

Piroxicam confer neuroprotection in Cerebral Ischemia by inhibiting Cyclooxygenases, Acid-Sensing Ion Channel-1a and Aquaporin-4: an *in silico* comparison with Aspirin and Nimesulide.

Muhammed Khairujaman Mazumder & Anupom Borah*

Received October 27, 2014; Accepted November 13, 2014; Published April 30, 2015

Cellular and Molecular Neurobiology Laboratory, Department of Life Science and Bioinformatics, Assam University, Silchar-788011, Assam, India; Anupom Borah - Email: anupomborahh@gmail.com; *Corresponding author

Abstract:

Cerebral ischemia (CI), caused by the deprivation of oxygen and glucose to the brain, is the leading cause of permanent disability. Neuronal demise in CI has been linked to several pathways which include cyclooxygenases (COX) - mediated production of prostaglandins (PGs) and subsequently reactive oxygen species (ROS), aquaporin-4 (AQ-4) - mediated brain edema and acid-sensing ion channel-1a (ASIC-1a) - mediated acidotoxicity, matrix remodeling, in addition to others. Several non-steroidal anti-inflammatory drugs (NSAIDs) are presently in use to prevent these pathways. However, owing to the large number of processes involved, there is high drug load. So, identifying drugs with multimodal role has always been a frequently sought venture. The present *in silico* study has been performed to find out the relative efficacy of three different NSAIDs (Piroxicam, Aspirin and Nimesulide) in preventing neurodegeneration in CI, with respect to their inhibitory potential on COXs, AQ-4 and ASIC-1a. We find that piroxicam is the most potent inhibitor of these receptors as compared to the NSAIDs under investigation. Since piroxicam has already been reported to inhibit N-methyl-D-aspartate (NMDA) receptor and matrix metalloproteinases (MMPs), which are also linked to CI-induced neurodegeneration, we hereby propose piroxicam to be a gold-standard drug in preventing neurodegeneration in CI.

Key Words: ASIC-1a, aquaporin-4, COXs, inflammation, neuroprotection, non-steroidal anti-inflammatory drug, Piroxicam

Background:

The pathological events involved in Cerebral ischemia (CI) include oxygen-glucose deprivation [1], inflammation [2], leukocyte infiltration, calcium overload and glutamate excitotoxicity that culminates in mitochondrial oxidative stress, apoptosis and neuronal cell death [3]. Hyperactivation of cyclooxygenase (COX) -1 and COX-2, which are constitutively expressed in brain, following CI increases the production of prostaglandins (PGs). PGs are further processed to produce several reactive oxygen species (ROS) including OH radicals. These molecules exaggerate the inflammatory cascade leading to neuronal demise [4]. In addition to COX, activation of

aquaporin-4 (AQ-4), the most common water channel of the brain, has been reported in CI, which leads to influx of water in the brain thereby causing brain edema [5, 6]. AQ-4 is thus regarded as a vital drug target against CI [5, 6]. Genetic deletion of AQ-4 has been found to prevent brain edema in CI [7]. Acid-sensing ion channel-1a (ASIC-1a), activation of which causes calcium overload into the neurons thereby eliciting excitotoxicity and acidotoxicity [6], is another target for preventing neurodegeneration in CI.

Non-steroidal anti-inflammatory drugs (NSAIDs) are known to exert their effect by inhibiting both COX-1 and COX-2 with

varying affinities [8], and therefore are among the most prescribed drugs for the treatment of inflammation and pain across the world. Several studies have reported the use of COX inhibitors (like nimesulide, aspirin) for the effective treatment of CI [9] and suggested that these inhibitors possess neuroprotective effect in addition to anti-inflammatory effect [10]. Piroxicam - which is also an NSAID - has been in use in clinics to treat rheumatoid arthritis, musculoskeletal disorders and postoperative pain. It was also reported that piroxicam was more effective than other NSAIDs such as ibuprofen or aspirin in preventing vascular abnormalities [11]. We previously reported that piroxicam can potentially inhibit matrix metalloproteinase 2/9 [12] and NMDA receptor [13]. However, the binding affinity and the interaction of piroxicam and the other two popular NSAIDs (Aspirin and Nimesulide) with COXs, AQ-4 and ASIC-1a, and its possible role in preventing neurodegeneration associated with CI are not yet reported. We performed a comparative study on inhibition of these receptors by the three NSAIDs.

