Synthesis, Biological Activity, Molecular Modelling Studies and 3D-QSAR Investigations of N-[2-(aryl/substituted aryl)-4-oxo-1, 3-thiazolidin-3-yl] pyridine-4-carboxamides

Asha B. Thomasa,*, Rabindra K. Nanda, Lata P. Kothapalli, Piyush A. Sharma and Kishori G. Apte

**Department of Pharmaceutical Chemistry, Padm. Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, Maharashtra, India**

**Department of Pharmaceutical Chemistry, Sri Aurobindo Institute of Pharmacy, Indore, Madhya Pradesh, India**

**National Toxicology Centre (NTC), Pune, Maharashtra, India**

**Abstract:** In the present research work new 4-thiazolidinones possessing dual cyclooxygenase/lipoxygenase inhibition were synthesized. Sixteen N-[2-(aryl/substitutedaryl)-4-oxo-1,3-thiazolidin-3-yl]pyridine-4-carboxamide derivatives(5a-q) differing by the phenyl group substitution were synthesized by sonication. Compounds N-[2-(4-chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl] pyridine-4-carboxamide (5d) and N-[2-(4-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl] pyridine-4-carboxamide (5f) showed promising anti-inflammatory activity investigated by the carrageenan induced edema model in rats. Compound 5f, was the most active in the series with low ulcerogenic index and good safety profile with highest in vitro inhibition of COX-1/COX-2 (89.28; 52.80%, p<0.01) with 27.36% inhibition of 15-LOX. Compounds 5d and 5f, at the dose of 100mg/kg p.o. were also effective in lowering the elevated levels of AST, ALT and ALP in serum and also lowered the elevated levels of MPO in edematous tissue comparable to standard anti-inflammatory drug, Indomethacin. The Autodock investigation of the synthesized compounds within the COX and LOX enzymes predicted fairly good correlation between the anti-inflammatory activities (IC50) and their binding affinities. The 3D-QSAR studies (V-Life Molecular Design Software) of the synthesized 4-thiazolidinones for their anti-inflammatory activity by the developed kNN, PLRS and MLR models suggested that presence of electron withdrawing functional groups like -NO2,-Cl on the phenyl ring were favourable for activity. The results of the present study may help to identify the relative positions and ranges of important electrostatic and steric fields at various positions around the pharmacophore that could be useful in the design of new molecules with improved anti-inflammatory activity.

**Keywords:** Anti-inflammatory activity, 3D-QSAR, dual COX/LOX inhibitors, microwave irradiation, Schiff’s bases, sonication, 4-thiazolidinones.

**INTRODUCTION**

Non-steroidal anti-inflammatory drugs are of huge therapeutic benefit in the treatment of rheumatoid arthritis and various types of inflammatory conditions by blocking the production of prostanoids from arachidonic acid through inhibition of cyclooxygenase (COX) activity. Recent evidence also suggests that anti-inflammatory drugs could be beneficial in a number of neurodegenerative disorders including Alzheimer’s and Parkinson’s diseases, which have a prominent neuroinflammatory component [1, 2]. However their long term clinical use is associated with significant side effects such as gastric ulcers, gastrointestinal lesions, bleeding and nephrotoxicity which limit their clinical applications [3]. Therefore, there is a constant need for development of novel drugs with better safety profiles that could be used long term to relieve various chronic inflammatory conditions. Besides COXs, 5-lipoxygenase (LOX) is an important metabolic enzyme for formation of leukotrienes from arachidonic acid (AA), leading to inflammation and other pathological responses. Combined with the earlier studies that COX inhibition alone may lead to an up regulation of AA metabolism by the 5-LOX pathway, it is now appreciated that dual inhibition against both COXs and 5-LOX might present an enhanced anti-inflammatory potency without risk of serious side effects including gastric damage. Thus, a single agent inhibiting both enzymes has been of interest to medicinal chemists.

In recent year’s computational chemistry, especially quantitative structure-activity relationship (QSAR) studies have become an integral part of the drug discovery process [4]. The 3D-QSAR predictions allow medicinal chemists to identify three dimensional pharmacophores which are essential and important for biological activity resulting in successful lead development [5].

Of particular importance are the 4-thiazolidinones because of their varied biologically properties [6]. Many 4-Thiazolidinone analogues show anticonvulsant [7, 8], antibacterial [9-11], antifungal [12, 13], ischemic [14], anti-
ONTOF Mass spectrometer using electrospray ionisation (EI source), Avance II 400 NMR) using dimethyl sulphoxide were obtained on a NMR Spectrophotometer (Bruker). Reflectance attachment (Shimadzu 8400S). The IR spectra (KBr) were recorded on a FTIR spectrophotometer with Diff. Reflectance attachment (Shimadzu 1700). The UV studies were carried out on a UV Visible spectrophotometer (VMP PM, 32/1104) and are uncorrected. Reactions were monitored by thin layer chromatography using precoated silica gel plates (E. Merck and Co., Darmstadt, Germany). UV studies were carried out on a UV Visible spectrophotometer (VMP PM, 32/1104) and are uncorrected. Reactions were monitored by thin layer chromatography using precoated silica gel plates (E. Merck and Co., Darmstadt, Germany). UV studies were carried out on a UV Visible spectrophotometer (VMP PM, 32/1104) and are uncorrected. Reactions were monitored by thin layer chromatography using precoated silica gel plates (E. Merck and Co., Darmstadt, Germany).

EXPERIMENTAL SECTION

All research chemicals were purchased from Acros organics (NY, USA), Sigma-Aldrich (St. Louis, Missouri, USA) and used as such for the reactions. Melting points (mp) were determined on a Veego melting point apparatus (VMP PM, 32/1104) and are uncorrected. Reactions were monitored by thin layer chromatography carried out using precoated silica gel plates (E. Merck and Co., Darmstadt, Germany). UV studies were carried out on a UV Visible spectrophotometer (Shimadzu 1700). The IR spectra (KBr) were recorded on a FTIR spectrophotometer with Diffuse Reflectance attachment (Shimadzu 8400S). 1H NMR spectra were obtained on a NMR Spectrophotometer (Bruker Avance II 400 NMR) using dimethyl sulphoxide-d6 as the solvent. The mass spectra were obtained on a Hewlett Packard Electron Impact mass spectrometer GCD-1800A (70 eV El source) using direct insertion probe and Quadrupole TOF Mass spectrometer using electrospray ionisation (Positive mode). Microanalyses were performed on a Thermo Finnigan C, H, N analyzer.

