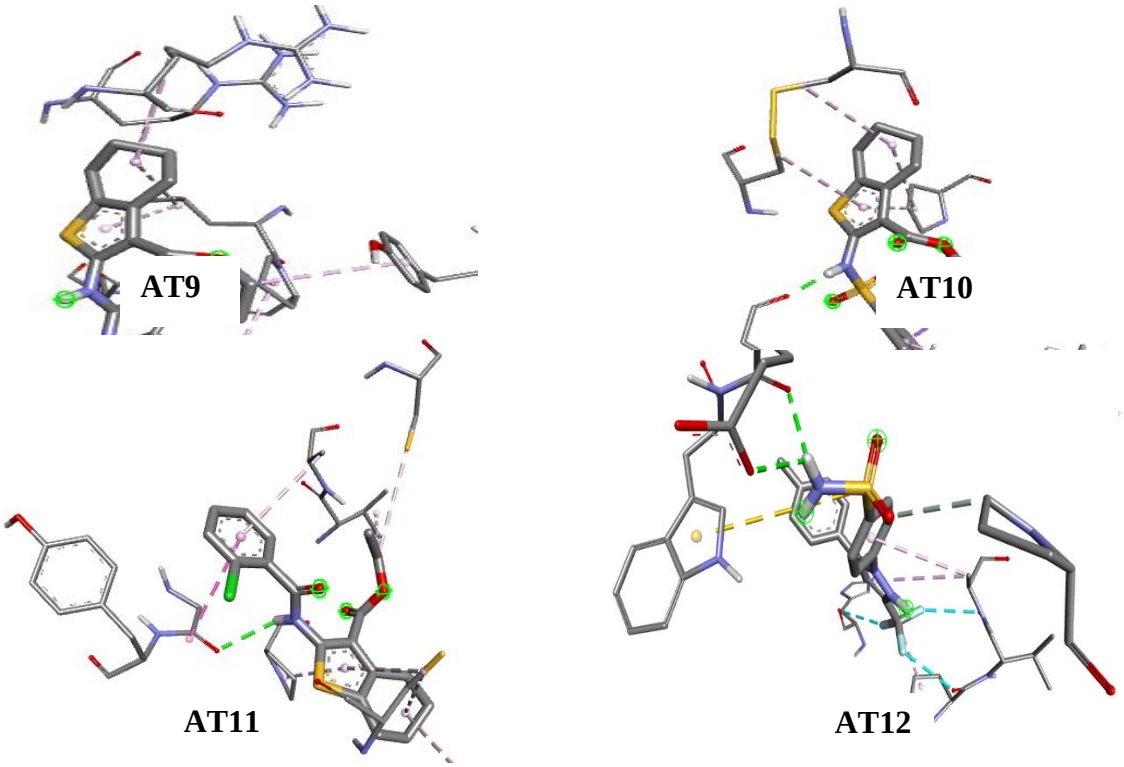
Docking studies:

Docking of all derivatives on 1PXX showed that all these derivatives are capable of inhibiting the enzyme to an extent. Among them AT9, AT11, AT12 were ranked highest having docking score -8.7, -8.1, -8.1 respectively. These scores were comparable with that of standard (Celecoxib) having score -8.4. Based on these assumptions AT9, AT10, AT11 were taken for synthesis and further studies.

Table 04: Docking score of the scaffolds

COMPOUND	**SCORE**
AT1	**-6.1**
AT2	**-6.2**
AT3	**-6.2**
AT4	**-6.3**
AT5	**-7.2**
AT6	**-7.1**
AT7	**-6.5**
AT8	**-6.7**
AT9	**-8.7**
AT10	**-8.1**
AT11	**-8.1**
Standard (Celecoxib)	**-8.4**

Figure 03: Docked images of AT9, AT10, AT11, Standard on the receptor



SYNTHESIS

All the materials of AR grade were used. Solvents were dried and purified using suitable methods. Melting point was recorded using Stuart smp50 automatic melting point apparatus. NMR data was recorded using Bruker 400. IR was recorded using Bruker Vertex 70.