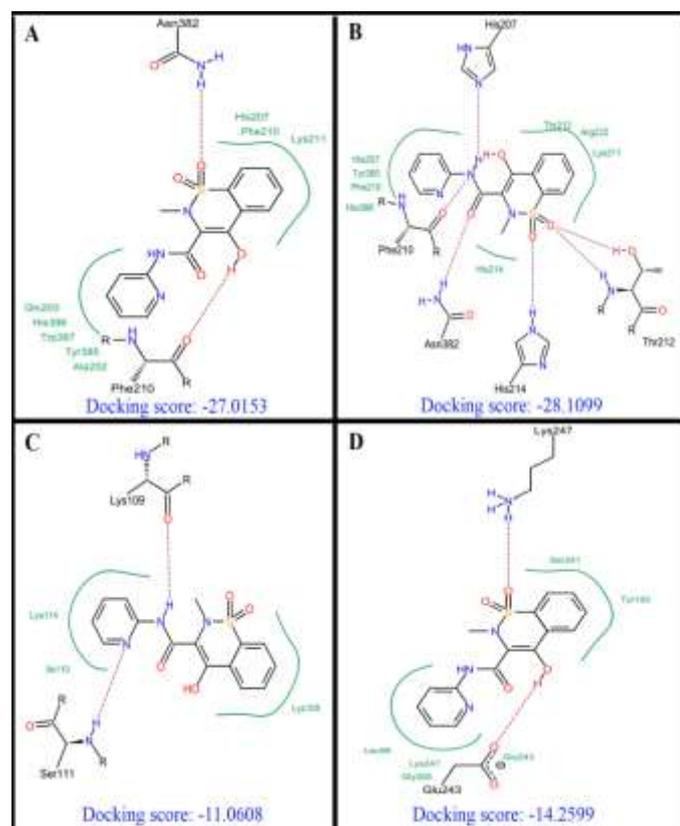


Figure 1: Docking pose of Piroxicam with (A) COX-1; (B) COX-2; (C) AQ-4 and (D) ASIC-1a, developed using FlexX. The dotted lines represent hydrogen bonds formed between the respective ligands and the receptors; the green lines represent weak interactions. Docking scores (free energy of binding), in kcal/mol, for each are shown against respective poses.

Methodology:

The Receptors

The 3-D structures of COX-1, COX-2, Aquaporin-4 and ASIC-1a were downloaded from Protein Data bank (<http://www.rcsb.org/pdb>) in .pdb format. We searched for 3-D structures of the receptors, and have selected one best fit

structure of each based on the completeness of the structure, bound ligand and the resolution.

The Ligands

The structures of Aspirin, Nimesulide, Piroxicam and Arachidonic acid (AA) were downloaded from NCBI PubChem Compounds (<http://www.ncbi.nlm.nih.gov/pccompound>) in .sdf format. The structures of ethoxolamide and hydroxamic acid were available with the respective receptors - AQ-4 and ASIC-1a respectively, and were used for reference of binding site and comparison of inhibitory potential, while AA - the natural substrate of COXs - was used for the same purpose in case of COXs.

Molecular Docking

Flips, rotations and protonations in the receptor molecules were corrected before docking by using FlexX, following Mazumder *et al.* [12]. The enzymes were docked using FlexX with their natural substrate and/or known inhibitors as well as with the NSAIDs under investigation. Known inhibitors of the receptors were used for reference of binding sites; and amino acids within a region of 20Å were included in the simulation following Mazumder and Borah [13]. The best poses, in terms of free energy of binding, were compared following Mazumder *et al.* [12, 14].

Results:

Interactions of ligands with COX-1: A total of 2 hydrogen bonds and 19 weak interactions are being formed between AA and COX-1. Aspirin forms 2 hydrogen bonds and 3 weak interactions; nimesulide forms 3 hydrogen bonds and 6 weak interactions (**Supplementary Figure 1**) while piroxicam forms 2 hydrogen bonds and 8 weak interactions (**Figure 1A**).