HIV [15] and anticancer activities [16]. In recent years, research has focused on the study of 4-thiazolidinones as anti-inflammatory and analgesic agents [17-20]. However there are no reports on the QSAR studies of 4-thiazolidinones for their anti-inflammatory activity.

Diarylheterocycles, and other central ring pharmacophore templates, have been extensively studied as cyclooxygenase inhibitors. All these tricyclic molecules possess 1, 2-diaryl substitution on a central four-, five- or six-membered ring system. Several derivatives containing pyrazoline, thiophene, di-tert-butylphenol, hydrazine, pyrroliidine and pyrazole subunits have been found to be dual COX/LOX inhibitors. A number of reported dual COX/LOX inhibitors like Licof (4-oxo-2-phenyl-1,3-thiazolidin-3-yl)pyridine-4-carboxamide(5a-p)

The synthesized Schiff’s bases (3a-p) were converted to their 4-thiazolidinones (5a-p) as per our reported green route method of sonication (Scheme 2) and characterized by their analytical and spectral data [24].

Reagents and conditions: i. H2O, MWI, Power level 3(240 W, 35% irradiation)

N-[2-(aryl/substitutedary)-4-oxo-1, 3-thiazolidin-3-yl]pyridine-4-carboxamide(5a-p)

The synthesized Schiff’s bases (3a-p) were converted to their 4-thiazolidinones (5a-p) as per our reported green route method of sonication (Scheme 2) and characterized by their analytical and spectral data [24].

Reagents and conditions: ii. Anhydrous ZnCl2, molecular sieves [MS (1-2 gms, 3A X 1.5 mm)], THF, sonication.

N-(4-oxo-2-phenyl-1,3-thiazolidin-3-yl)pyridine-4-carboxamide(5a).

White powder, yield 92.0%, mp 250-253°C, IR (νmax cm⁻¹, KBr): 3256 (-NH), 3028 (-CH), 1743 (ring - C=O), 1650 (amide-I C=O). 1H NMR (400 MHz, DMSO-d6): δ ppm 8.77,8.68 (d,Pyridine 2H); 8.75 (s, NH 1H); 7.75,7.73 (d, Pyridine 2H); 7.51(s, Aromatic 5H); 5.96 (s, CH 1H); 3.40, 3.36 (d, CH2 1H); 3.27,3.23 (d, CH2 1H). MS: m/z 299(M)⁺. Anal. Calcd for C15H13N2O2S (299.35): C, 59.82; H, 4.42; N, 14.22%.

N-(2(2-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl)pyridine-4-carboxamide(5b).

White powder, yield 89.7%, mp 250-253°C, IR (νmax cm⁻¹, KBr): 3344 (-OH), 3217 (-NH), 3039 (-CH), 1739 (ring - C=O), 1697 (amide I C=O). 1H NMR (400 MHz, DMSO-d6): δ ppm 9.04,8.98 (d, Pyridine 2H); 8.12,8.11 (d, Pyridine 2H); 7.54 (s, NH 1H);7.47-6.87 (m, Aromatic 4H); 6.22 (s, OH 1H); 5.69 (s, CH 1H); 3.48, 3.44 (d, CH2 1H); 3.29,3.25 (d, CH2 1H). MS: m/z 316.6(M+ H)⁺. Anal. Calcd for C15H15N2O2S (316.35): C, 57.13; H, 4.16; N, 13.33%. Found: C, 57.27; H, 4.12; N, 13.19%.
N-[2-(4-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-ylpyridine-4-carboxamide(5c). Yellow powder, yield 90.2%, mp >290°C, IR (ν_{max}, cm^{-1}, KBr): 3445 (OH), 3217 (-NH), 3052 (-CH), 1724 (ring -C=O),1668 (amide I,C=O). 1H NMR (400 MHz, DMSO-d6): δ ppm 8.77,7.85 (d, Pyridine 2H); 8.06 (s,NH 1H); 7.85,7.84 (d, Pyridine 2H); 7.75,7.73 (d, Aromatic 2H); 7.34,7.29 (d, Aromatic 2H); 6.05 (s, CH 1H); 5.36 (s, OH 1H); 3.70, 3.66 (d, CH 3H); 3.59,3.54 (d, CH 2H). MS: m/z 315(M^+). Anal. Calcd. for C_{15}H_{18}N_{2}O_{3}S (315.35): C, 57.13; H, 3.62; N, 12.59%. Found: C, 57.38; H, 3.92; N, 13.66%.

N-[2-(4-chlorophenyl)-4-oxo-1,3-thiazolidin-3-ylpyridine-4-carboxamide(5d). White crystals, yield 96.8%, mp 239-242°C, IR (ν_{max}, cm^{-1}, KBr): 3159 (-NH), 3082 (-CH), 1728 (ring -C=O), 1674 (amide I,C=O), 1083 (-C=O). 1H NMR (400 MHz, DMSO-d6): δ ppm 8.77,7.85 (d, Pyridine 2H); 8.07 (s, NH 1H); 7.85,7.84 (d, Pyridine 2H); 7.75,7.73 (d, Aromatic 2H); 7.71,7.69 (d, Aromatic 2H); 6.05 (s, CH 1H); 3.70,3.77 (d, CH 2H); 3.60,3.64 (d, CH 3H). MS: m/z 318.5 (M+H)^+. Anal. Calcd. for C_{15}H_{18}FN_{2}O_{3}S (317.34): C, 56.77; H, 3.81; N, 13.24%. Found: C, 56.82; H, 3.87; N, 13.61%.

N-[2-(4-fluorophenyl)-4-oxo-1,3-thiazolidin-3-ylpyridine-4-carboxamide(5i). Yellow powder, yield 94.2%, mp 240-242°C, IR (ν_{max}, cm^{-1}, KBr): 3147 (-NH), 3082(-CH), 1731 (ring C=O), 1666(amide I, C=O), 1222 (-C=O). 1H NMR (400 MHz, DMSO-d6): δ ppm 8.89,8.88 (d, Pyridine 2H); 8.03,7.98 (d, Pyridine 2H); 7.66 (s, NH 1H); 7.45,7.44 (d, Aromatic 2H); 7.06,7.05 (d, Aromatic 2H); 5.33 (s, CH 1H). 3.41, 3.37 (s, CH 3H). 3.18, 3.14(d, CH 2H). MS: m/z 318.5 (M+H)^+. Anal. Calcd. for C_{15}H_{18}F_{2}N_{2}O_{3}S (317.34) .

