Broad spectrum antimicrobial, anti-inflammatory and peripheral analgesic activities of heteroaryl nitazoxanide analogs

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Background
The antiparasitic drug nitazoxanide possesses diverse biological activity. However, very few investigation was accomplished with nitazoxanide analogs. Therefore, herein we focused on the screening of bioactivities using some nitazoxanide-like synthesized molecules.
Objectives
Four heteroaryl nitazoxanide analogs synthesized in our laboratory were investigated for antimicrobial, anti-inflammatory, and analgesic activity.
Materials and methods
Disc diffusion method was used for assessing antimicrobial potency against several Gram-positive bacteria, Gram-negative bacteria, and fungi. Carrageenan-induced rat paw edema model was performed to evaluate anti-inflammatory activity. The analgesic property was evaluated using the acetic acid-induced writing inhibition method in the mice model. Molecular docking simulations against cyclooxygenase-1, cyclooxygenase-2, phospholipase A2, NF-κB inducing kinase, and interleukin-1 receptor-associated kinase 4 were also performed.
Results and conclusion
All the synthesized compounds exhibited broad spectrum antimicrobial property against a number of Gram-positive, Gram-negative species and unicellular fungi. Compound 4 or *N*-(5-nitrothiazol-2-yl)-furan-3-carboxamide emerged as the most prominent antimicrobial agent exhibiting zone of inhibition ranging in 14–22 mm. These zone diameters are sometimes greater than that displayed by nitazoxanide. Compounds 2 and 3 also showed remarkable broad-spectrum antimicrobial activity with a zone of inhibition 10–20 mm and 12–20 mm, respectively. Compound 4 also displayed potential anti-inflammatory activity which is comparable to standard aceclofenac. Compound 4 also showed mild analgesic effects. The compounds also exhibited moderate binding affinities against the selected target receptors and enzymes during *in silico* molecular docking. Heteroaryl nitazoxanide analogs showed prominent broad-spectrum antimicrobial, anti-inflammatory, and mild analgesic properties. This study indicates that heteroaryl nitazoxanide analogs might be interesting candidates for new drug discovery.

Keywords:
anti-inflammatory, antimicrobial, broad-spectrum, nitazoxanide, peripheral analgesic

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Introduction

Discovery of new medicinal agents is becoming more important because existing therapeutic agents are continuously being ineffective due to emergence of drug resistance. In the era of modern drug discovery scientists are continuously making effort to synthesize drugs with maximum drug efficacy, minimal adverse effects and as much as possible without incidence of drug resistance. A possible solution to combat the alarming situation about global antibiotic resistance scenario could be designing and exploring the new heterocyclic scaffolds possessing unique mode of activity and broad spectrum of pharmacological activity. Although, recently several natural products with significant antimicrobial properties have been developed, many of them lacks broad spectrum activity [1]. Synthesized heterocyclic molecules are continuously showing promising bioactivities including antimicrobial activity against a range of organisms. In this context, five membered heterocyclic ring systems, such as 'thiazole' and 'imidazole' derivatives played a crucial role in synthetic medicinal chemistry because of their diversified bioactivity [2–4]. Thiazole moiety have been represented as a key pharmacophore for synthesizing numerous biologically important compounds. Substituted thiazole analogs reportedly

possess a wide range of activity such as antibacterial [5], antifungal, antiviral [6], anthelmintic [7], antitubercular, antiplasmodial [8], anti-inflammatory [9], anticancer [10], antiallergic [9], antiarrhythmic and anticoagulant [11], analgesic, anticonvulsant, antioxidant [12], antipsychotropic [13], antiarthritic [14], hypolipidemic [12], etc. There are many important drugs with chemical structure consisting of 'thiazole' nucleus, for example antiparasitic agents nitazoxanide (NTZ), aminitrozole and tenonitrozole [15].

Drug discovery process nowadays has expedited significantly due to incorporation of computer-aided drug design, making it much more cost-efficient. Challenges faced during various stages of drug development and formulation processing can be overcome using *in silico* molecular docking strategies [16]. Potent drug-like compounds can be designed based on drug-receptor interactions and by identifying the drug inclusion complex that is most stable. Hence, it is regarded as an essential technique for lead compound optimization and can provide insights behind the molecular mechanistic pathway of pharmacological activity demonstrated by novel lead compounds.

In our previous study [17], we presented the synthetic routes to four heteroaryl amide analogs of NTZ (Fig. 1). We also reported the cytotoxic effects and *in silico* binding activities of these analogs against some putative molecular targets. We have recently explored some interesting pharmacological screening with the synthesized molecules. Here, we present the evidence of some other significant biological activities demonstrated by these synthetic molecules including antimicrobial, anti-inflammatory and analgesic activities.

Materials and methods

We investigated the antimicrobial, anti-inflammatory as well as peripheral analgesic activities of the synthesized analogs of nitazoxanide namely *N*-(5-nitrothiazol-2-yl)-thiophene-2-carboxamide (1), *N*-(5-nitrothiazol-2-yl)-thiophene-3-carboxamide (2), *N*-(5-nitrothiazol-2-yl)-furan-2-carboxamide (3) and *N*-(5-nitrothiazol-2-yl)-furan-3-carboxamide (4) (Fig. 1). In each experiment, we took respective standard and controls in order to compare these activities for the synthetic compounds.

Ethical approval

Ethical approval was obtained from the Ethical Review Committee of Animal and Human Studies of Faculty of Biological Sciences, University of Dhaka (208/Bio/SCS).

