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Analgesic, anti-inflammatory activity and docking study of 2-(substituted phenyl)-3-(naphthalen-1-yl)thiazolidin-4-ones

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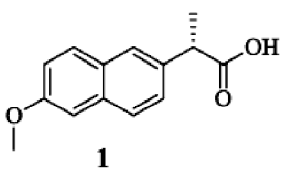
A potential analgesic and anti-inflammatory activities have been reported in 4-thiazolidinone analogs as well as some drugs bearing naphthyl moiety such as naproxen. Thus a reported series of 2-(substituted phenyl)-3-(naphthalen-1-yl)thiazolidin-4-ones (Tz_{1-10}) was explored for analgesic and anti-inflammatory activities. The analgesic activity was determined using tail immersion and acetic acid-induced writhing model while the anti-inflammatory activity was determined using carageenan-induced paw edema method. The docking studies were also carried out to gain understanding of binding modes of the potent compounds in the COX-2 enzyme active cavity. The compounds Tz_1, Tz_5 and Tz_6 emerged as potent analgesic and anti-inflammatory agents. The compounds displayed excellent binding interaction with the receptor with Tz_6 showing the highest binding energy (–11.08 kcal/mol). Moreover, the synthesized compounds are predicted to have good oral bioavailability as shown by their physicochemical properties calculated using software. The naphthalenyl substituted thiazolidinones reported in the present study provide basis for further studies and future prospects for development of new anti-inflammatory and analgesic agents.

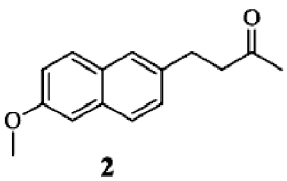
Keywords: 4-Thiazolidinone, analgesic, anti-inflammatory, naphthylamine, Schiff's base.

Introduction

Non-steroidal anti-inflammatory agents (NSAIDs) are the important type of compounds possessing analgesic, antipyretic and anti-inflammatory activities. Generally, NSAIDs work by inhibiting cyclooxygenases (COX-1 and COX-2), thereby reducing the production of prostaglandins and thromboxanes. But the inhibition of COX-1 leads to serious complications like gastrointestinal pain, bleeding, kidney problems and CNS effects. Thus the COX-2 selective inhibitors such as rofecoxib, celecoxib and valdecoxib are highly effective as anti-inflammatory agents[1–3] but they cause myocardial infarctions. The other important types of NSAIDs are naphthalene derivatives such as naproxen (**1**), nabumetone (**2**) and other aroyl propionic acid[4] but they are nonspecific in action and have reported gastrointestinal problems. The literature reveals that the substitutions at the α-position[5] and β-position[6,7] of the naphthalene moiety significantly affects anti-inflammatory activity. Further the thiazolidinone analogs also exhibit remarkable anti-inflammatory and analgesic[8–10] activities. The compounds containing a combination of naphthalene and thiazolidinone moiety had never been studied for analgesic and anti-inflammatory activity. Thus in the present study, it was thought to explore the derivatives possessing thiazolidinone and naphthalene moiety to find more effective analgesic and anti-inflammatory agents than the existing drugs.



1
Naproxen



2
Nabumetone

In the light of this fact, in the present study, ten reported naphthalene derivatives possessing substituted

thiazolidinone moiety at α-position were screened for analgesic activity and anti-inflammatory activity. This reported series has been previously synthesized and evaluated for antidiabetic activity by our lab[11]. The potent analgesic and anti-inflammatory derivatives were docked on COX-2 target to find binding interactions and comparing their binding mode with the related drug naproxen. The naphthalene moiety of naproxen and reported compounds fits well in the hydrophobic cavity of COX-2 enzyme. Furthermore, oral bioavailability and drug likeness of said compounds has also been predicted by computing physicochemical properties.