N-[2-(4-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-ylpyridine-4-carboxamide(5j). Brown powder, yield 90.3%, mp 278-281°C, IR (ν_{max}, cm^{-1}, KBr): 3267 (-NH), 3040(-CH), 1710 (ring C=O), 1685(amide I,C=O), 1448 (-C=H). 1H NMR (400 MHz, DMSO-d6): δ ppm 8.75,8.74 (d, Pyridine 2H); 8.42 (s,NH 1H); 7.83,7.85 (d, Pyridine 2H); 7.74,7.72 (d, Aromatic 2H); 6.95,6.93 (d, Aromatic 2H); 5.89(s, CH 3H); 3.86,3.82 (d, CH 2H); 3.76, 3.75 (d, CH 3H), 3.59(-OCH_{3} 3H). MS: m/z 330.6 (M + H)^+. Anal. Calcd. for C_{15}H_{18}O_{3}N_{2}S (329.37): C, 58.34; H, 4.59; N, 12.76%. Found: C, 58.58; H, 4.63; N, 12.80%.

N-[4-oxo-2-[[E]-2-phenylthiophenyl]-1,3-thiazolidin-3-ylpyridine-4-carboxamide(5k). Yellow powder, yield 87.2%, mp 247-250°C, IR (ν_{max}, cm^{-1}, KBr): 3148 (-NH), 3010(-CH), 1718 (ring C=O), 1660(amide I,C=O), 1622(-C=C),1421(-CH). 1H NMR (400 MHz, DMSO-d6): δ ppm 8.76,8.75 (d, Pyridine 2H); 8.27 (s, NH 1H); 7.85,7.84 (d, Pyridine 2H);5.72-7.30,m(Aromatic 5H); 7.02, 6.98(d, alkenic 2H); 5.43 (s, CH 1H); 3.71,3.69(d, CH 2H); 3.68,3.65 (d, CH 3H). MS: m/z 342.6 (M+H)^+. Anal. Calcd. for C_{16}H_{14}N_{2}O_{3}S (341.43): C, 63.32; H, 5.61; N, 12.31%. Found: C, 63.49; H, 5.65; N, 12.33%.

N-[2-(4-hydroxy-3-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-ylpyridine-4-carboxamide(5l). Pale yellow powder, yield 80.3%,mp 217-219°C, IR (ν_{max}, cm^{-1}, KBr): 3400(OH),3149 (-NH), 3008(-CH), 1710 (ring C=O), 1658(amide I, C=O),1392(-OH). 1H NMR (400 MHz, DMSO-d6): δ ppm 7.85,7.84 (d, Pyridine 2H);8.75,8.74(d,Pyrindine 2H); 8.38 (s, NH 1H); 7.42(s,-OH 1H); 7.08-6.86,m(Aromatic 3H); 5.85 (s, CH 1H); 3.91 (-SOCH_{3} 3H); 3.68,3.66 (d, CH 2H); 3.56,3.54(d, CH 1H). MS: m/z 346.5 (M + H)^+. Anal. Calcd. for C_{16}H_{16}N_{2}O_{3}S (345.37): C, 55.64; H, 4.38; N, 12.17%.Found: C, 55.79; H, 4.47; N, 12.20%.

N-[2-furan-2-yl-4-oxo-1,3-thiazolidin-3-ylpyridine-4-carboxamide(5m).Brown powder, yield 81.0%, mp 198-201°C, IR (ν_{max}, cm^{-1}, KBr): 3271 (-NH), 3112(Furan ring -CH), 3066(-CH-), 1714 (ring C=O), 1664(amide I, C=O), 1542,1404 (Furan ring C=C). 1H NMR (400 MHz, DMSO-d6): δ ppm 9.71,9.70 (d, Pyridine 2H); 8.11(s, NH 1H); 7.83,7.80 (d, Pyridine 2H); 7.45-6.41(m,Furfural ring 3H); 5.99(s,CH 1H); 3.94,3.90(d, CH 3H); 3.66,3.62 (d, CH 3H). MS: m/z 290.4 (M + H)^+. Anal. Calcd. for C_{15}H_{12}N_{2}O_{3}S (289.31): C, 53.97; H, 3.83; N, 14.52%. Found: C, 54.12; H, 3.88; N, 14.61%.

Synthesis, Biological Activity, Molecular Modelling Studies

The Open Conference Proceedings Journal, 2013, Volume 4 101
N-[2-[4-(dimethylamino)phenyl]-4-oxo-1,3-thiazolidin-3-yl]pyridine-4-carboxamide(5n). Brown powder, yield 91.6%, mp 224-226°C, IR (vmax, cm⁻¹, KB): 3434 (-NH), 3190 (-CH), 1700 (ring C=O), 1664(amide IC=O), 1367(-CH₂), 1 H NMR (400 MHz, DMSO-d₆): δ ppm 8.76, 8.75 (d, Pyridine 2H); 8.34 (s, NH 1H); 7.86, 7.85 (d, Pyridine 2H); 7.71, 7.69 (d, Aromatic 2H); 6.71, 6.68 (d, Aromatic 2H); 5.22 (s, CH 1H); 4.39, 4.38 (d, CH₂ 1H); 3.85, 3.84 (d, CH₂ 1H); 3.10 (s, OCH₃ 3H); MS: m/z 343.6(M + H)⁺. Anal. Calcd for C₁₇H₁₀N₂O₅S (344.22): C, 59.63; H, 5.30; N, 16.36%. Found: C, 59.78; H, 5.41; N, 16.44%.

N-[2-(5-nitrothiobenzyl-2-yl)-4-oxo-1,3-thiazolidin-3-yl]pyridine-4-carboxamide(5o). Blackish brown powder, yield 94.3%, mp 174-176°C, IR (vmax, cm⁻¹, KB): 3120 (-NH), 3082 (thiophene -CH), 1712 (ring C=O), 1672 (amide I, C=O), 1500, 1536 (-CH₃), 1458 (-OH), 6.9, 8.67. δ ppm 3.54, 3.53, 7.44, 7.43 (d, Pyridine 2H); 7.97 (s, NH 1H); 7.86, 7.85 (d, Pyridine 2H); 7.44, 7.43 (d, Thiophene 2H); 5.69 (s, CH 1H); 3.53, 3.54 (d, CH₂ 1H); 3.45, 3.46 (d, CH₂ 1H); MS: m/z 351.5(M + H)⁺. Anal. Calcd for C₁₇H₁₀N₂O₅S (350.73): C, 44.56; H, 2.88; N, 15.99%. Found: C, 44.70; H, 2.95; N, 16.04%.

