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SYNTHESIS, ANTIINFLAMMATORY AND ANALGESIC ACTIVITIES OF SOME NOVEL 1, 3, 4 THIADIAZOLES BEARING BIPHENYL MOIETY

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ABSTRACT

In the present study, a series of novel thiadiazole derivatives were synthesized, with the goal of developing more potent anti-inflammatory compounds with fewer side effects. As per the synthetic protocol, in the presence of triethylamine and dichloromethane, the designed 1,3,4-thiadiazole derivatives of biphenyl-4-yloxy acetic acid were synthesized by conjugating 5-substituted phenyl-1,3,4-thiadiazole -2- amines and biphenyl-4-yloxy acetic acid. The structures of all the novel compounds are defined and tested by the spectral analysis and screened with carrageenan-induced paw edema method for *in-vivo* antiinflammatory activity. *In silico* evaluation was also carried out by docking the engineered ligands on the COX-2 receptor to obtain further insight into the protein-ligand interactions. In addition to the anti-inflammatory activity, a form of writhing caused by acetic acid was used to screen the title compounds for analgesic activity. Compounds **3f** (71%) with 2,6-dichlorophenyl substitution resulted in extraordinary anti-inflammatory activity that was found to be equipotent with the standard drug Indomethacin (69%). The **3c** (69 %), **3j** (67 %) and **3d** (64 %) derivatives exhibited substantial behavior and the findings obtained are in agreement with the findings of molecular docking. In comparison to standard drug aspirin, derivatives **3f** (73 %), **3c** (64 %), **3j** (67 %) produced significant analgesic activity. These findings reveal that **3f** is a promising, anti-inflammatory, and analgesic pharmacophore.

Keywords: Thiadiazole, Antiinflammatory activity, *In Silico* evaluation, Analgesic activity, biphenyl derivatives, Spectral analysis

1. INTRODUCTION

The currently available non-steroidal anti-inflammatory drugs (NSAIDs) like flurbiprofen, fenbufen and naproxen are the drugs of choice for the treatment of inflammation for the last few decades. Though its proven that the long-term usage of these drugs will cause gastro-intestinal ulceration, bleeding and nephrotoxicity, they are still considered as first drugs of choice and are extensively used worldwide for the treatment of inflammation, pain, fever and for the prevention of thrombosis. Inflammation is a complex process depicted by COX-2 (cyclooxygenase-2) enzyme which is involved in the conversion of the arachidonic acid to prostaglandin pathway, responsible for the inflammation process **[1, 2]**. It is also well documented that the enzyme COX-2 enzyme is over expressed in various human cancer cells like colorectal, gastric and breast cancer **[3-5]**.

Many compounds have been designed, synthesized, and proved as selective COX-2 inhibitors like celecoxib, roficoxib, etoricoxib these molecules are termed as coxibs which display improved gastrointestinal safety profile compared to the traditional NSAIDs. Other than the celecoxib, the selective inhibitors are either withdrawn or not launched due to their adverse side effects. For example, roficoxib because of its increased risk of cardiovascular side effects, it is withdrawn from the market. Further, the investigational studies confirmed that the drugs withdrawn possess more selectivity than celecoxib towards the COX-2. Therefore, it is not clear that how much degree of selectivity should be considered as safe. Recent studies also highlighted the potential applications as COX-2 inhibitors in treatment of cancers and neurodegenerative orders such as Alzheimer's disease and Parkinson's disease. So, identification of novel inhibitors with new scaffold with the structural features other than that of known inhibitors could be beneficial and desirable in the treatment of inflammatory diseases.

Heterocycles containing Sulphur and Nitrogen bearing a symmetrical 1,3,4 – thiadiazole moieties were proven to be promising in medicinal chemistry **[6-7]**, associated with a wide spectrum of pharmacological activities such as antiinflammatory **[7-10]**, anticancer **[11-15]**, antimicrobial **[16-20]**, antidepressant **[21-22]**, antidiabetic **[23-24]**, analgesic **[25]**, leishmanicidal activity **[26]**, antioxidant activity **[27-28]**, anti-HIV activity **[29]**, diuretic activity **[30]**, antitubercular activity **[31]** and antifungal activity **[32]** . Therefore, based on our interest in heterocyclic synthesis and based on the reported studies, we planned to

synthesize 1,3,4 thiadiazole derivatives of biphenyl-4-yloxy acetic acid. Furthermore, literature survey revealed that the modification of the carboxyl functional group of representative NSAIDs resulted in the increased inflammatory activity with reduced ulcerogenic effect **[33-35]**. Hence, it was considered worthwhile to replace the carboxylic acid group of biphenyl-4-yloxy acetic acid by a thiadiazole nucleus ring system to give a compact and planar structure. The study was further extended to predict the analgesic activity of the target compounds. The design strategy followed for the designing of title compounds is depicted in the **Figure 1**.