Interactions of ligands with COX-2: AA forms 1 hydrogen bond and 13 weak interactions with COX-2; aspirin forms two hydrogen bonds and five weak interactions; nimesulide forms four hydrogen bonds and seven weak interactions (**Supplementary Figure 2**); while piroxicam forms six hydrogen bonds and eight weak interactions (**Figure 1B**).

Interactions of Aquaporin-4: With the inhibitor ethoxolamide, AQ-4 forms 2 hydrogen bonds and 2 weak interactions; with aspirin, it forms 1 hydrogen bond and 3 weak interactions; with nimesulide, 1 hydrogen bond and 5 weak interactions are formed (**Supplementary Figure 3**); while with piroxicam, 2 hydrogen bonds and 3 weak interactions (**Figure 1C**).

Interactions of ligands with ASIC-1a: The ASIC-1a inhibitor - sinomenine - forms 3 hydrogen bonds and 4 weak interactions with the receptor; aspirin forms 4 hydrogen bonds and 3 weak interactions; nimesulide forms 2 hydrogen bonds and 3 weak interactions (**Supplementary Figure 4**); while piroxicam forms 2 hydrogen bonds and 6 weak interactions (**Figure 1D**).

Inhibition of the receptor activities

From our study, we find that piroxicam has highest free energy of binding with both COX-1 and COX-2 as well as with Aquaporin -4 as compared to the other NSAIDs in question and the natural substrate AA. In case of ASIC-1a, aspirin shows highest free energy of binding and Piroxicam has the second highest score **Table 1** (see **supplementary material**).

When a ligand binds with the active site of a receptor with higher free energy of binding as compared to the natural

substrate and/or other inhibitors, the activity of the receptor is inhibited [12, 14].