N-[2-(3-hydroxynaphthalen-2-yl)-4-oxo-1,3-thiazolidin-3-yl]pyridine-4-carboxamide(5p). Brown powder, yield 93.9%, mp 258-260°C, IR (vmax, cm⁻¹, KB): 3300(-OH), 3200 (-NH), 3076(-CH), 1716 (ring C=O), 1650 (amide I, C=O), 1458(-OH). 1H NMR (400 MHz, DMSO-d₆): δ ppm 10.85(s, OH); 69, 68.67 (d, Pyridine 2H); 7.96 (s, NH 1H); 8.05, 8.03 (d, Pyridine 2H); 7.84-7.82 (m, Aromatic 2H); 7.63-7.59 (m, Aromatic 3H); 7.44-7.40 (m, Aromatic 1H); 5.95 (s, CH 1H); 3.53, 3.54 (d, CH₂ 1H); 3.48, 3.47 (d, CH₂ 1H); MS: m/z 366.6(M + H)⁺. Anal. Calcd for C₁₉H₁₃N₂O₅S (365.41): C, 62.45; H, 4.14; N, 11.50%. Found: C, 62.58; H, 4.18; N, 11.60%.

Biological Investigations

Experimental Animals

Swiss albino mice of either sex (20-30 g) were used for the biological studies. They were housed under standard laboratory conditions, maintained on a natural dark and light cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experimentation. The screening protocol was approved by the Institutional Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals (Protocol Nos.: DYPIPSR/586-25/2010;RP.No.048).

Acute Toxicity Study

The acute toxicity study for test 4-thiazolidinones were carried out in mice according to OECD guidelines [25]. Different doses of test drugs were administered at graded doses up to 2000 mg/kg, p.o. and animals were monitored individually and continuously for a period of up to 14 days for behavioral changes, toxic reactions and mortality.

Evaluation of Anti-Inflammatory Activity

Carrageenan Induced Paw Edema Inhibition Model in Rats

The study was performed by the procedure of Winter et al. [26]. Albino rats received a subplanter injection of 0.05 ml saline containing 1% carrageenan in the right hind paw of each rat. A suspension of tested compounds (graded doses of 25, 50, 100 and 200 mg/kg p.o.), standard drug Indomethacin (10 mg/kg, p.o.), or an equivalent volume of vehicle (gum acacia 2%) were administered intraperitoneally 1 hr before carrageenan injection. Control animals received the same volume of vehicle. The paw volume was measured by plethysmometry (UGO Basile 71400) immediately after the injection. Subsequent readings of the volume of the same paw were carried out at 60 min intervals and compared to the initial readings.

Measurement of Serum Levels of Marker Enzymes (AST, ALT and ALP)

The biochemical changes were investigated at the end of the 5 hrs study; the animals were sacrificed under light ether anaesthesia. Blood samples were collected by cardiac puncture and serum was separated by centrifugation at 2500 rpm below 30°C for 30 min. The levels of marker enzymes; aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were determined using biochemical kits [27, 28].

Myeloperoxidase Levels in Edematous Tissue in the Carrageenan Induced Paw Edema Model

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation was determined following the procedure of Mullane et al. [29, 30]. After 5 hrs following injection of carrageenan, the hind paw edematous tissue were obtained and weighed. Each piece of tissue was homogenised in a solution containing 0.5%(w/v) hexadecyltrimethyl-ammonium bromide dissolved in 10mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 g at 4°C. An aliquot of the supernatant was then allowed to react with a solution of ortho- dianisidine dihydrochloride (1.6mM) and 0.1mM hydrogen peroxide. The rate of change in absorbance was measured spectrophotometrically at 650 nm. Optical density units were converted into units of concentration using the molar absorptivity coefficient for the oxidized form of o-dianisidine [ε = 10,062 (M cm⁻¹) 1]. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide/min at 37°C and expressed in U/100 mg of wet tissue.

Ulcerogenic Study in Mice

Acute ulcerogenic effect was investigated as described by Barf et al. in the same animals that were submitted to the anti-inflammatory evaluation. Briefly, animals were euthanized at the end of the experiments and the stomachs were excised along its greater curvature for visualization of gastric lesions with a stereomicroscope [31].

Assessment of COX Inhibitory Activity (COX-1/2 Assay)

The COX-1/2 inhibitory activities of the test thiazolidinones were measured using ovine COX-1/2 enzymes included in the COX inhibitory screening assay kit provided by Cayman (Item No.760111; Cayman Chemical Co., MI). The inhibitor screening assay is based on the measurement of the peroxidase component of COXs assayed colorimetrically by monitoring the appearance of oxidized N,N',N',N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm [32]. The inhibitory activity were tested at an arachidonic
acid substrate concentration of 1 µM and 0.1 µM respectively for COX-1/2 assay. The test compounds and standard Indomethacin were added to the reaction mixture at a concentration of 200 µM.

**Assessment of Soyabean Lipoygenase Inhibitory Activity**

The Lipoxygenase (LOs) inhibitory activity was assessed using the Cayman’s Lipoxygenase Inhibitor Screening Assay Kit (Item No.:760700) which detects and measures the hydroperoxides produced in the lipoxygenation reaction and is a general detection method for LO, and can be used to screen libraries of compounds which inhibit LO enzymes [33].

**Molecular Docking Study**

The advanced docking program AutoDock 4.2 was used to evaluate the binding free energy of the inhibitors within the macromolecules and to gain insight into the binding modes of active 4-thiazolidinones.

**Selection and Preparation of Ligands and Target Protein Crystal Structures**

The ligands (4-thiazolidinones) were studied for their binding activities into COX-1/2 and 15-LOX enzymes. The three dimensional structures of the aforementioned compounds were drawn in PRODRG server using JME Molecular Editor; then energy minimization was performed with the ffGmx GROMACS force field by steepest descent for at most 50,000 steps, extended by 11 additional atom hybridized atoms and other chemical features. The crystal structure of COX-1 in complex with Ibuprofen (1EQG; resolution: 2.61Å), COX-2 in complex with SC-558(1CX2; resolution:3.0 Å) and LOX (1LOX; resolution:2.4 Å) was extracted from the RCSB Protein Data Bank [34]. All bound water molecules, hetero atoms and ligands were removed from the proteins and polar hydrogens were added. For the targets, the amino acids of the ligand binding sites were defined using data in pdbsum [35].