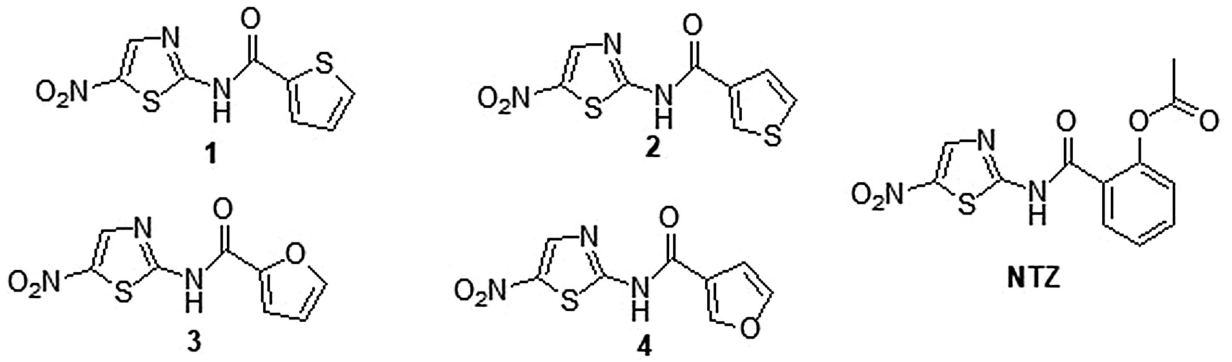
Investigation of antibacterial activity

In vitro antibacterial property was evaluated using Kirby-Bauer's agar diffusion susceptibility test [18], and performed as per recommendation stated in the latest CLSI (Clinical and Laboratory Standard Institute) [19]. The test was conducted in the food microbiology lab of Advanced Research Centre in Sciences located at University of Dhaka. In short, measured amount of each test sample (Compound 1–4 and NTZ) was dissolved in specific volume of solvent (0.1% DMSO) and loaded on sterile blank discs (6 mm) to obtain the desired concentrations (200 mg/disc) to be tested. A total of 11 bacteria and 3 fungi were used in antimicrobial screening. Ciprofloxacin (5 μg/disc) was utilized as positive control and for negative control a blank disc was used. The discs were kept at 4°C for 24 h upside down in order to diffuse the materials to the medium with subsequent incubation at 37°C for 24 h. The antimicrobial activity was obtained via measuring zone diameters (in mm) and termed as zone of inhibition (ZOI).

Evaluation of *in vivo* anti-inflammatory activity

Anti-inflammatory potency of synthesized heteroaryl NTZ analogs 1–4 were investigated by performing the

Figure 1



Structures of synthetic analogs and nitazoxanide.

carrageenan-induced paw edema model in rat [20]. For this test, Wister rats (*Rattus norvigicus*) of both sex, each weighing 100–150 gm were used. The rats were kept in a standard environmental condition and fed with standard diet in the 'Animal Husbandry Room' of Institute of Food and Nutrition (INFS), University of Dhaka. Carrageenan (100 mg) was accurately weighed and dissolved in saline solution by gentle heating in a water bath. The final volume was diluted up to 10 ml using saline solution to make a 1% solution of carrageenan. As standard, the reference standard drug aceclofenac (100 mg) was accurately weighed and transferred to a graduated test tube where it was dissolved in 5 ml of normal saline (0.9% NaCl solution). For all test compounds, a dose of 100 mg/kg (P.O) body weight of rats was selected for administering. For this purpose, 70 mg of each synthesized molecule was triturated in unidirectional way with 2-Tween-80 (2–3 drops) and DMSO (1–2 drops). After proper mixing, the volume of the suspension was made to 5 ml via adding normal saline water and mixed properly by a vortex mixer. The method was essentially same as that of Winter *et al*. [20] with minor modifications. After keeping the animal in normal husbandry conditions for few days, the animals were randomly subdivided into 5 groups, at a ratio of 3 rats/group. Aceclofenac (100 mg/kg body wt.) [21] was administered as standard anti-inflammatory agent for this test. One hour after the oral administration of test materials, 1% carrageenan solution (0.1 ml) was administered to each rat of all groups through a 26-gauge needle inserted into the subplanar surface of the right hind paw of the rats. A plethysmometer (Ugo Basile, 7140, Italy) was used to measure the paw volume at different time intervals after the carrageenan administration and the mean increase in volume were recorded at each of those time intervals. Percentage inhibition of paw edema was determined using the following formula:

$$\% \text{ inhibition of paw edema} = [\{(Vt - Vo)\text{control} - (Vt - Vo)\text{treated}\}/(Vt - Vo)\text{control}] \times 100$$

Where, Vo = paw volume at 0 time (prior to carrageenan administration),

Vt = paw volumes at the t time.

Vt-Vo = paw edema volume

Assessment of *in vivo* peripheral analgesic activity

Acetic acid induced writhing method [22,23] was performed for the assessment of peripheral analgesic activity. Swiss-albino mice (*Mus musculus*) of either sex weighing 25–30 g, aged 4–5 weeks, were used for the experiment. They were maintained in standard husbandry condition in the Animal husbandry room of INFS, University of Dhaka and fed standard diet. Fifty randomly selected mice were divided into ten groups designated as control receiving only acetic acid, standard receiving aceclofenac (25 mg/kg), compounds 1 to 4 (25 mg/kg and 50 mg/kg) with 5 mice in each group. Doses of the all test samples were adjusted according to the weight of each mouse. Dose selection was accomplished based on the aceclofenac dose used and according to the dose level used for synthesized compounds in our previous studies [3,24]. To identify the individual mice, the animals were marked from M-1 to M-5. After proper mixing of compounds (3.125 and 6.25 mg for 25 mg and 50 mg/kg dose, respectively) with small amount of normal saline, minimal amount of Tween-80 (suspending agent) and 1–2 drops of Dimethyl sulfoxide (DMSO) was added as solubilizing agent and the final volume of the prepared suspension was adjusted to 3.0 ml by normal saline. The sample solutions were vortexed to stabilize the suspension. A standard sample of aceclofenac was prepared by taking 3.125 mg (for 25 mg/kg bw) and adjusting the volume to 3.0 ml similarly. The control group received normal saline (0.1 ml/10 g of body weight) P.O. (oral). After 30 min of administration of samples and the standard aceclofenac to the respective groups, 1 ml of 0.7% acetic acid was injected intraperitoneally to each mouse. Then, number of squirms or writhing was counted for each mouse for up to 15 min.

Statistical analysis

In all cases, the data were presented as mean±SEM. Statistical evaluation was carried out applying one-way ANOVA using IBM SPSS Statistics package for Windows, Version 25.0 (Armonk, NY, USA) followed by Dunnett's test used to measure *P* values at a Confidence interval (CI) of 95%.