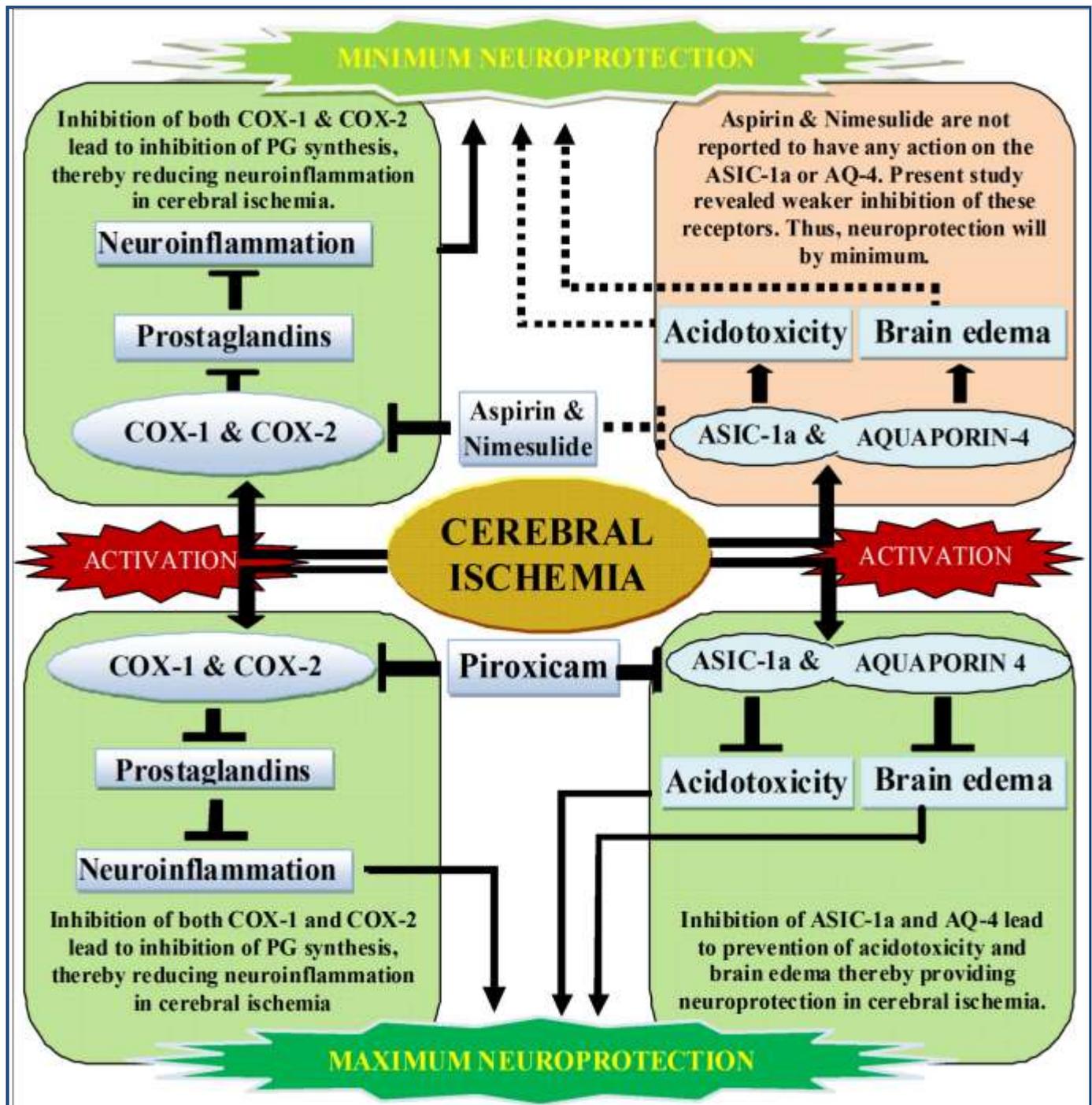


Figure 2: Different pathways leading to neurodegeneration in CI and the differential role of aspirin, nimesulide and piroxicam in neuroprotection. CI induces expression and activation of COX-1, COX-2, AQ-4 and ASIC-1a. Although Aspirin and Nimesulide are inhibitors of COXs, Piroxicam is a better inhibitor of all the four receptors and thus will provide more neuroprotection in CI. There is no report of the inhibition of AQ-4 by Nimesulide and Aspirin, and the present study revealed weaker inhibition (denoted by dotted lines) of the receptors by the two drugs. Thus, Piroxicam is the most potent drug in preventing neurodegeneration in CI, owing to its multi-modal action.

Discussion:

From our study, we find that the active site residues of COX-1 and COX-2 with which piroxicam interacts are those which were reported to be responsible for catalytic activity of COX-1 [15] and COX-2 [16]. The catalytic domain of COX-1 and COX-

2 comprises of the residues 117-587 [17] with which piroxicam, aspirin and nimesulide have been found to interact directly (Figure 1, Supplementary Figure 1 & 2 (see supplementary material)). This suggests that the binding sites are similar as reported for other drugs, thereby bringing about similar mode

of inhibition. The finding that piroxicam can potentially inhibit both COX-1 and COX-2; and has higher affinity for both as compared to nimesulide and aspirin is attributed to the presence of more number of interacting groups in piroxicam. Although, aspirin and piroxicam form same number of hydrogen bonds, piroxicam forms more number of weak interactions with COX-1 (**Figure 1; supplementary Figure 1 (see supplementary material)**); while forming hydrogen bonds and weak interactions with more number of residues of COX-2 (**Figure 1**) than the other two NSAIDs shown in **supplementary Figure 2 (see Supplementary material)**, thereby showing greatest affinity for both COX-1 and COX-2, and potentially interacting with the residues in the receptor which are involved in catalytic activity [17]. As aspirin is a smaller molecule, it showed lowest affinity and lesser interactions with both COXs (**supplementary Figure 1 & 2 (see supplementary material)**). Thus, it may be concluded that piroxicam - by virtue of its larger size and more interacting groups - can interact more efficiently with COXs than aspirin and nimesulide.