**Grid Generation and Run of Molecular Docking**

The grid maps representing the native ligands in the actual docking target sites were calculated with AutoGrid. The grids were chosen to be sufficiently large to include not only the active site but also significant portions of the surrounding surface. The three dimensional grids, 60 Å grid size (x, y, z) with a spacing of 0.375 Å, were created. The cubic grid box was centered in the active region and encompassed the binding site where the ligands were embedded. Of the three different search algorithms offered by AutoDock, the GA-LS search algorithm (Genetic algorithm with local search) was chosen to search for the best conformers [36]. The parameters were set using ADT (Autodock Tool Kit) on PC associated with Autodock 4.2. For all docking parameters, default values were used with 50 independent docking runs for each docking case.

**Molecular Docking and Analysis of the Docked Results**

In the molecular docking studies, both the binding free energy and docking energy generated by Autodock was employed as the criterion for ranking. Cluster analysis was performed on the docked results using a root mean square (RMS) tolerance of 0.5. Each of the clusters that exhibited significant negative interaction energies was examined by Accelrys, Discovery Studio Visualizer v2.0. [Accelrys Inc., San Diego, CA (2007)] to determine their binding orientations, molecular modeling, evaluation of the hydrogen bonds and for measuring RMSD, which was measured as distance between the centroids of the docked inhibitor and the native ligand. The mode of interaction of the native ligand within enzymes was used as the standard docked model as well as for RMSD calculation. The correct hydrogen bond interaction was considered according to Spoel et al. [37].

**3D-QSAR Studies**

The 3D-QSAR analysis of the synthesized N-[2-(aryl/substituted aryl)-4-oxo-1, 3-thiazolidin-3-yl]pyridine-4-carboxamide derivatives (5a-p) for their anti-inflammatory activity was performed using VLife MDS (Version 3.5, V Life Sciences) [38].

**Biological Data**

Data sets of 4-Thiazolidinones with sufficient range of anti-inflammatory activity were used for the present 3D-QSAR investigations. The biological activity (IC\textsubscript{50} µM) were converted to logarithmic values and subsequently used as dependent variables for the QSAR analysis.

**Energy Minimization and Alignment of Molecules**

All compounds were built on workspace of molecular modelling software VLife MDS 3.5. The structures were then converted to three-dimensional space for further analysis. All molecules were batch optimized for minimization of energies using Merck molecular force field (MMFF) followed by considering distance-dependent dielectric constant of 1.0, convergence criterion or root-mean-square (RMS) gradient at 0.01kcal/mol Å\textsuperscript{2} and the iteration limit set to 10,000. The energy-minimized geometry was used for calculation of various 3D descriptors. All molecules were subsequently aligned by an atom based alignment technique using the unsubstituted thiazolidinone derivative 5a (Fig. 1).

**3D-Descriptor Calculations**

After suitable alignment of the selected molecules, a common rectangular grid (lattice) was generated using the 3D-QSAR module of V Life MDS. The steric, electrostatic and hydrophobic interaction energies were computed at the lattice points of the grid using a methyl probe of charge +1. These interaction energy values were considered for relationship generation and utilized as descriptors. The pre-processing of the independent variables (3D descriptors) was done by removing invariant columns. The QSAR models were obtained by manual data selection type algorithm of training and test set data. The training and test set were selected such that they followed the unicolumn statistics, i.e., maximum of the test was less than maximum of training set and minimum of the test set was greater than of training set, which is a prerequisite for further QSAR analysis. This indicated that the test is interpolative i.e., derived from the min-max range of training set.

For the SW- k Nearest Neighbour Molecular Field Analysis (SW-kN NN; Model 1), five molecules, namely 5d-5f, 5j, 5l were selected as the test set while the remaining 11
molecules (5a-c,g-i,k,m-p) were used as the training set. In the Partial Least Square Linear Regression Analysis (PLRS; Model 2) and Multiple Regression Analysis (MLR, Model 3), compounds 5c, 5d and 5f were used as test set while 5a-b, c, g-p were used as the training test.

**Feature Selection and Model Development**

An integral aspect of any model-building exercise is the selection of appropriate set of features with low complexity and good predictive accuracy [39]. For all the developed QSAR models, the Stepwise (SW) forward-backward variable selection method was employed to derive the best equations [40]. For the QSAR models, the cross-correlation limit was set at 0.5, number of variables at 4, and the term selection criteria at r² for model 1 and q² for models 2 and 3 respectively. An F value was specified to evaluate the significance of a variable. The variance cut-off was set at 2, with autoscaling in which the number of random iterations was set at 10. Additionally for the kNN model, the number of maximum to minimum neighbours was set at 5:2. The 3D-descriptors were considered as independent variables and biological activity as dependent variables for the QSAR models.

**Statistical Analysis**

The SW- kNN MFA, PLSR and MLR methods of analysis were used to derive the 3D QSAR equations. Statistical parameters employed were n number of compounds in regression, the regression coefficient r², r²se (standard error of squared correlation coefficient), the F-test (Fischer's value) for statistical significance, the cross-validated correlation coefficient q² and q²se (standard error of cross-validated squared correlation coefficient). Regression correlation coefficient values close to 1.0 represent the best fit of the regressions [41]. The predictive ability (q²) of the generated models was evaluated by cross validation using a 'leave one-out' (LOO) method [42]. The external predictive power pred r² of the model was also assessed by predicting log IC₅₀ value of test set molecules, which were not included in the QSAR model development. The QSAR investigations resulted in several 3D QSAR equations, the best three are discussed.

**RESULTS AND DISCUSSION**

**Synthesis**

The synthetic strategies adopted to obtain the intermediate and target compounds are illustrated in Schemes 1 and 2 respectively. The microwave assisted synthesis of the Schiff’s base intermediates (3a-p) involved the reaction of isonicotinyl hydrazone (INH,1) with various substituted aryl/hetero aryl aldehydes (2a-p) in water in shorter reaction times (6-9 mins) with improved yields (86.7-99.2%) as compared to the reported conventional methods. The intermediates were converted into 4-thiazolidinones (5a-p) on ultrasound assisted reaction with mercaptoacetic acid(4) in presence of anhydrous ZnCl₂ and molecular sieves in good yields (80.3-96.8%) in reduced reaction times (30-45 min). Satisfactory IR, ¹H-NMR and MS characteristics were obtained. The ¹H-NMR spectra of 3a-p showed a singlet signal for =CH proton between 7.4-9.48 ppm, confirming formation of Schiff’s bases. The ¹H-NMR spectra also exhibited a signal between 7.01-8.66 ppm for the N-H proton. The ³J coupling constants of these two protons were almost negligible. However, the theoretical 3D optimisations of compounds 3a-p using ChemDraw Ultra 8.0, V-Life MDS and ACD/ChemSketch indicated that the bulky substituted phenyl and pyridine carboxamide groups are at the same side of the N=C bond, indicating the Z-configuration of Schiff’s bases. The Z-configuration will be further confirmed through NOE (Nuclear Overhauser effect) experiments. However, in the ¹H-NMR spectra of 3k, the coupling constant of 16 Hz states that the two olefinic protons are located on opposite sides of the C=C bond. In the ¹H-NMR spectra of all compounds of series 5, a singlet signal equivalent to 1 proton between 5.22-6.38 ppm (C-2, CH) and a doublet of doublet signal equivalent to 2 protons between 3.36-3.94 ppm;3.14- 3.85ppm (C-5, CH₂) was observed, considered to be a strong confirmation of ring closure representing the formation of thiazolidinone nucleus. The calculated J value in the range of 8-16 Hz justified the presence of the geminal protons at the C-5 position of the thiazolidinone ring. The elemental analysis results of all compounds were within ± 0.4% of the theoretical values.