In silico molecular docking studies

The heteroaryl NTZ analogues were subjected to molecular docking simulation using previously established methodologies [25,26]. The structure of the compounds and standards were drawn and energy optimized based on the MMF94 level of theory using Avogadro 1.2. Aceclofenac (PubChem CID 71771) was selected as the standard for this assay and its structure was obtained from PubChem. For study of analgesic activity, cyclooxygenase-1 (PDB: 1EQG) enzyme and cyclooxygenase-2 (PDB: 5IKT) enzyme were selected and obtained from the RCSB protein

data bank. Similarly, phospholipase A2/PLA2 (PDB: 4UY1), NF-κB inducing kinase/NIK (PDB: 4IDV) and interleukin-1 receptor-associated kinase 4/IRAK-4 (PDB: 5KX7) were selected for assessing anti-inflammatory activity. The proteins were downloaded in PDB format and all unwanted ligands, water molecules and heteroatoms were removed using PyMOL 2.4.1 to prepare them for docking. Coordinates of the active site of the target proteins were also determined using PyMOL. Swiss-PdB Viewer version 4.1 was then used to add hydrogen atoms to the proteins and minimize their energy levels using the GROMOS 96 43B1 parameters set where *in vacuo* computations were performed without any reaction field. The energy optimized compounds were then docked against the target proteins using PyRx 0.8 and the ligand-protein interactions were compared with standard drugs. AutoDock Vina feature of PyRx was used for docking simulations where the grid box was positioned at the center of the protein's active site coordinates. AutoDock Vina's semiflexible modeling feature was used to determine the binding affinities of drug-protein linkage. Discovery Studio Visualizer 2016 (BIOVIA) was used to analyze the best ligand-protein interaction from two-dimensional and three-dimensional images of the docking site.

Results

The synthetic analogs of nitazoxanide showed broad spectrum antimicrobial activities

All the compounds exhibited antimicrobial activity against all types of microorganisms. Compound 1 displayed mild activity with ZOI ranges from 10 to 14 mm (Table 1). Compound 4 demonstrated significant antimicrobial activity against all types of organisms (ZOI ranging 14-22 mm). The activity of 4 was sometimes superior to that of NTZ (entry 3, 4, 5, 8, 9, 13, and 14). Compound 3 also showed remarkable activity (ZOI:12–20 mm) which is comparable or sometimes better (entry 10, 13) than that of NTZ. Interestingly, both 3 and 4 showed potent antifungal activity which is similar or sometimes better than that of NTZ. Compound 2 also showed significant antimicrobial activity against *Bacillus megaterium*, *Bacillus subtilis, Shigella dysenteriae*, *Vibrio mimicus* (ZOI 18, 20, 18, and 18 mm, respectively) and fungal strains such as *Aspergillus niger* and *Candida albicans* (ZOI 17 mm for each).

Table 1 Zone of inhibition (mm) determination of test samples against bacteria and fungi

		Zone diameter (mm)				
Sl.	Name of the organism	1	2	3	4	NTZ
Gram positive Bacteria:						
1	*Bacillus cereus*	10	12	16	15	17
2	*Bacillus megaterium*	14	18	18	17	19
3	*Bacillus subtilis*	11	20	15	20	16
4	*Sarcina lutea*	10	10	13	17	15
Gram negative Bacteria:						
5	*Pseudomonas aeruginosa*	12	15	12	19	18
6	*Salmonella paratyphi*	10	11	15	20	22
7	*Salmonella typhi*	11	15	17	15	20
8	*Shigella boydii*	10	10	15	22	15
9	*Shigella dysenteriae*	12	18	14	16	14
10	*Vibrio mimicus*	10	18	18	14	16
11	*Vibrio parahemolyticus*	10	11	15	22	23
Fungi:						
12	*Saccharomyces cerevisiae*	10	10	14	14	14
13	*Candida albicans*	16	17	20	22	19
14	*Aspergillus niger*	16	17	20	22	20

Ciprofloxacin: 40 mm-45 mm.

Synthetic analogs of NTZ showed remarkable anti-inflammatory activities

The anti-inflammatory activities of synthetic NTZ analogs are summarized in Table 2. It is evident that compound 4 or *N*-(5-nitrothiazol-2-yl)-furan-3-carboxamide, showed significant anti-inflammatory effect at a dose of 100 mg/kg from the first hour and onward, with 55.7%, 40%, 57%, 40.7% inhibition of paw edema at first, second, third and fourth hour, respectively. Furan 2-carboxamide derivative 3 showed moderate anti-inflammatory effect at 100 mg/kg dose starting from the first hour and onwards having highest inhibition in 3rd hour. The % paw edema inhibition was 20, 37.5, 57.14, and 1.11% in first, second, third and fourth hour, respectively. However, corresponding thiophene derivatives 1 and 2, exhibited mild anti-inflammatory activity in comparison with the standard aceclofenac, which showed significant anti-inflammatory effect (% paw edema inhibition of aceclofenac was 74, 50, 47, 44% in first, second, third, and fourth hour, respectively).

Nitazoxanide analogs exhibited peripheral analgesic activities

Compound 4 showed mild peripheral analgesic activity with 28.1% (25 mg/kg) and 31.7% (50 mg/kg) percent inhibition of writhing responses. The percent inhibition of standard aceclofenac was 74.83% at 25 mg/kg body weight. However, compounds 1, 2, and 3 showed poor analgesic activity compared with standard drug (Table 3).

Docking simulations predicted probable biological target sites of the Nitazoxanide analogs

In silico docking simulations revealed probable target sites of the synthesized NTZ analogs and gave insignts to their mechanism of action. Docking scores (kcal/

Table 2 Assessment of anti-inflammatory activity of the synthesized analogs by carrageenan-induced rat paw edema model

	Mean Paw Volume (ml) (mean±SEM)				% Paw edema inhibition			
Group	1st hour	2nd hour	3rd hour	4th hour	1st hour	2nd hour	3rd hour	4th hour
Control	0.05±0.08	0.08±0.21	0.21±0.08	0.27±0.10	-	-	-	-
Aceclofenac (100 mg/kg)	0.013**±0.10	0.04***±0.07	0.11***±0.08	0.15**±0.11	74.0	50.0	47.0	44.0
1 (100 mg/kg)	0.03±0.01	0.07±0.04	0.13***±0.06	0.18***±0.05	40.0	12.5	30.0	33.0
2(100 mg/kg)	0.03**±0.01	0.07±0.01	0.16***±0.04	0.22*±0.04	40.0	12.5	23.80	18.51
3(100 mg/kg)	0.04**±0.01	0.05***±0.01	0.08***±0.04	0.24±0.02	20.0	37.5	57.14	11.11
4(100 mg/kg)	0.01***±0.12	0.07*±0.11	0.09***±0.12	0.16***±0.10	55.7	40	57.0	40.7

Each value represents the Mean Paw Volume of rats±SEM, (n=5). ***P less than 0.001, **P less than 0.01, *P less than 0.05 compared with control group induced with carrageenan. Aceclofenac was used as standard.