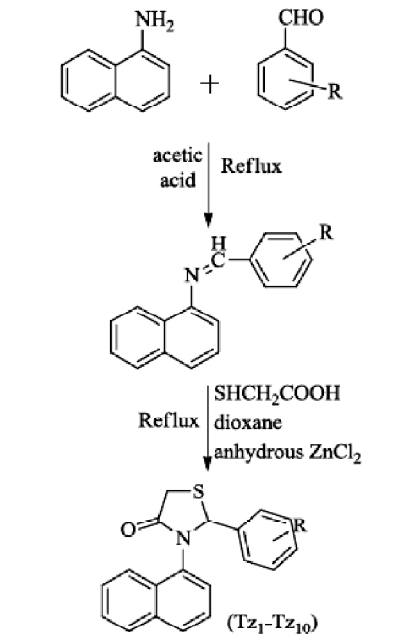


Fig. 1. Scheme of synthesis for compounds Tz_1-Tz_{10}.

Material and methods:

Experimental procedures:

The synthetic scheme and structures of the title compounds are presented in Fig. 1, the procedure of which has been previously reported by our lab[11].

Biological activity:

Experimental animals:

Wistar rats (100–150 g) and mice (20–30 g) were acquired and used in the research from Animal House of Institute of Pharmaceutical Research, GLA University, Mathura. Animals were maintained under standard environmental conditions, i.e. temperature of 25±1°C, relative humidity of 45–55%, and 12 h light and 12 h dark cycle. They were fed with standard pellet diet and water was supplied *ad libitum*. All the experimental processes used have been carried out under the strict complying rules of CPCSEA under the authorization of the Institutional Animal Ethics Committee (GLAIPR/CPCSEA/IAEC/2013/P.Chem/5).

Analgesic activity:

In the present work, the central and the peripheral analgesic activity of the synthesized compounds were determined using the tail immersion method and acetic acid-induced writhing method in mice, respectively. The mice were divided into 12 groups (control, standard, 10 test groups (Tz_1-Tz_{10})), comprising 6 in each group, for the study.

Tail immersion method:

The study protocol was conducted according to the method explained by Shah and Alagawadi[12]. Freshwater was filled in a beaker and heated to 55±1°C. The standard drug Pentazocine (35 mg/kg b.w.) and test compounds (100 mg/kg b.w.) were suspended in 0.5% CMC and administered orally to mice. The control group received only vehicle[13]. A mark was made above the lower 5 cm portion of the tail and the tail was immersed up to that mark in the water maintained at 55±1°C. The mice react within a few seconds by moving back the tail which was noted. The immersion cut off time was 15 s. This reaction time was determined before and at regular intervals after 0.5, 1, 2, 3 and 4 h of oral administration of the test compounds and the standard drug.

Acetic acid-induced writhing method:

The standard drug, Diclofenac sodium (25 mg/kg b.w.) and test compounds (100 mg/kg b.w.) were suspended in 0.5% CMC and administered orally to mice 30 min prior to acetic acid administration. The control group received vehicle only. The 0.6% acetic acid (10 mL/kg; i.p.) was administered, after the 30 min of oral administration of standard drug and test compounds to the respective group mice. For 5–15 min of acetic acid injection, stretching movements con-

sisting of arching of the back, elongation of body and extension of hind limbs were counted. The analgesic activity is expressed in terms of percentage inhibition[14,15] that was calculated using the following formula:

% Inhibition =

100 – [(Experimental writh count/control writh count)×100]

Anti-inflammatory activity:

In the present work, the anti-inflammatory activity was evaluated using carrageenan-induced paw edema method in Wistar rats of either sex (100–150 g) using plethysmograph according to Winter's method[16]. The rats were divided into twelve groups (control, standard and test groups (Tz_1-Tz_{10})), comprising six rats in each group for the study. A 1% w/v suspension of carrageenan was freshly prepared in 0.9% w/v NaCl. The standard drug, Diclofenac sodium (25 mg/kg b.w.) and test compounds (100 mg/kg b.w.) were suspended in 0.5% CMC and administered orally to rats 30 min prior to carrageenan injection. The control group received only vehicle. The 0.1 mL of carrageenan suspension was injected into left hind paw sub-plantar region, 30 min after the oral administration of standard drug and test compounds to the respective group rats. The ankle joint of left paw of each experimental animal was marked to ensure constant paw volume. The paw volumes of animals of each group were measured up to the fixed mark made, at 30 min, 1 h, 2 h, and 3 h after the administration of carrageenan. The difference between the paw volumes of drug-treated animals and that of the control group was calculated and the mean edema volume was calculated. The percent inhibition of edema was calculated as compared to the control using the following formula:

% Inhibition of oedema = $(V_c - V_t/V_c)\times100$

where, V_t = paw volume of drug-treated animals, V_c = paw volume of control group.