The molecular docking and modelling studies are valid authentic approaches in the drug design and discovery process to the study the molecular interactions of the small molecules. So, docking studies were performed to prove whether COX – 2 is a possible target for the antiinflammatory activity of the synthesized compounds or not and the results obtained were correlated with the results obtained in *in vivo* method. The Schrodinger software was used for docking the synthesized compounds on COX-2 crystal structures and we calculated then compared the binding affinities & modes of these compounds to the active site to establish the importance of the structural components for the anti-inflammatory activity.

2. Experimental Protocols:

2.1. General:

All chemicals and solvents used in the experimental study were purchased from S.D fine, Merck, HIMEDIA and Sigma Aldrich. On thin layer chromatography, the progression of the reaction, purity of the intermediate and final compounds was tested using silica gel pre-coated plates as stationary phase in the solvent method-Dichloromethane (DCM): methanol (MeOH) (7:3 v / v). Spot identification is achieved by observing under UV light and the spectral experiments were used to classify the compounds. The title compounds IR spectra have been recorded on the Bruker FTIR spectrophotometer and are given in KBr in cm^{-1}. The ^{1}H NMR was measured in generated chloroform and deuterated DMSO on the Bruker AM-400 NMR spectrometer. The chemical shifts are reported in δ (ppm) relative to tetramethyl silane as internal standard. Mass spectra analyses was performed with an Agilent 6400 Series equipped with an electrospray ionization source (capillary voltage at 4000V, nebulizing gas temperature at 300 °C, nebulizing gas flow at 12 L/min). The elemental analysis of the title compounds was performed on Perkin Elmer C, H and N model 240 C analyzer.

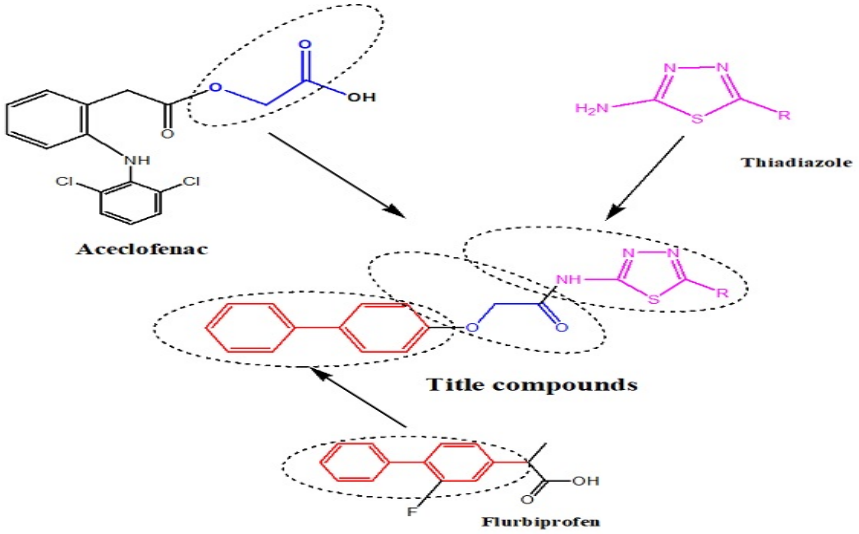

Figure 1: Design strategy adopted for the designing of title compounds

2.2. Chemical Synthesis:

2.2.1. General procedure for preparation of 5-substitutedphenyl-1,3,4-thiadiazol-2-amine(1a-i): Specific substituted carboxylic acids (0.05mol) and thiosemicarbazide (1.2m.eq) in phosphorous oxychloride (10mL) have been refluxed for 8h and cooled, accompanied by ice quenching. The separated solid was filtered and suspended in water and based on aqueous potassium hydroxide followed by filtration, drying and crystallization from the mixture of dimethyl formamide (DMF) and ethanol (9:1) to produce colorless solid with a yield of 40-65 %.