Table 3 Assessment of analgesic activity of the compounds by acetic acid induced writhing method

		Writhing count							
Test Samples	Dose (mg/kg)	M1	M2	M3	M4	M5	Number of Writhing (Mean±SEM)	Writhing (%)	Inhibition (%)
Control	0	27	27	28	28	29	27.8±0.374	100.0	_
Aceclofenac (25 mg/kg)	50	1	14	7	9	4	7.0±2.214***	25.17	74.83
1 (25 mg/kg)	25	25	27	29	25	28	26.8±0.800	96.4	3.66
1 (50 mg/kg)	50	25	27	26	23	27	25.6±0.748**	92.08	7.92
2 (25 mg/kg)	25	26	27	28	28	26	27.0±0.447	97.12	2.88
2 (50 mg/kg)	50	26	26	28	28	26	26.8±0.490	96.4	3.66
3 (25 mg/kg)	25	25	25	24	24	26	24.8±0.374**	71.9	18
3 (50 mg/kg)	50	23	25	26	20	20	22.8±1.241	68.3	10.8
4 (25 mg/kg)	25	25	25	24	24	26	20.0±1.140***	82.2	28.1
4 (50 mg/kg)	50	17	19	20	22	17	19.0±0.949***	89.01	31.7

Number of writhing is represented as the Mean±SEM (n=5), M1, M2, M3, M4 and M5 represents the mice number. Writhing inhibition was calculated based on the mean value. ***P less than 0.001, ** P less than 0.01, * P less than 0.05 compared with the control. Control received acetic acid only. Aceclofenac was used as standard.

mol) were used to assess the target site specificity of the NTZ analogs and ligand-protein interactions were utilized to measure the stability of the docked compound with the protein's active site. For predicting analgesic activity, the compounds were docked with cyclooxygenase-1 (PDB: 1EQG) and cyclooxygenase-2 (PDB: 5IKT) enzymes. For predicting anti-inflammatory property, the compounds were docked with phospholipase A2 (PDB: 4UY1), NF-κB inducing kinase (NIK) (PDB: 4IDV) and interleukin-1 receptor-associated kinase 4 (IRAK-4) (PDB: 5KX7). In both cases, aceclofenac was used as the standard ligand. Results of the docking simulations have been presented in Table 4.

Against the active site of cyclooxygenase-1, compound 4 had the highest docking score of −6.6 kcal/mol among the synthesized NTZ analogs. 2D and 3D pictures of the binding interactions reveals that compound 4 formed strong conventional hydrogen bond with ARG120, as well as carbon hydrogen bonds with TYR355 and ILE523 residues in the chain B of COX-1 enyzme. Aceclofenac also forms strong hydrogen bond with TYR355, indicating that compound 4 docks with the same binding pocket as aceclofenac. Compound **4** also had other hydrophobic interactions with COX-1 enzyme and it has been presented in Fig. 2.

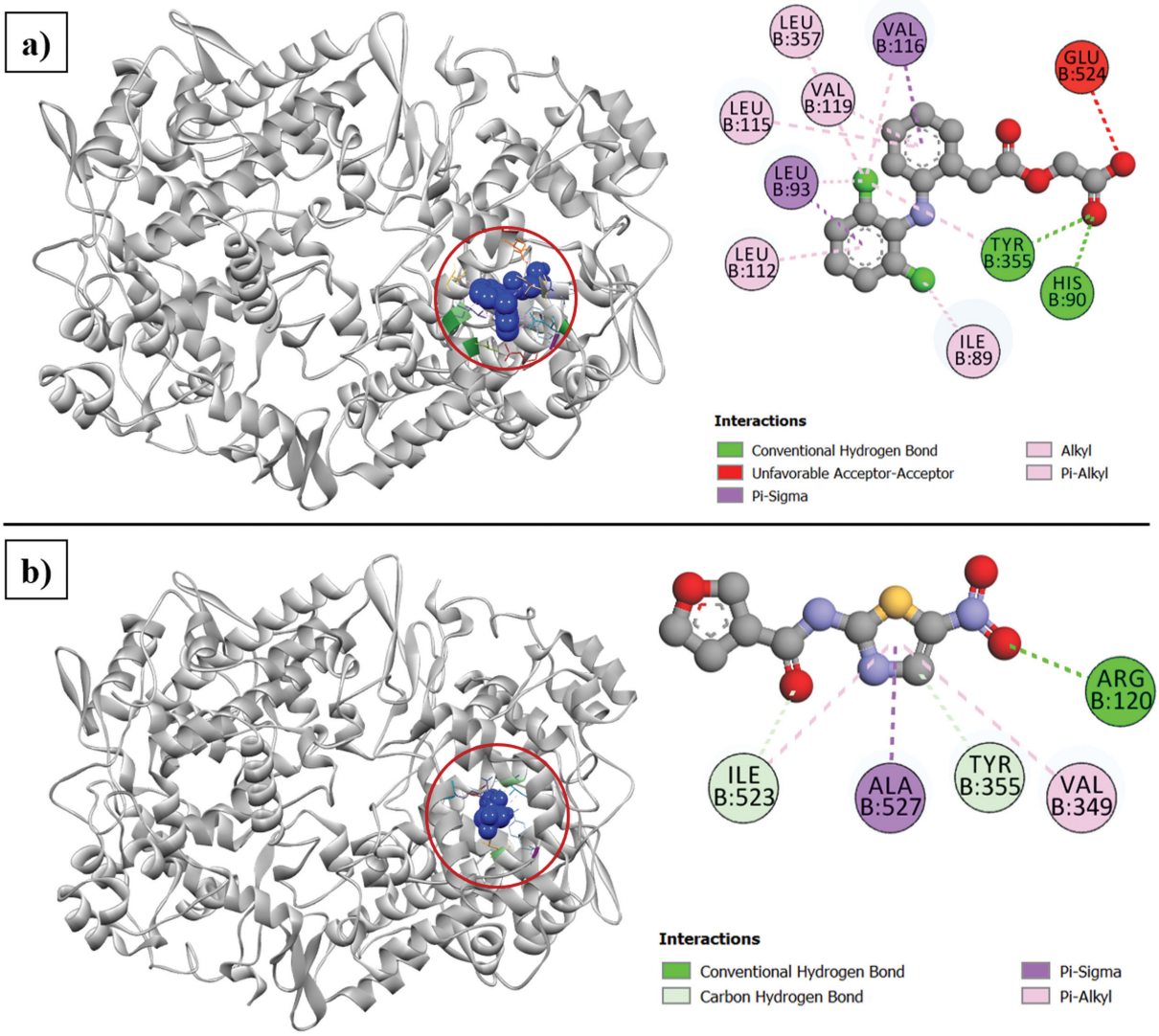
Compound 3 demonstrated strongest binding affinity (−7.2 kcal/mol) towards cyclooxygenase-2 enzyme. This strong affinity could be because of the four strong hydrogen bonds formed with GLN203, ASN382, TYR385 and ASN382 residues located at the active site of COX-2 enzyme. Interestingly, aceclofenac does not have similarity to any of the binding interactions of compound 3, though their region of binding is quite similar (Fig. 3).