Statistical analysis: The data is expressed as mean±standard deviation. The statistical analysis was performed using one-way ANOVA followed by Dunnet's test and $p < 0.05$ was considered significant.

Molecular docking study:

The *in silico* molecular docking of the reported naphthylthiazolidinone derivatives having analgesic and anti-inflammatory activity was performed against human cyclooxygenase-2 (COX-2) enzyme to understand the binding interactions. The X-ray crystal structure of human COX-2 enzyme (PDB ID-5IKR; resolution-2.342 Å) bound with mefenamic acid was obtained[17] from the RCSB protein data bank (http://www.rcsb.org/pdb).

The structural model of COX-2 enzyme is a dimer of polypeptide chains having 551 amino acids and bound ligand mefenamic acid in each chain. Out of the two chains, Chain A was utilized in the current study. The bound ligand mefenamic acid was removed from the active binding site of the protein model using Chimera and redocked using AutoDock program suite (http://autodock.scripps.edu) to validate molecular docking simulation of human COX-2. Similar docking parameters were used to perform molecular docking of ten thiazolidinone derivatives (Tz_1-Tz_{10}) and naproxen.

Results and discussion

In this study, the compounds from the previously reported series of naphthalene containing thiazolidin-4-one moiety possessing substituted phenyl ring at 2-position were assessed for their analgesic and anti-inflammatory activity.

Analgesic activity:

The central analgesic activity of the synthesized compounds was determined using the tail immersion method. The withdrawal of the tail from hot water (55±1°C) was taken as the reaction against painful stimuli. The compounds Tz_1, Tz_5, and Tz_6 showed excellent central analgesic activity (Fig. 2) as indicated by increase in the reaction time. For peripheral analgesic activity, acetic acid-induced writhing model in mice was used. The analgesic activity was expressed in terms of percentage inhibition of writhes count (Fig. 3). The compounds Tz_1, Tz_5, and Tz_6 were found to be effective in limiting the stretching movements induced by acetic acid. Thus the results were parallel to the central analgesic activity.

Anti-inflammatory activity:

For anti-inflammatory activity, the carrageenan-induced paw edema model in rats was used for screening of synthesized compounds. The paw volumes of rats from control, standard, and test groups were measured at 0.5, 1, 2, and 3