**Biological Investigations**

The acute toxicity studies of the synthesized compounds did not produce any morbidity and mortality up to a maximum dose of 2000 mg/kg body weight in mice. The maximum dose of up to 200 mg/kg of test compounds was selected for further biological studies in animal models.

**Evaluation of Anti-Inflammatory Activity: Carrageenan Induced Paw Edema Inhibition Model in Rats**

The *in vivo* anti-inflammatory activity of synthesized intermediates (3a-p) by carrageenan induced paw edema test
in rats showed varying degree of anti-inflammatory activity from 15.05% - 55.91% at 200mg/kg p.o. However, all the synthesized Schiff’s bases were less active as compared to standard indomethacin (10 mg/kg p.o., 86.02%, p<0.01).

As compared to the intermediates, the 4-thiazolidinones (5a-p) at graded doses of 25, 50,100 and 200 mg/kg p.o. exhibited significant anti-inflammatory activity with dose dependency. Among the synthesized 4-thiazolidinones, the introduction of electron withdrawing nitro substituent at para/ortho positions of phenyl ring as in 5f, and 5h, resulted in significant inhibition of edema in comparison to the meta substituted derivative 5g(80.64%, p<0.01).

As these compounds exhibited increasing anti-inflammatory activity in the second phase of carrageenan induced edema, they may exert their activity through inhibition of enzymes which are important in the arachidonic acid cascade, thereby preventing the formation of inflammatory prostaglandins from arachidonic acid. The ulcerogenic liabilities of tested compounds were either absent or much less than that of indomethacin at all graded doses. The results of the anti-inflammatory screening of the test compounds are summarized in Figs. (2a-c) respectively.

Serum Levels of Marker Enzymes in Carrageenan Induced Paw Edema Model

In the control carrageenan treated group, significant increase in the levels of AST, ALT and ALP in serum were observed. However compounds 5d and 5f, at the dose of 100mg/kg p.o. were effective in lowering the elevated levels of enzymes in serum comparable to standard Indomethacin thereby suggesting their membrane stabilising potential (Fig. 3).

Myeloperoxidase Level in Edematous Tissue in Carrageenan Induced Paw Edema Model

A significant increase in myeloperoxidase activity in edematous tissue in the control carrageenan treated group was observed which was effectively inhibited by the tested 4-thiazolidinones. Compound 5f was found to significantly
The Open Conference Proceedings Journal, 2013, Volume 4

Thomas et al.

(p<0.01) lower elevated levels of MPO in edematous tissue when compared to indomethacin. The inhibition of MPO can be well correlated with the reduction of edema formation (Fig. 4). COX Inhibitory Screening Assay (COX-1/2)

**In Vitro COX-1 Inhibition**

All compounds inhibited COX-1 in the range of 22.61% to 83.33 % when added to the assay mixture at 200 µM and act as competitive inhibitors. N-[2-(4-nitrophenyl)-4-oxo-1, 3-thiazolidin-3-yl] pyridine-4-carboxamide (5f) exhibited the highest inhibitory activity (% inhibition: 89.28%, p<0.01). The ortho/meta substituted nitro derivatives with lower inhibitory activity (64.28%, p<0.01 and 78.57%, p<0.01 respectively) suggests that the addition of the nitro substituent at the para position of the phenyl ring is favourable for activity. Addition of the hydroxyl group at para/ortho position of the phenyl ring as in compounds 5c and 5b resulted in less active compounds[p-OH(36.90%, p<0.01); o-OH(22.61%, p>0.05)] compared to compound 5f. When a methoxy substituent was added to the meta position of the para hydroxyl derivative, the inhibition was significantly improved (71.42%, p<0.01). It was evident from the data that the addition of methoxy substituent as in 5i and in the p-methoxy derivative 5j (% inhibition:70.23%, p<0.01) was favourable for activity. Compound 5d with a para chloro substituent on the phenyl ring showed dramatic improvement in the inhibitory activity (83.33%, p<0.01) which is comparable to 5f, the most active compound in the series.

**In Vitro COX-2 Inhibition**

Tested compounds showed low to moderate COX-2 inhibition at 200 µM acting as competitive inhibitors of the enzyme. Compound 5f exhibited the highest inhibitory activity (52.80%, p<0.01). Substitution of the nitro group at the meta/ortho position resulted in reduction in inhibitory activity [m-nitro(45.20%, p<0.01); o-nitro(42.00%, p<0.01)]. In the halogen series, interestingly the addition of fluoro substituent at the para position of the phenyl ring in 5i exhibited significant improvement in the inhibitory activity (43.20%, p<0.01) as compared to the para/ meta substituted chloro analogues. Standard indomethacin at 200 µM exhibited 64.28% inhibition of COX-1 and 75.60 % inhibition of COX-2.

**Soybean Lipoygenase Inhibitor Screening Assay**

In the 5-LOX inhibitory assay, most compounds showed low to moderate inhibitory activity. The p-hydroxy compound 5c was the most active in the series with % inhibition of 39.80% (p<0.01). In addition the p-chloro (5d) and m-chloro derivatives (5e) also showed moderate LOX inhibitory activity (38.30%, p<0.01 and 37.31%, p<0.01)
respectively) suggesting that \( p \)-hydroxy, \( p \)-chloro and \( m \)-chloro groups may be important for significant LOX inhibitory activity (Fig. 5).