Table 4 Binding affinities (kcal/mol) towards target proteins of standard ligands and synthesized compounds for prediction of biological activities

	Analgesic		Anti-inflammatory		
Compounds	1EQG	5IKT	4UY1	4IDV	5KX7
Standard	−7.6	−8.0	−7.6	−8.4	−7.7
1	−6.3	−6.5	−6.6	−6.8	−6.3
2	−6.1	−6.1	−6.6	−6.9	−6.4
3	−6.4	−7.2	−6.7	−6.7	−6.5
4	−6.6	−6.5	−6.6	−6.8	−6.6

Standard ligand for cyclooxygenase-1 (PDB: 1EQG), cyclooxygenase-2 (PDB: 5IKT), phospholipase A2 (PDB: 4UY1), NF-κB inducing kinase (NIK) (PDB: 4IDV) and interleukin-1 receptor-associated kinase 4 (IRAK-4) (PDB: 5KX7) is

Figure 2



Two-dimensional and three-dimensional pictorial representation of key interactions between ligands and binding pocket of cyclooxygenase 1 (PDB: 1EQG). (a) Aceclofenac with 1EQG; (b) Compound 4 with 1EQG.

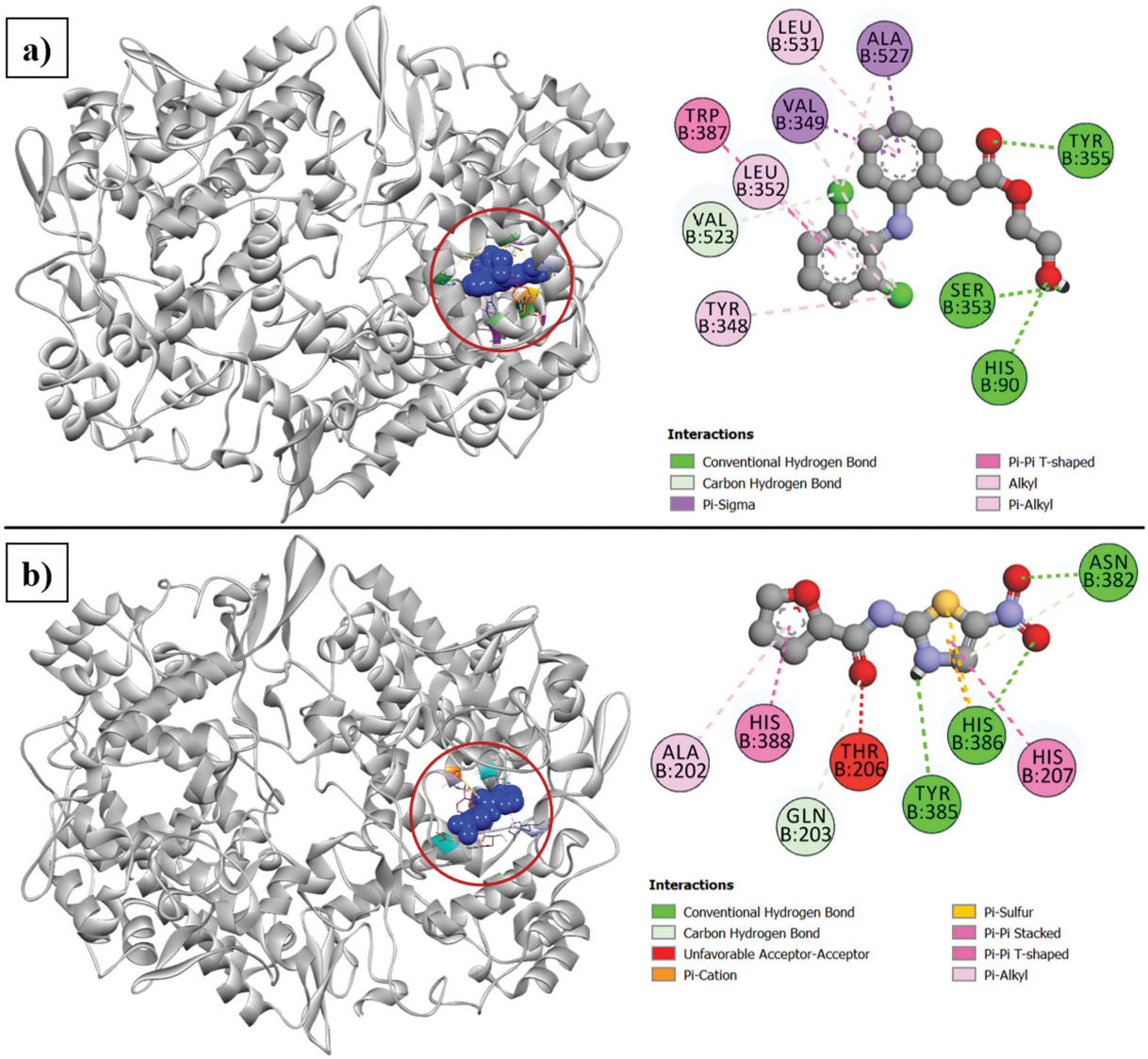
Against phospholipase A2 enzyme, compound 3 showed the highest docking score of −6.7 kcal/mol with strong conventional hydrogen bonds created with GLY28 and GLY30 residues near the protein's active site (Fig. 4). Against NF-κB inducing kinase (NIK), compound 2 had the highest binding affinity of −6.9 kcal/mol due to hydrogen bonds formed with ARG408 and ARG416 residues. Standard ligand aceclofenac and compound 2 both formed hydrogen bonds with ARG408, pi-sulfur bond with MET469, pi-sigma/pi-alkyl bonds with VAL414 and LEU522 residues. This indicates that both compound 2 and aceclofenac had quite indentical docking position. The interactions have been shown in Fig. 5.