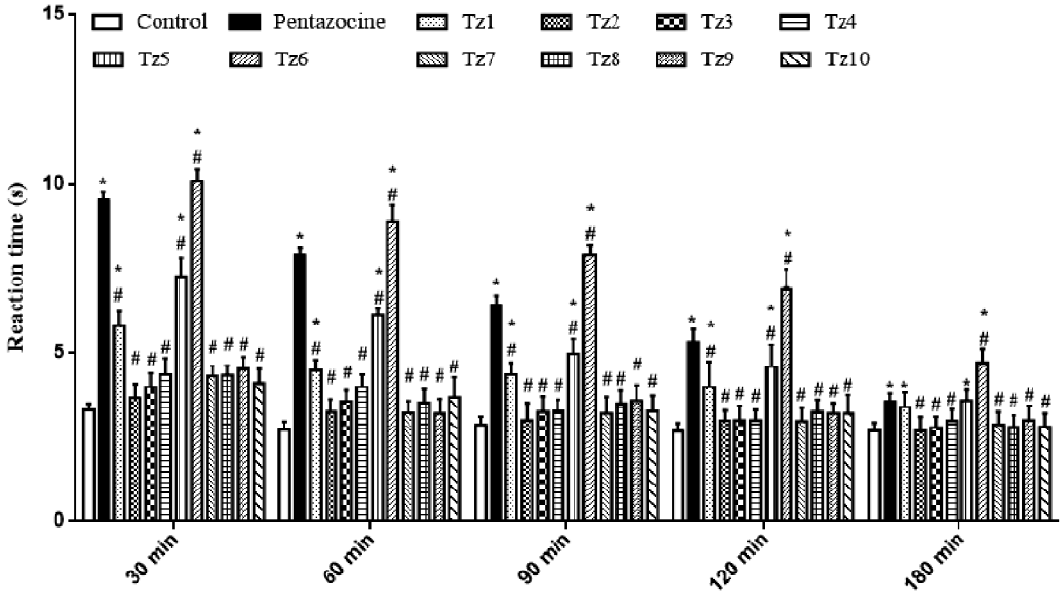


Fig. 2. Central analgesic activity of test compounds (100 mg/kg b.w.) and pentazocine (35 mg/kg b.w.) using tail immersion method, * represents $p < 0.05$ as compared to control, # represents $p < 0.05$ as compared to pentazocine.

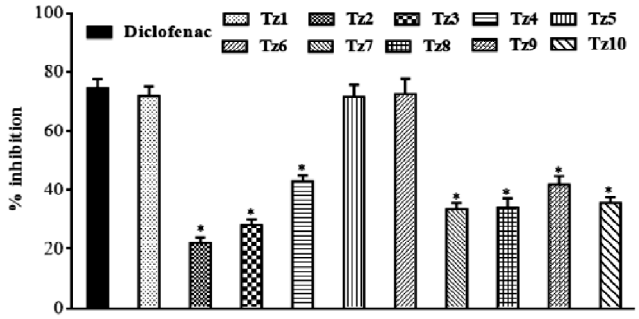


Fig. 3. Peripheral analgesic activity of test compounds and Diclofenac sodium using acetic acid induced Writhing method (% inhibition are expressed as mean ± S.D, * = $p < 0.0001$ vs standard drug (Diclofenac)).

h after the administration of carrageenan plethysmographically. The results are expressed as percentage inhibition of the edema. For all the compounds maximum edema inhibition was observed after 2 h. Fig. 4 indicates that among all the compounds, Tz_5 and Tz_6 showed excellent anti-inflammatory activity while compounds Tz_1, and Tz_8 showed moderate activity.

The closer examination of structures of potent compounds shows that these compounds are having an electron withdrawing group i.e. nitro, fluoro or chloro on phenyl ring attached to 2nd position of thiazolidinone ring system. Thus for structure-activity relationship, the presence of electron-withdrawing groups on phenyl ring attached to 2nd position of thiazolidinone ring system is favourable for the analgesic and anti-inflammatory activity. Thus this study opens the gateway to explore more such substituents taking this lead molecule for development of novel and more potent analgesic and anti-inflammatory agents.

Molecular docking study:

To gain further insight into the binding interaction of the potent compounds with the target enzyme for analgesic and anti-inflammatory activities, molecular docking studies were performed. The molecular docking simulation process for human COX-2 enzyme was validated by redocking the bound ligand mefenamic acid to the active binding site of the protein. The mefenamic acid successfully fulfills the desired criteria for the validation of molecular docking like chemical resemblance and similar overlay as that of the bioactive conformation with the binding energy in the given range. The docking of these compounds revealed that they can occupy the same binding site as co-crystallized ligand (mefenamic