Our finding that piroxicam has better ability to inhibit AQ-4 and ASIC-1a, compared to the other NSAIDs (except for Aspirin with ASIC-1a), is of immense importance, since these channels are known to be associated with the pathophysiology of CI [5, 6]. Similar to the AQ-4 inhibitor- acetazolamide [18] (**supplementary Figure 3 (see supplementary material)**), piroxicam forms hydrogen bonds and weak interactions. With ASIC-1a, aspirin shows a bit more affinity but piroxicam shows more affinity than the others, including sinomenine (**Supplementary Figure 4 (see supplementary material)**) [19]. While our study confirms the findings of Bhattacharya *et al.* [20] who previously reported inhibition of AQ-4 and ASIC-1a by Piroxicam, we report for the first time a comparative study of inhibition of these receptors by the three NSAIDs. We also report that piroxicam has better ability to inhibit both COX-1 and COX-2, AQ-4 and ASIC-1a (**Table 1 (see supplementary material)**), and the mode of inhibition of the receptors is similar to their known inhibitors involving same residues. Thus our findings elucidated a novel mechanism of neuroprotection in CI by piroxicam as compared to other NSAIDs. Therefore, piroxicam may be a drug of choice to counter neurodegeneration in CI (**Figure 2**).

Conclusion:

Our study confirms that piroxicam may confer neuroprotection by multiple mechanisms, owing to its unique property - unlike other NSAIDs - to prevent brain edema and acidotoxicity, in addition to inflammation; and thus may be the drug of choice to prevent neurodegeneration in CI (**Figure 2**). In addition, as the intrinsic causes of most of the neurodegenerative disorders are neuroinflammation, oxidative stress, acidotoxicity and excitotoxicity; piroxicam may be a potential drug in these conditions as well. Since CI has become

a major global health issue, our finding is of immense significance.

Acknowledgement:

We thank the Bioinformatics Centre, Assam University, Silchar (INDIA) for software (under DBT-BIF) and e-journal (under DBT-DeLCON) facilities. MKM is recipient of Maulana Azad National Senior Research Fellowship (MAN-SRF) from University Grants Commission (Govt. of India).

Author's Contribution:

MKM performed the molecular docking and contributed to the acquisition, assessment and interpretation of data, and drafting the MS. AB contributed to the overall design and conception of idea, and final approval of the MS.

Conflict of interest: None declared.

References:

- [1] del Zoppo GJ, *Cerebrovasc Dis.* 2009 **27**: 65 [PMID: 19342834]
- [2] Brea D *et al.* *Cerebrovasc Dis.* 2009 **27**: 48 [PMID: 19342833]
- [3] Kato H & Kogure K, *Cell Mol Neurobiol.* 1999 **19**: 93 [PMID: 10079969]
- [4] Candelario-Jalil E *et al.* *J Neurochem.* 2003 **86**: 545 [PMID: 12859668]
- [5] Papadopoulos MC & Verkman AS, *Pediatr Nephrol.* 2007 **22**: 778 [PMID: 17347837]
- [6] Pignataro G *et al.* *Brain.* 2007 **130**: 151 [PMID: 17114797]
- [7] Manley GT *et al.* *Nat Med.* 2000 **6**: 159 [PMID: 10655103]
- [8] Patrono C & Rocca B, *Pharmacol Res.* 2009 **59**: 285 [PMID: 19416627]
- [9] Sacco RL *et al.* *Lancet.* 2007 **369**: 331 [PMID: 17258673]
- [10] Hurlley SD *et al.* *J Neurotraum.* 2002 **19**: 1 [PMID: 11852973]
- [11] White RP & Robertson JT, *Neurosurg.* 1983 **12**: 40 [PMID: 6338410]
- [12] Mazumder MK *et al.* *Med Hypotheses.* 2014 **83**: 697 [PMID: 25459137]
- [13] Mazumder MK & Borah A, *Med Hypotheses.* 2014 **83**: 740 [PMID: 25459147]
- [14] Mazumder MK *et al.* *CNS Neurosci Therap.* 2013 **19**: 596 [PMID: 23638910]
- [15] Thuresson ED *et al.* *J Biol Chem.* 2001 **276**: 10347 [PMID: 11121412]
- [16] Rowlinson SW *et al.* *J Biol Chem.* 1999 **274**: 23305 [PMID: 10438506]
- [17] Gierse JK *et al.* *J Biol Chem.* 1996 **271**: 15810 [PMID: 8663121]
- [18] Wu WN *et al.* *Brit J Pharmacol.* 2011 **164**: 1445 [PMID: 21585344]
- [19] Huber VJ *et al.* *Bioorg Med Chem Lett.* 2007 **17**: 1270 [PMID: 17178220]
- [20] Bhattacharya P *et al.* *Med Hypotheses.* 2012 **79**: 352 [PMID: 22795752]