**Molecular Docking Study**

In an attempt to further corroborate the design of new isoniazid analogues, aimed at better COX-1/2 and LOX activity, the binding affinities of the active 4-thiazolidinones were investigated using the advanced docking program AutoDock 4.2. For the COX-1 enzyme, good correlation between experimental (IC\(_{50}\)) and predicted *in vivo* anti-inflammatory results were obtained for compounds (r\(^2\) of 0.676). In the COX-1 studies (PDB code: 1EQQ), ligands 5f and 5h exhibited binding and docking energies comparable to standard drugs, Ibuprofen and Indomethacin respectively. The obtained COX-1 complex with Ibuprofen (\( \Delta G_b \): -8.39 Kcal/mol; Docking energy: -9.90 Kcal/mol) reveals a binding pose in which the carboxylate group forms three hydrogen bonds; two bonds with Arg120 and one hydrogen bond with Tyr 355 residues present at the mouth of the cyclooxygenase active site which is in good agreement with the crystal structure of COX-1. In addition, hydrophobic residues such as Val349, Met522, Gly526, Ala527, Phe518 and Leu531 surround the phenyl isopropyl group, within van der Waals contact distance. The main interactions observed for standard Indomethacin and COX-1 are two hydrogen bonds involving the carboxylate group with Arg120. Visual inspection of the COX-1 complex with 5h (\( \Delta G_b \): -8.14 Kcal/mol; Docking energy: -9.25 Kcal/mol) reveals similar binding orientation as

---

**Fig. 5.** Effect of 4-thiazolidinones on *in vitro* COX-1/2 and 15-LOX inhibitory assay.

**Fig. 6.** Binding into active site of COX-1 enzyme.

(Hydrogen bonds shown as Green Dashed Lines)
the complex of Ibuprofen with COX-1, involving hydrogen bonding interactions of one of the oxygen atom of the nitro group with Arg120 and Tyr355 respectively (Fig. 6).

(Hydrogen Bonds Shown as Green Dashed Lines)

From the docking studies of 4-thiazolidinones on 1CX2, it was observed that the computed binding energies showed good correlation with the in vivo anti-inflammatory activity ($r^2$ of 0.642). The co-crystallized ligand SC-558 displayed lowest binding (-10.66 Kcal/mol) and docking energies (-12.63 Kcal/mol) which confirms that it is a selective COX-2 inhibitor. The COX-2 complex with SC-558 reveals a binding pose involving hydrogen bonding interactions of oxygen at atoms of the sulfone group at the mouth of the binding channel with His90 and Arg513. The sulfone substituted phenyl ring is surrounded by Phe518, Ile517, Tyr355, Ser353 and Leu352 in hydrophobic contacts. Additional hydrophobic interactions involving Arg120, Tyr385, Trp387, Ala527, Met522 and Leu531 are observed which surround the bromophenyl ring. All the other studied ligands exhibited higher energies as compared to SC-558. Ligands 5d, e, f, h, o exhibited binding and docking energies comparable to standard Indomethacin. The effective fit of these compounds into the COX-2 active site showed good co-relation of the experimental biological activities with their $\Delta G_b$ values comparable to standard Indomethacin (Fig. 7).

In the LOX binding studies, ligand 5d exhibited most favourable binding within both the $\beta$-barrel and catalytic domain of 1LOX ($\Delta G_b$: -8.20 Kcal/mol, Docking energy: -9.50 Kcal/mol; $\Delta G_b$: -8.22 Kcal/mol, Docking energy: -9.07 Kcal/mol respectively) through hydrogen bond interaction with Cys 97 through the -NH atom of its amide group within the $\beta$-barrel domain. Furthermore, hydrophobic residues, such as Met377 and Ser382 surround the chlorophenyl ring within van der Waals contact distance indicating that they may be important for activity. The effective fit of ligands 5d and 5e into the LOX active site showed good correlation of the in vitro LOX enzyme activities with their $\Delta G_b$ values.

3D-QSAR Study

To elucidate the effect of the aromatic substitutions at the C-2 position of the thiazolidinone ring on the anti-inflammatory activity, their activities were converted into logarithmic values. Using the QSAR module of V- Life MDS, the 3D-QSAR investigations were performed using the SW-kNN, PLRS and MLR models. The training set/test set selections were done manually such that the compounds populate the wide range of anti-inflammatory activities in similar proportions. The validation parameters for the 3D QSAR models are illustrated in Table 1.

The developed QSAR models were found to be statistically significant and predictive in terms of $r^2$, $q^2$, F and pred_r^2 values. SW-kNN model showed good internal
predictive ability \((q^2 = 0.5568)\) and a good fitness plot with an optimal ability to predict the activities of test set molecules \((\text{pred}_r^2 = 0.7859)\) which have not been included to build the QSAR model. Model 2 and 3 also displayed excellent fit \((r^2 = 0.9630, 0.9856)\) with good internal predictive abilities \((q^2 = 0.8408, 0.8988)\). However, the ability of MLR model to predict the activities of test set molecules was lower \((\text{pred}_r^2 = 0.5884)\) as compared to the PLSR model \((\text{pred}_r^2 = 0.7393)\).

**Interpretation of QSAR Models**

The local electrostatic and steric fields around aligned molecules found to be important for anti-inflammatory activity variation by the developed models are shown in Fig. (8).

---

**Table 1. Comparative Data of Validation Parameters for the 3D-QSAR Models**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model 1 (kNN)</th>
<th>Model 2 (PLSR)</th>
<th>Model 3 (MLR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contributing descriptor/Equation</td>
<td>Electrostatic (E_{545}(0.9050-1.2450))</td>
<td>1) (\log \text{IC}<em>{50}=0.0396 E</em>{609} - 0.012 S_{697} - 0.0107 E_{546} - 0.0068 S_{771} - 0.3499)</td>
<td>2) (\log \text{IC}<em>{50} = -0.0342 E</em>{609} - 0.0166 S_{697} - 0.0219 E_{590} - 0.0097 S_{771} - 0.2131)</td>
</tr>
<tr>
<td>Training set (N)</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Test set (N)</td>
<td>5(5d-f,5i,5l)</td>
<td>3 (5c,5d,5f)</td>
<td>3(5c,5d,5f)</td>
</tr>
<tr>
<td>(r^2)</td>
<td>--</td>
<td>0.9630</td>
<td>0.9856</td>
</tr>
<tr>
<td>(r^2) se</td>
<td>--</td>
<td>0.0727</td>
<td>0.0524</td>
</tr>
<tr>
<td>(q^2)</td>
<td>0.5568</td>
<td>0.8408</td>
<td>0.8988</td>
</tr>
<tr>
<td>(q^2) se</td>
<td>0.2500</td>
<td>0.1507</td>
<td>0.1388</td>
</tr>
<tr>
<td>(\text{pred}_r^2)</td>
<td>0.7859</td>
<td>0.7393</td>
<td>0.5884</td>
</tr>
<tr>
<td>(\text{pred}_r^2) se</td>
<td>0.1918</td>
<td>0.2646</td>
<td>0.3325</td>
</tr>
<tr>
<td>F test</td>
<td>--</td>
<td>104.0577</td>
<td>102.5127</td>
</tr>
<tr>
<td>Knearest neighbour</td>
<td>2</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**Fig. (8).** Relative positions of local fields around 4-thiazolidinones.
In model 1, the electrostatic field descriptor $E_{545}$ at the para position of C-2 substituted aromatic ring on the thiazolidinone nucleus has positive coefficient in the range of 0.9050 to 1.5130 and was found to contribute significantly for anti-inflammatory activity of the molecules. The nitro (5f-h) and p-fluoro substituted (5i) 4-thiadolidinones displayed positive values for the descriptor $E_{545}$ within the range as derived from model 1 indicating that the presence of electron withdrawing functional groups like $-\text{NO}_2$ or $-\text{F}$ at para position are favourable for increase in anti-inflammatory activity.