Compound 4 had the strongest binding affinity against IRAK-4 with a docking score −6.6 kcal/mol. This strong affinity to the protein could be because of the carbon hydrogen bonds formed with GLY195 and ASP329 residues. Compound 4 also shares similar hydrophobic interactions as aceclofenac with VAL200, ALA211, VAL246, TYR262 and LEU318 residues of IRAK-4, which means the they have identical docking site (Fig. 6).

Discussion

NTZ is well known for its antiparasitic, antibacterial and antiviral activities [27–29]. Numerous clinical trials have been accomplished with NTZ to validate its utility as antiparasitic, antibacterial and antiviral agents including SARS-CoV-2. In our study, the synthesized NTZ analogs exhibited potential broad-spectrum antimicrobial activity, anti-inflammatory activity and analgesic property. Among the synthesized analogs *N*-(5-nitrothiazol-2-yl)-furan-3-

Figure 3

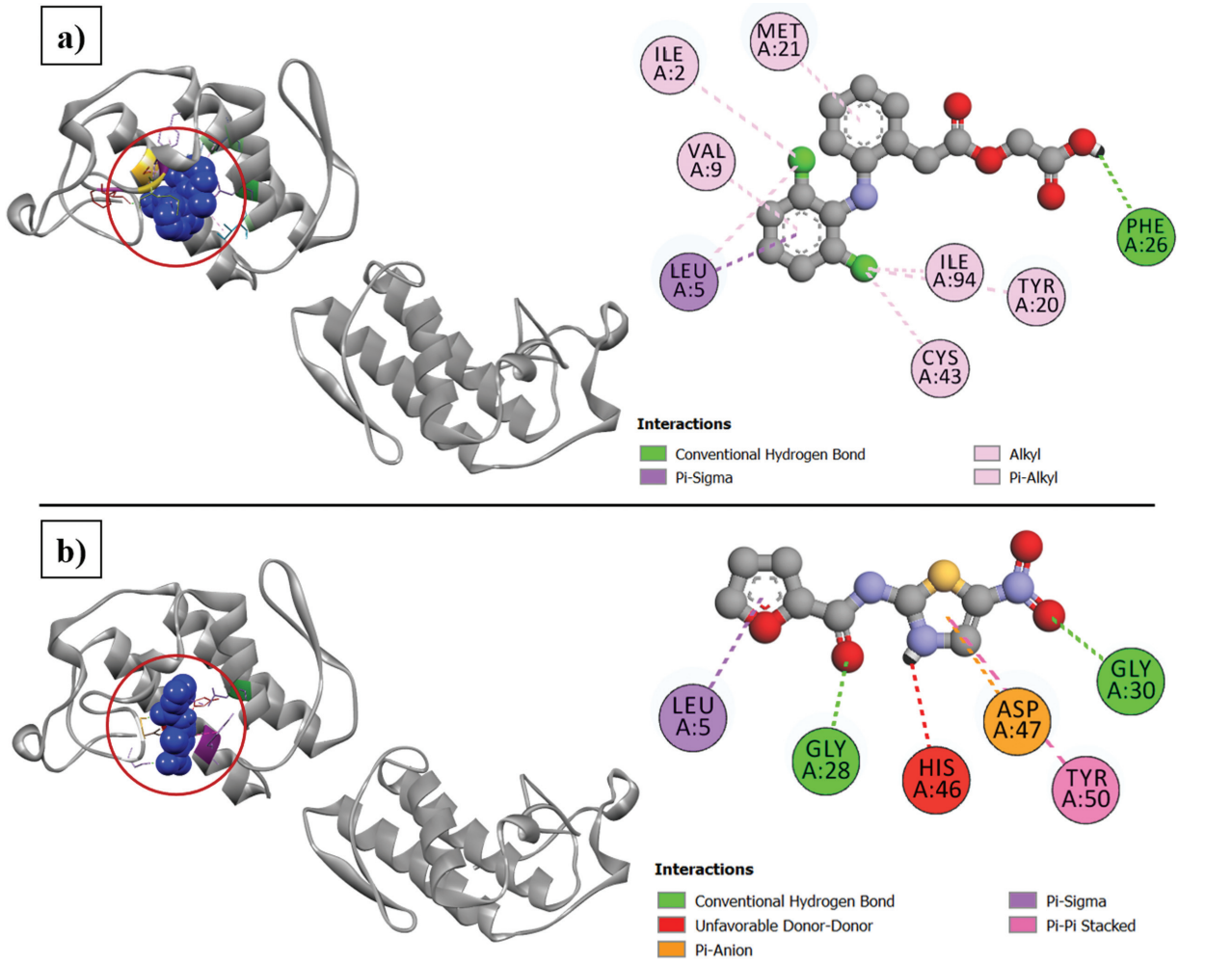


Two-dimensional and three-dimensional pictorial representation of key interactions between ligands and binding pocket of cyclooxygenase 2 (PDB: 5IKT). (a) Aceclofenac with 5IKT; (b) Compound 3 with 5IKT.

carboxamide (4) showed highest antimicrobial property against all types of microorganisms. Compounds 2 and 3 also showed remarkable antimicrobial activity. From the present study, a very simple structure activity relationship can be noticed. Furan carboxamide derivatives displayed superior activity than that of the thiophene analogs and carboxamide functionality at 3-position rather than 2-position of the heterocyclic ring always produced better results. The synthesized analogs 2, 3 and 4 demonstrated higher antibacterial activity than that of the parent compound NTZ against some gram- positive organisms such as *Bacillus subtilis, Sarcina lutea*, gram-negative bacteria such as *Shigella dysenteriae, Shigella boydii, Vibrio mimicus* etc. and the fungi *Aspergillus niger* and *Candida albicans*. The obtained activity of the NTZ analogs might be due to interaction of the compounds with the bacterial target proteins such as Glucosemine-6-phosphate synthase (G6PS), pyruvate ferredoxin oxidoreductase (PFOR), β-ketoacyl-acyl carrier protein synthase III (FabH), pyruvate dehydrogenase (PDH), etc [17,30,31].