Scheme 01: Synthetic scheme followed for the N-Substituted Amino Thiophenes

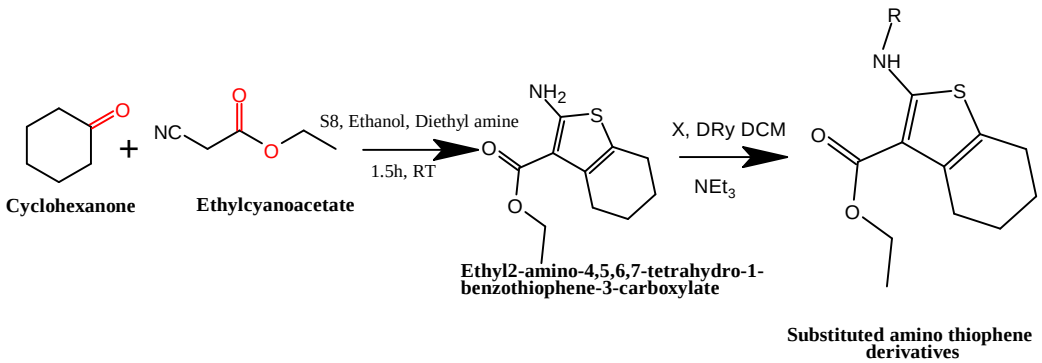
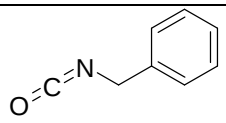
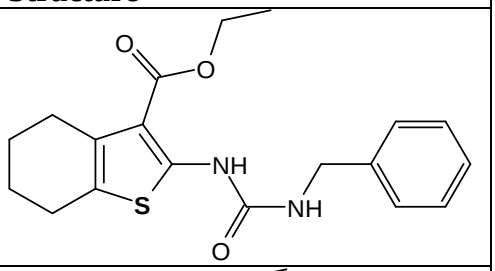
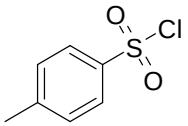
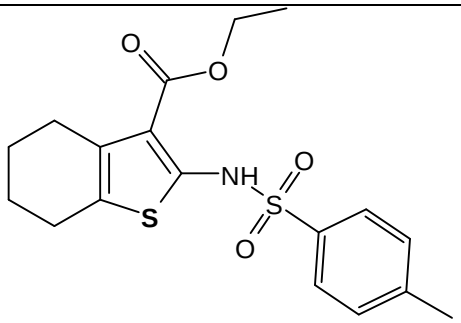
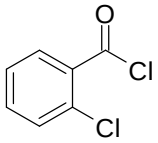
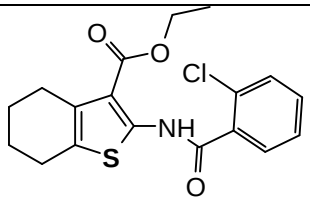


Table 05: Potent derivatives synthesized based on the docking result

Compound code	X	Structure
AT9	 Benzyl isocyanate	
AT10	 Tosylchloride	
AT11	 2-chloro benzoyl chloride	

Synthesis of ethyl 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (GV_1)

Sulfur (3.2g, 0.1mol) was dissolved in Cyclohexanone (9.8g. 0.1mol) and Ethylcyano acetate (11.g, 0.1mol) and stirred for 30mins. To this well stirred mixture, Diethylamine (9.14g, 0.125mol) was added dropwise in 30mins. Continue the reaction on stirring for 3hrs under inert atmosphere. Monitor the reaction by using TLC, after completion keep the reaction mixture in refrigerator for overnight. Add chilled methanol to the reaction mixture and the precipitate was filtered under vacuum and washed with chilled methanol. Recrystallized from suitable solvent. A filter column was done for purification using pure DCM as eluent.