Edited by P Kanguane

Citation: Mazumder & Borah, *Bioinformation* 11(4): 217-222 (2015)

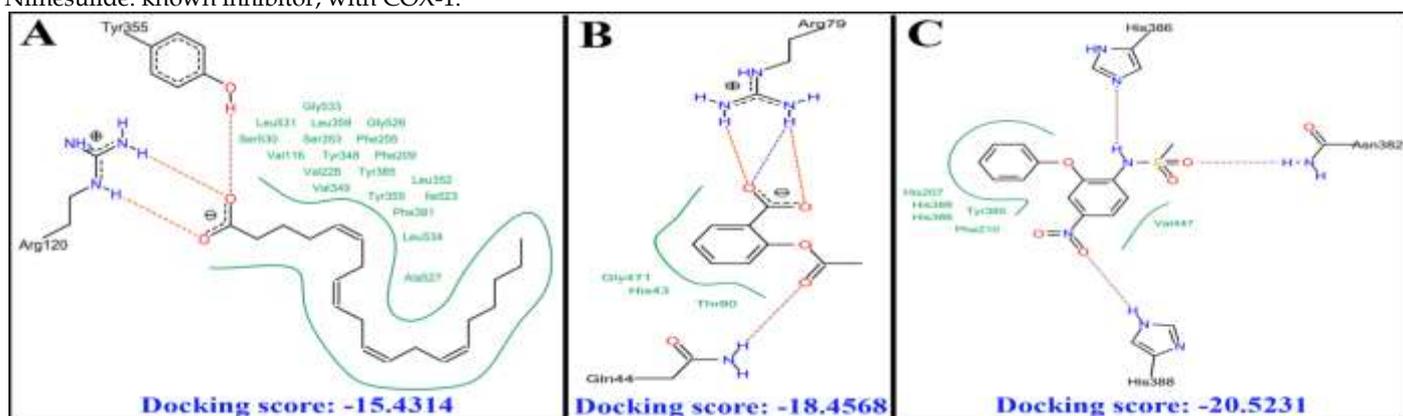
License statement: This is an open-access article, which permits unrestricted use, distribution, and reproduction in any medium, for non-commercial purposes, provided the original author and source are credited

Supplementary material:

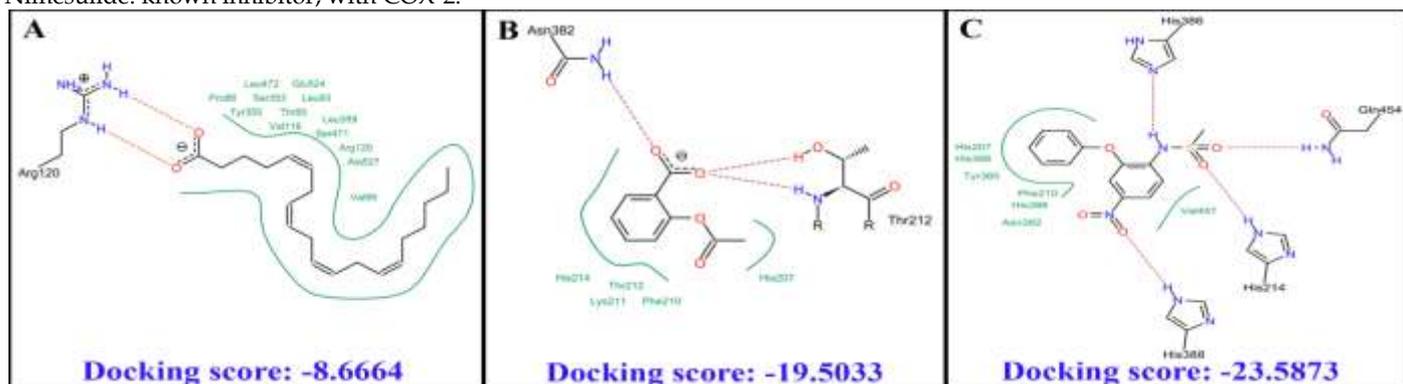
Table 1: Docking scores of different ligands with the receptors. Scores were determined using LeadIT (FlexX). AA: Arachidonic acid the natural substrate of the COXs, is used as reference ligand. Both Ethoxolamide and Sinomenine are known inhibitors of AQ-4 and ASIC-1a respectively and are used as reference ligands.