In anti-inflammatory activity screening, again the furan 3-carboxamide analog 4 exhibited prominent activity which is comparable to that observed by standard aceclofenac (Table 2). Furan-2 carboxamide analog also provided remarkable anti-inflammatory property. The thienyl analogs are less active than the furan analogs. The present activity of the furan carboxamide analogs can be rationalized from a published report where some synthesized benzofuran carboxamide displayed prominent anti-inflammatory activity [32]. The observed anti-inflammatory activity

Figure 4



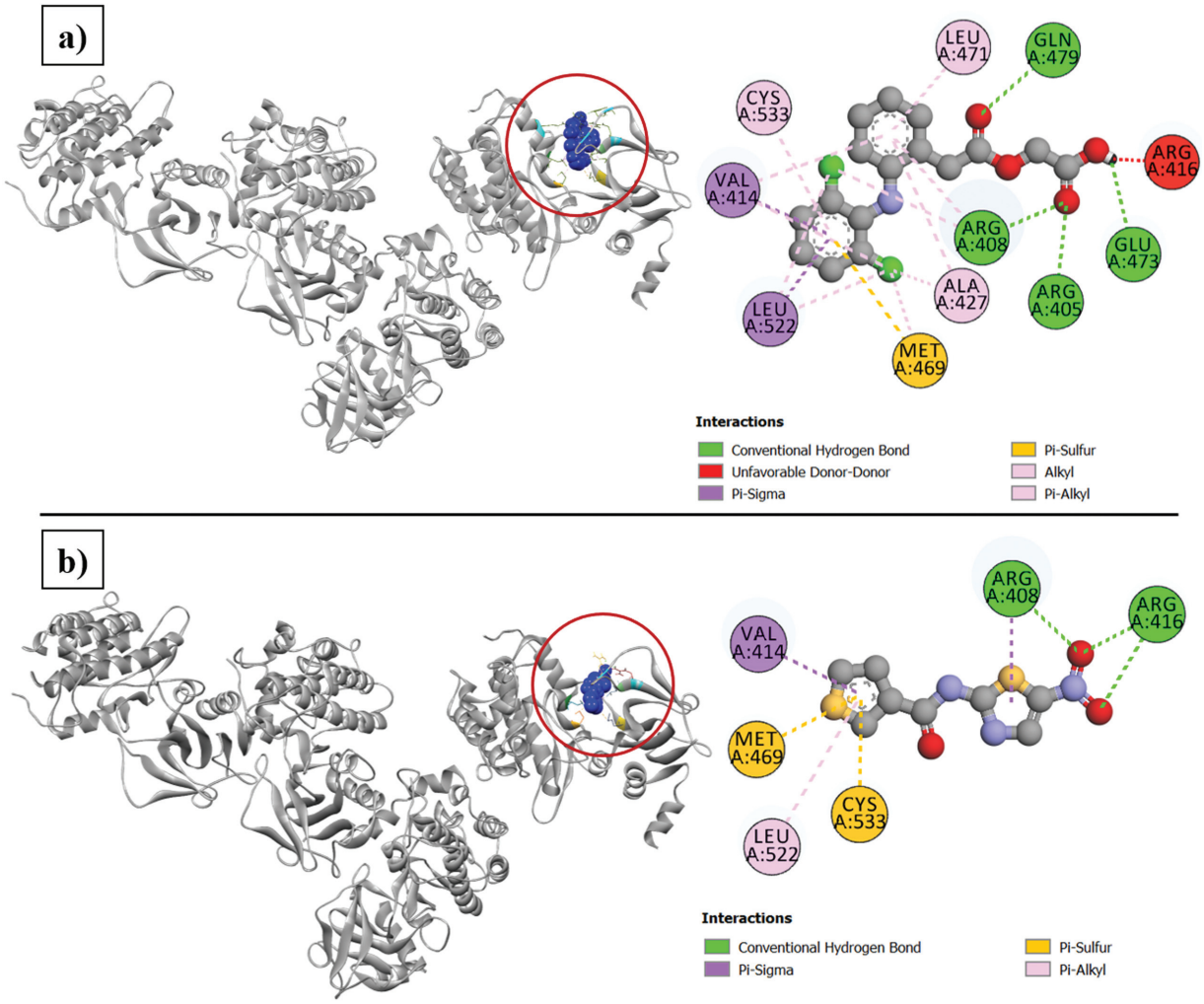
Two-dimensional and three-dimensional pictorial representation of key interactions between ligands and binding pocket of phospholipase A2 (PDB: 4UY1). (a) Aceclofenac with 4UY1; (b) Compound 3 with 4UY1.

of the NTZ analogs might arise from blockade of the biosynthesis of several mediators responsible for inflammatory reactions. The synthesized compounds might interact with several enzymes such as phospholipase A2, COX-1, COX-2, IRAK-4, etc. All of these enzymes possess a significant role in mediating inflammation and pain [33].

The synthesized compounds also demonstrated peripheral analgesic activity measured *via* acetic acid induced writhing inhibition method. Again, the furan carboxamide derivative 3 and 4 exhibited mild analgesic activity compared with the standard drug. This activity might be a consequence of the interaction of the compounds with the same enzymes stated above as we know that pain and inflammation usually occur via the same biochemical pathway resulting the synthesis of prostaglandin, thromboxane and leucotrienes [34,35].

To determine the molecular mechanism of action behind demonstrated anti-inflammatory and analgesic activity, we conducted docking simulations on COX-1, COX-2, phospholipase A2, NIK and IRAK-4 enzymes and proteins, and their docking scores were compared with their potent inhibitor aceclofenac. Firing threshold of nociceptors are sometimes directly stimulated due to inflammatory mediators such as prostaglandin, which are usually released during trauma. Inhibition of phospholipase A2, COX-1 and COX-2 enzymes thus play a significant role in inducing analgesic activity [36]. Similarly, NIK and IRAK-4 are also responsible for releasing various inflammatory mediators such as interleukin, which also contribute to inflammation. Our molecular docking simulation revealed that compound 4 had highest docking scores among the synthesized compounds against COX-1 and IRAK-4. It also had significantly strong binding affinities

Figure 5



Two-dimensional and three-dimensional pictorial representation of key interactions between ligands and binding pocket of NF-κB inducing kinase (NIK) (PDB: 4IDV). (a) Aceclofenac with 4IDV; (b) Compound 2 with 4IDV.