Yield: 72%, Melting point: 117.6º C

^{1}H NMR δ (ppm): 4.2 (m, 2H), 2.7 (t, 2H), 2.5 (t, 2H), 1.7(m, 4H). ^{13}C NMR δ (ppm): 166.2, 165.8, 145.18, 132.98, 110.10, 60.32, 27.49, 25.50, 23.25, 22.89, 14.27. AT-IR (cm^{-1}): 3300Cm-1 (NH stretching), 1641Cm-1 (Carbonyl stretching), 1448 Cm-1 (Ester stretching), 1240 Cm-1 (C-N stretching).

Synthesis of ethyl 2-(3-benzylureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (AT9)

In a RBF compound GV_1 (0.5g, 2.22mmol) was dissolved in dry DCM under N_2 atmosphere and added bezylisocyanate (0.29g, 2.22mmol) in dry DCM. Stirred over a night. The precipitated product was collected and recrystallized from rectified spirit. Further purified by column chromatography using n-Hexane – Ethyl acetate system.

Yield: 68%, Melting point:140.3 º C

^{1}H NMR δ (ppm): 7.34 (m, 4H), 7.23 (m, 1H), 4.35 (m, 2H), 4.28 (d, 2H), 2.76, 2.72 (m, 4H), 1.78 (m, 4H), 1.40 (t, 3H). ^{13}C NMR δ (ppm): 165.69, 156.49, 149.29, 138.71, 138.70, 128.68, 127.46, 127.27, 122.65, 131.95, 60.60, 44.60, 26.22, 22.29, 22.59, 14.29. AT-IR (cm^{-1}): 3313Cm-1 (NH stretching), 1666 Cm-1 (Carbonyl stretching), 1338Cm-1 (Ester stretching), 1338Cm-1 (Ester stretching).

Synthesis of ethyl 2-[(4-methylbenzene-1-sulfonyl) amino]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (AT 10)

In RBF compound GV_1 (0.5g, 2.22mmol) was dissolved in dry DCM under N_2 and add Para toluene sulfonyl chloride (0.42g,2.22mmol) in dry DCM. Stirred overnight. Reaction was monitored using TLC. At the end reaction mixture was acidified and allowed for precipitation. Solvent was removed by rotary evaporator. Crude product was recrystallized from ethanol and purified by silica gel chromatography.

Yield: 79%, Melting point: 225.1 º C

^{1}H NMR δ (ppm): 7.46 (d, 2H), 7.30 (d, 2H), 4.25 (m, 2H), 2.76 (t, 4H), 2.40 (s, 3H), 1.77 (m, 2H), 1.38 (t, 3H). ^{13}C NMR δ (ppm): 165.84, 148.73, 142.89, 142.89, 138.04, 136.84, 128.99, 127.62, 114.42, 60.79, 26.22, 26.21, 22.59, 22.29, 21.52, 14.28

Synthesis of ethyl 2-(2-chlorobenzamido)-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (AT11)

In RBF compound GV_1 (0.5g, 2.22mmol) was dissolved in dry DCM under N_2 atmosphere and added 2-chloro benzoyl chloride (0.38g, 2.22mmol) in DCM. Stirred the reaction in ambient temperature for 6hrs. Reaction was monitored using TLC. Solvent was removed by rotary evaporator. Crude product was recrystallized from ethanol and purified by silica gel chromatography.

Yield: 86%, Melting point: 112.5º C

^{1}H NMR δ (ppm): 7.34 (m, 3H), 7.24 (m, 1H), 6.95 (t, 1H), 4.35 (m, 2H), 4.28 (d, 2H), 2.74 (m, 4H), 1.78 (m, 4H), 1.40 (t, 3H). ^{13}C NMR δ (ppm): 165.71, 156.48, 149.29, 138.69, 128.68, 127.27, 122.65, 113.94. AT-IR (cm^{-1}): 1706 Cm-1 (Carbonyl stretching), 1442 Cm-1 (Ester stretching),757 Cm-1 (C-Cl stretching).

CONCLUSION:

In silico screening performed on the N-substituted amino thiophene scaffolds revealed that the selected derivatives (AT09, AT10, AT11) have the potential to form synthons for novel drug development and docking results underlines their ability to inhibit COX-2 enzyme. The synthetic route followed gives an easy way of synthesis of these compounds with sufficient yield.

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