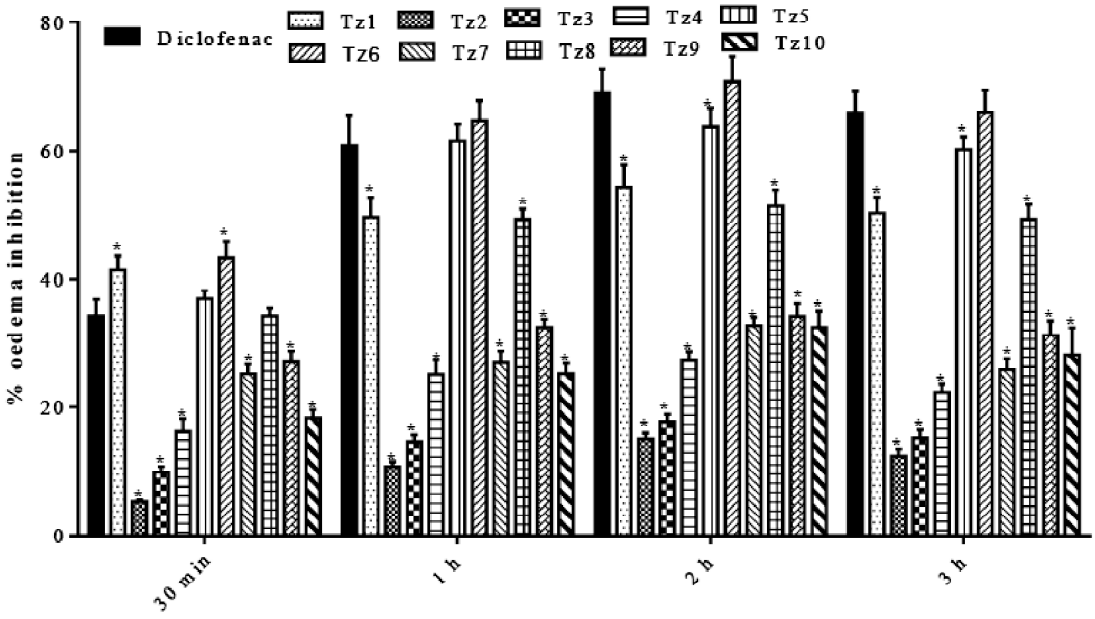


Fig. 4. Anti-inflammatory activity of test compounds and Diclofenac sodium using carrageenan induced paw edema method (% inhibition are expressed as mean ± S.D, * = $p < 0.0001$ vs standard drug (Diclofenac)).

acid) and their naphthalene moiety is orientated in the hydrophobic cavity of the COX-2 enzyme similarly as that of naproxen. The compounds displayed significant docking score and the highest binding energy was observed for Tz_6 (–11.08 kcal/mol) which was significantly better than that of the naproxen (–7.55 kcal/mol). Thus the mechanism of action of the compounds could be COX-2 inhibition.

The potent compounds Tz_1, Tz_5 and Tz_6 were further analyzed for binding modes in the receptor cavity (Fig. 5) to understand the nature and type of interactions between protein and ligand. The naphthalene moiety was found to be embedded in the hydrophobic pocket of the COX-2 enzyme as that of naproxen (Fig. 6). The naphthalene moiety of ligand Tz_1 (–9.86 kcal/mol) was involved in pi-sigma interactions with Ala527 and Val349 residues and in pi-alkyl interactions with Leu531 and Leu352 residues. The other important interactions of this ligand were pi-sulphur interaction with amino acid Trp387, pi-alkyl interactions of chloro with Tyr348, Val344 and Phe205 residues. The naphthalene moiety of ligand Tz_5 (–9.15 kcal/mol) was found to have pi-sigma interactions with Ala527 and Val349, pi-alkyl interactions with Leu531 and Leu352. The thiazolidinone sulphur participated in pi-sulphur interaction with Trp387 and Phe381 residues. The docking pose of compound Tz_6 (–11.08 kcal/mol) in the enzyme cavity revealed the presence of three important hydrogen bonding interaction of nitro group with Ser353, His90 and Arg513 residues. Both the rings of naphthalene moiety were involved in pi-sigma interaction with Leu352, pi-alkyl interactions with Val349 and Ala527 residues and pi-amide interaction with Gly526 residue.

In silico ADME study:

Poor pharmacokinetic properties are one of the main reasons why drug candidates have failed to develop in the early stages. Thus in the present study, *in silico* ADME properties and drug-likeness for the compounds Tz_1-Tz_{10} were predicted using MedChem Designer 3.0 (http://www.simulations-plus.com). The values of predicted parameters are present in Table 1. According to this data, none of the compounds (Tz_1-Tz_{10}) is having more than one violation of Lipinski's rule (Lipinski *et al.*, 2012). Thus the reported compounds are predicted to have good oral bioavailability.