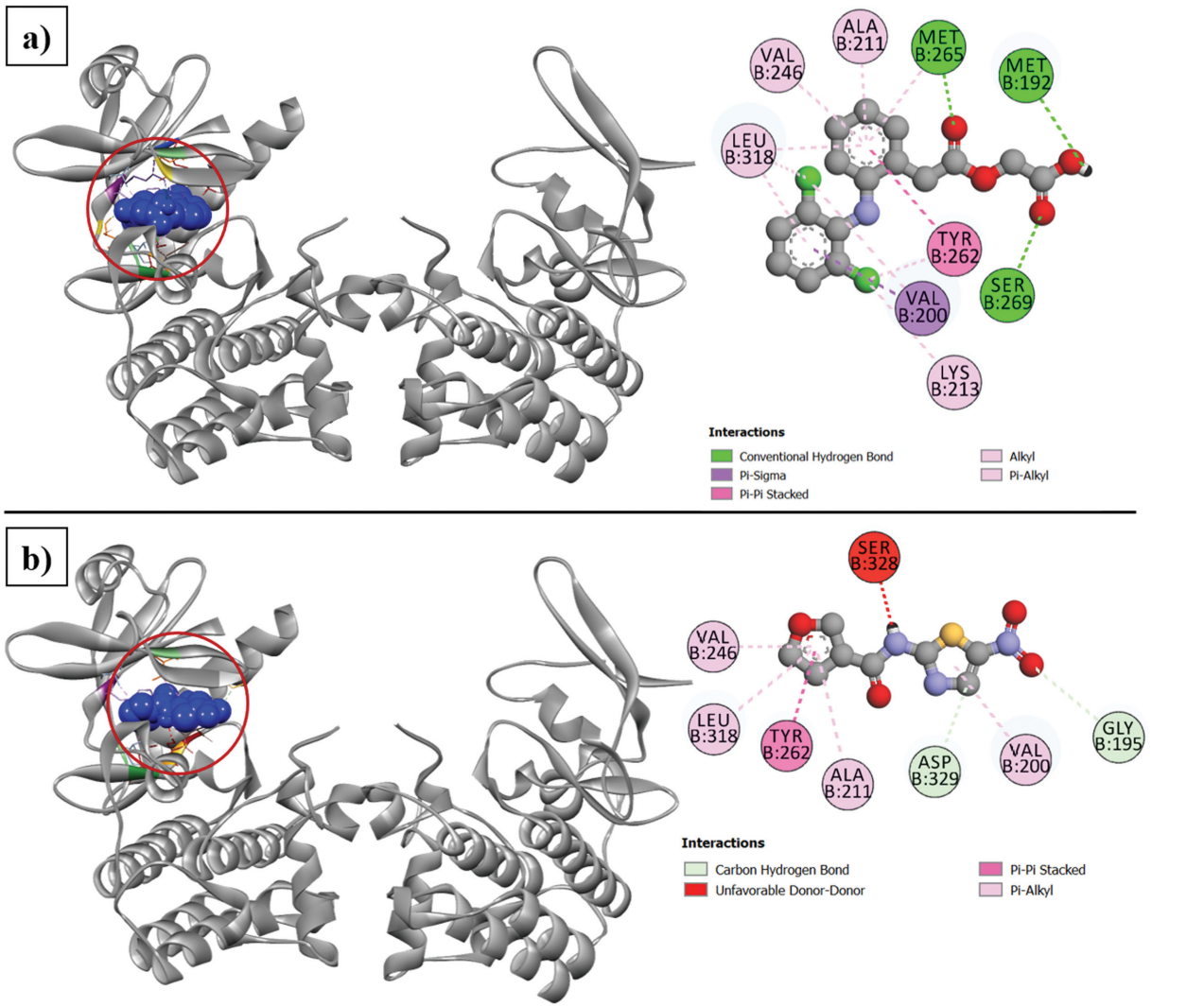
towards COX-2, phospholipase A2 and NIK. This is in accordance with the strong anti-inflammatory and analgesic property demonstrated by compound 4 in the *in vivo* assay. Compound 3 demonstrated strongest docking scores against COX-2 and phospholipase A2, which might contribute to the mild analgesic activity demonstrated by the compound.

We could not explore the antiviral testing of our analogs due to lack of laboratory facilities for antiviral testing and this can be considered as a limitation of this study. However, the present study signifies the synthesis of more analogs and especially furan-3-carboxamide analogs would be more interesting to explore further for discovering novel therapeutic agents with potent pharmacological activity such as antibacterial and anti-inflammatory activities.

Conclusion

Herein, we have reported the antibacterial, anti-inflammatory and peripheral analgesic properties of synthesized four thiophene and furan analogs namely, *N*-(5-nitrothiazol-2-yl)-thiophene-2-carboxamide, *N*-(5-nitrothiazol-2-yl)-thiophene-3-carboxamide, *N*-(5-nitrothiazol-2-yl)-furan-2-carboxamide and *N*-(5-nitrothiazol-2-yl)-furan-3-carboxamide. These analogs showed prominent pharmacological activities such as broad-spectrum antimicrobial, anti-inflammatory and mild analgesic activity. Among the synthesized compounds compound 4 having furan 3-carboxamide moiety showed most promising biological activities. Molecular docking simulation also revealed that compound 4 had strong binding affinity towards various target proteins and enzymes related to analgesic and anti-inflammatory activity. There is

Figure 6



Two-dimensional and three-dimensional pictorial representation of key interactions between ligands and binding pocket of interleukin-1 receptor-associated kinase 4 (IRAK-4) (PDB: 5KX7). (a) Aceclofenac with 5KX7; (b) Compound 4 with 5KX7.

potential scope for more synthetic analogs of nitazoxanide to be discovered with both on label (conventional) and off label activities and the research activity in this direction is underway.

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Conflicts of interest

Declaration of Competing Interest: The author declares that there is no conflict of interest.

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