In models 2 and 3, three electrostatic field descriptors $E_{609}$, $E_{546}$ and $E_{590}$ (blue points) and two steric field descriptors $S_{697}$ and $S_{771}$ (green points) contribute for the anti-inflammatory activity of the molecules. Descriptors $E_{609}$, $S_{697}$ and $S_{771}$ are common in both models indicating their significance for the anti-inflammatory activity.

In model 2, the negative coefficients for the electrostatic parameters $E_{609}$(-50.80%) and $E_{546}$(-10.84%) indicate that negative electrostatic potential is favoured for increase in activity and hence more electronegative substituents should be preferred in these regions (meta position of the phenyl ring at C-2 position of the thiazolidinone ring). The steric field descriptors $S_{697}$ and $S_{771}$ contributed negatively (-27.47% and -10.89% respectively) indicating that less bulky substituents are preferred in these positions (meta and para position of phenyl ring at C-2 position of the thiazolidinone ring) for optimal biological activity. In model 3, the electrostatic parameter $E_{609}$ also correlated negatively (-39.70%) with biological activity similar to model 2, suggesting that more electronegative substituents should be preferred in these regions (meta position of the phenyl ring at C-2 position of the thiazolidinone ring).

However, electrostatic descriptor $E_{590}$ contributed positively (12.08%), indicating that less electronegative groups may be favoured at the ortho position of the phenyl ring at C-2 position of the thiazolidinone ring. The negative coefficients for the steric descriptors $S_{697}$(-34.31%) and $S_{771}$ (-13.90%) as in model 2 showed that more bulky substituents at the meta and para positions of the phenyl ring at C-2 position of the thiazolidinone ring are detrimental for the anti-inflammatory activity.

The results of the 3D-QSAR analysis of the 4-thiadolidinones by the kNN, PLSR and MLR models evidenced that the substituents similar to electron withdrawing functional groups like $-\text{NO}_2\text{-Cl}$ which were found to be favourable for the activity and contributing to the electronic descriptors $E_{609}$, $E_{545}$ and $E_{546}$ and possessing similar $+\sigma$ and $+\pi$ values like $-\text{Br}$-$\text{OCF}_3$-$\text{CF}_3$, $-\text{CF}_2\text{SO}_2$ may be substituted in these regions to increase the activity. Also substituents possessing $+\sigma$ and $-\pi$ values like $-\text{COOH}$, $\text{CH}_2\text{CO}$-$\text{CN}$, $-\text{CONH}_2$-$\text{CH}_2\text{SO}_2$-$\text{SO}_2\text{NH}_2$ can also be substituted to study their effect on the activity. Further, the appearance of steric descriptors in both the PLSR and MLR models indicated that less bulky substituents are favoured at these positions for optimal biological activity.

Among the models studied, the kNN model was found to be the best model with good prediction capabilities while the studied regression models, the PLSR model was found to be more suitable with significant $r^2$, $q^2$ and $\text{pred}_r^2$ values as compared to the MLR model.

MLR and PLSR are the most fundamental and common modelling methods for regression QSAR and are favoured due to their simplicity and ease of interpretations. However, the interpretations by MLR may not be accurate as collinear descriptors have the potential to influence the coefficients such that erroneous values may be assigned as reflected in the lower $\text{pred}_r^2$ as compared to the PLSR model. PLSR model is more appropriate when the number of features greatly exceed the number of samples and when features are highly collinear. Amongst the derived QSAR models, the KNN model is the advanced model that requires practically no training and is asymptotically optimal. However these classification methods are suitable for the prediction of compound class (active vs. nonactive) when the availability of activity information is limited. On the other hand, regression methods are suitable for quantitative (activity values e.g., IC50 or EC50) prediction when sufficient activity information of compounds possessing the same property is available.

**CONCLUSIONS**

NSAIDs are widely used for treatment of inflammatory disorders. However as their chronic use has shown adverse effects, especially gastric disturbances, attempts have been made to synthesize 4-thiadolidinones as dual COX/LOX inhibitors, preventing the biosynthesis of both prostanooids and leukotrienes, thereby acting as potent anti-inflammatory agents. The anti-inflammatory assay measured by inhibition of carrageenan induced rat paw edema, showed that compounds 5d, 5f and 5h were the most active compounds with low gastric ulcerogenic effects as compared to standard indomethacin. Molecular modelling studies with COX-1/2 and LOX revealed good binding affinities comparable to the known NSAIDs like indomethacin and ibuprofen. 3D-QSAR studies of the synthesized compounds employing kNN, PLSR and MLR models indicated the presence of electron withdrawing functional groups like $-\text{NO}_2\text{-Cl}$ contributing significantly for the anti-inflammatory activity. In addition, decrease in steric interactions at certain lattice point could be beneficial for improved potency of the 4-thiadolidinone derivatives. Thus the developed 3D-QSAR models could be useful for predicting activity of structurally similar analogs and could also guide in design of new molecules with improved anti-inflammatory potential with low ulcerogenic activity. Finally as both COX and LOX are up-regulated in various cancers, development of drugs targeting both enzymes would be useful to further design drug for chemoprevention.

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflicts of interest.
would like to specially mention the contribution of Rasa Life Sciences, Pune (India) for providing the spectroscopic data of compounds. The authors would like to specially mention the contribution of Rasa Life Sciences, Pune (India) for the Molecular Docking studies.

REFERENCES


Received: September 24, 2013
Revised: October 12, 2013
Accepted: November 04, 2013

© Thomas et al.; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.