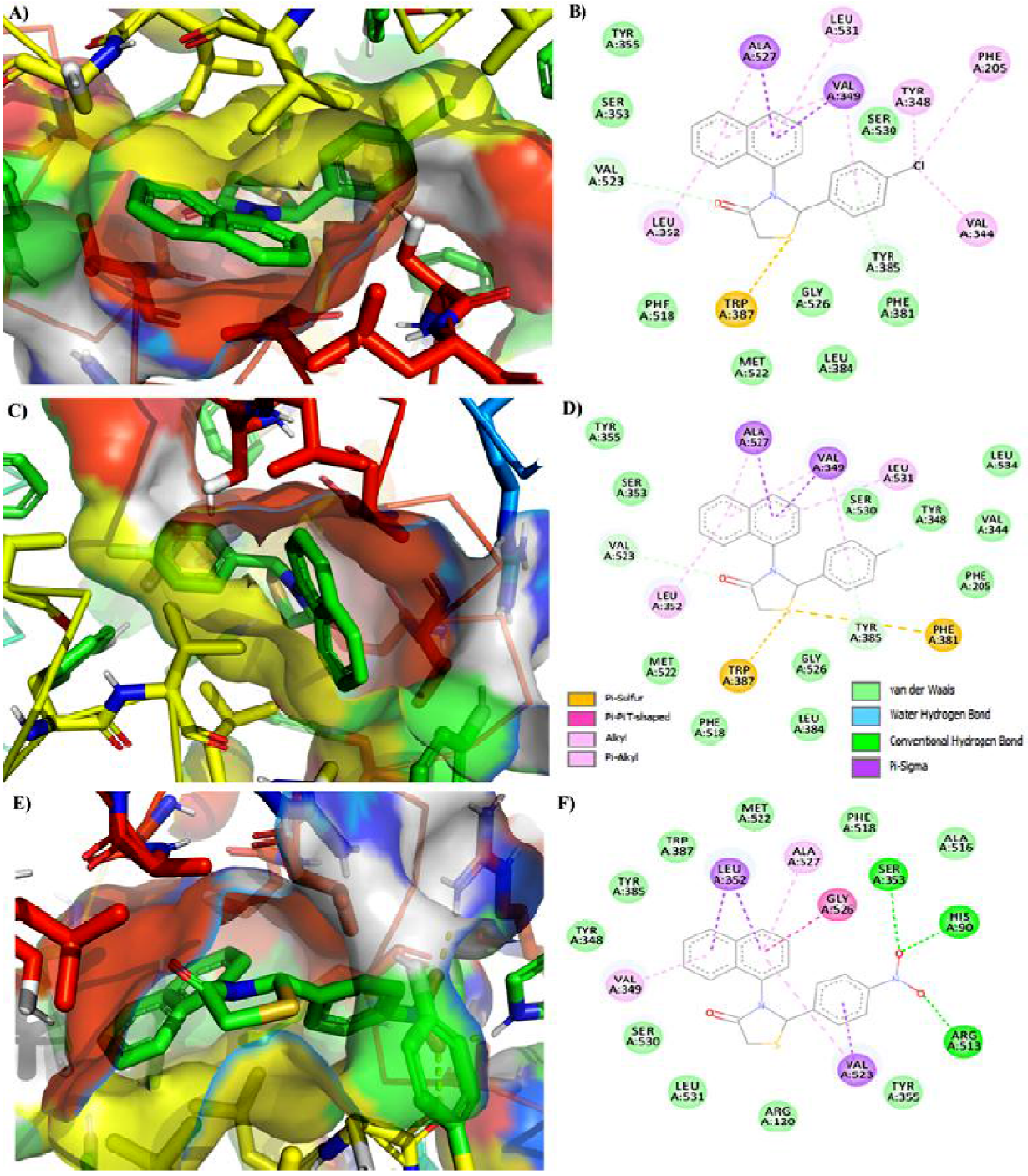


Fig. 5. Binding mode of compounds Tz_1 (A), Tz_5 (C) and Tz_6 (E) in the COX-2 binding pocket. 2D binding interactions of compounds Tz_1 (B), Tz_5 (D) and Tz_6 (F) with the active site of COX-2.