Receptors	Docking scores with different ligands (in kcal/mol)			
	Reference Ligand	Aspirin	Nimesulide	Piroxicam
COX-1	AA: -15.4314	-18.4568	-20.5231	-27.0153
COX-2	AA: -8.6664	-19.5033	-23.5873	-28.1099
AQ-4	Ethoxolamide: -8.0418	-6.6465	-9.3964	-11.0608
ASIC-1a	Sinomenine: -13.9839	-16.0001	-12.2049	-14.2599

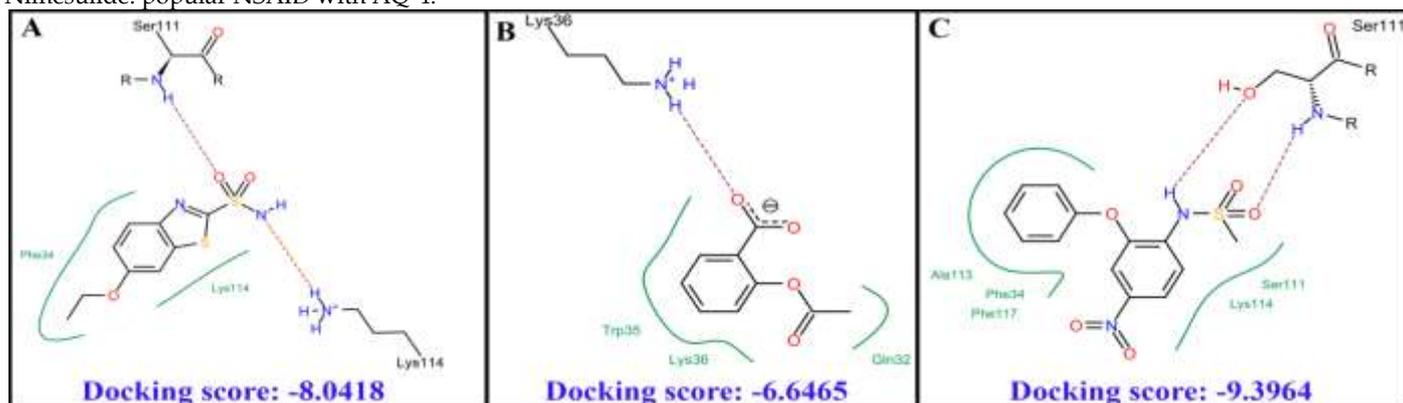
Supplementary Figure 1: Docking poses of (A) arachidonic acid: the natural substrate, (B) Aspirin: known inhibitor and (C) Nimesulide: known inhibitor, with COX-1.



Supplementary Figure 2: Docking poses of (A) arachidonic acid: the natural substrate, (B) Aspirin: known inhibitor and (C) Nimesulide: known inhibitor, with COX-2.



Supplementary Figure 3: Docking poses of (A) Ethoxolamide: the natural inhibitor, (B) Aspirin: popular NSAID and (C) Nimesulide: popular NSAID with AQ-4.



Supplementary Figure 4: Docking poses of (A) Sinomenine: the natural inhibitor, (B) Aspirin: popular NSAID and (C) Nimesulide: popular NSAID with ASIC-1a.