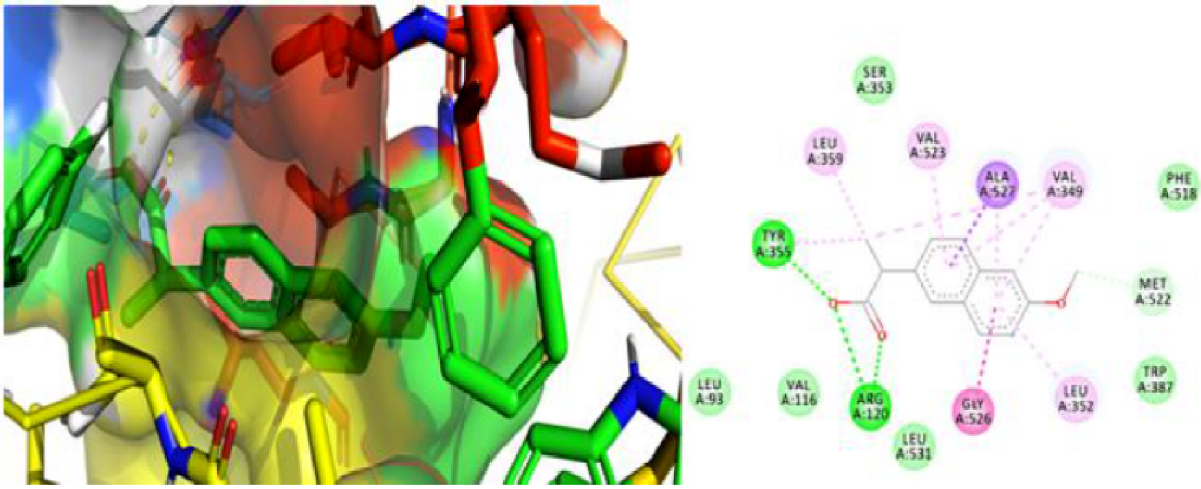


Fig. 6. 3D and 2D binding mode of naproxen in the COX-2 active pocket displaying orientation in cavity and various interactions with amino acid residues, respectively.

Table 1. Calculated ADME properties of compounds Tz_1-Tz_{10}

Compound code	*M* log *P*[a]	*S* + log *P*[b]	*S* + log *D*[c]	MWt[d]	MNO[e]	TPSA[f]	HBDH[g]	Rule of 5[h]
Tz_1	4.277	4.342	4.342	339.846	2	20.31	0	1
Tz_2	3.214	3.347	3.344	321.4	3	40.54	1	0
Tz_3	4.009	4.1	4.1	319.428	2	20.31	0	0
Tz_4	3.441	3.864	3.864	335.427	3	29.54	0	0
Tz_5	4.164	3.948	3.948	323.391	2	20.31	0	1
Tz_6	3.716	3.609	3.609	350.398	5	66.13	0	0
Tz_7	3.664	4.417	4.416	348.469	3	23.55	0	0
Tz_8	3.124	3.68	3.68	365.453	4	38.77	0	0
Tz_9	3.124	3.77	3.77	365.453	4	38.77	0	0
Tz_{10}	3.412	3.401	3.395	351.426	4	49.77	1	0

[a]Predicted log *P* using Moriguchi's model, [b]predicted log of the octanol/water partition coefficient using the Simulation Plus ANNE model, [c]predicted log *D* at pH 7.4 using the Simulation Plus ANNE model, [d]molecular weight, [e]the number of hydrogen bond acceptors (total number of nitrogen and oxygen atoms), [f]topological polar surface area, [g]number of hydrogen bond donor protons, [h]number of Lipinski's rule of 5 violations.

Conclusion

Compounds having *p*-chlorophenyl (Tz_1), *p*-fluorophenyl (Tz_5) and *p*-nitrophenyl (Tz_6) at 2-position of thiazolidin-4-one attached to α-position of naphthalene emerged as potentially active anti-inflammatory and analgesic agents. The docking studies further unlocked the binding site interactions of the potent inhibitors, and it was found that compounds fit well within the COX-2 enzyme's active site. Moreover, these compounds displayed better binding energy than the naproxen with similar binding orientation inside the active cavity space. Thus the substitution with electron withdrawing groups on 2-position of thiazolidinone moiety of the title compounds give potent therapeutic compounds. The *in silico* ADME evaluation of the compounds revealed that the compounds are having drug-likeness properties and thus they may have good oral bioavailability. So, it is concluded that naphthyl substituted thiazolidinone derivatives impact on medicinal chemists and biochemists for further search of better therapeutic agents taking these compounds as a lead molecule.

Acknowledgement

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