2.2.2. *General experimental procedure for 2-(biphenyl-4-yloxy)-N-(5-substituted phenyl [1,3,4] thiadiazol-2-yl)-acetamide (3a- n):*

To the 2-(4-phenylphenoxy) acetic acid (0.044mol), a mixture of toluene (80mL) and thionyl chloride (30mL), DMF (0.5mL) were added and the mixture was heated at 108-110^{o}C and stirred for 3h. Toluene and thionyl chloride were completely dissolved under vacuum and added to the residue of methylene chloride (20mL) from this process. Then the 5-substitutedphenyl-1,3,4-thiadiazol-2-amine (1.03m.eq), methylene chloride (50mL) and triethyl amine(10mL) were taken into the 250mL round bottom flask, cooled to 5-10^{o}C, then 2-(4-phenylphenoxy)acetyl chloride in methylene chloride was added below 10^{o}C slowly for 30min and after complete addition the temperature is raised to 25-30^{o}C. The reaction mixture is stirred at this temperature for 6h, checked the TLC (DCM: MeOH, 7:3) for the completion of the reaction, then water was added to the mixture and again stirred for 5min and extracted the aqueous layer with DMC (3x80mL). Then finally washed the

combined DMC layers with 20%(w/v) sodium chloride solution(30mL) and distilled off the solvent completely under reduced pressure and solid obtained was purified by column chromatography. This synthetic route followed for the synthesis of the designed compound is represented in **Figure 2**.

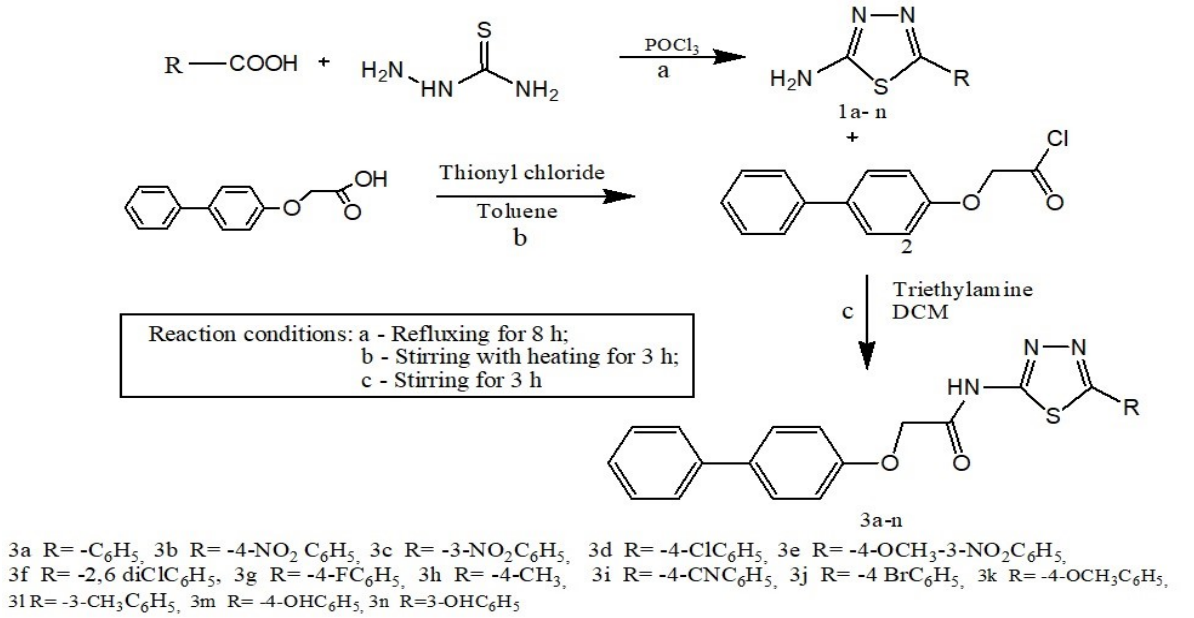

Figure 2: Scheme of synthesis involved in this study

Physical and Spectral data of the compounds:

2-(biphenyl-4-yloxy)-N-(5-phenyl- [1,3,4] thiadiazol-2-yl)-acetamide: 3a

Yield: 60%, MP:214-216°C, IR (KBr in cm^{-1}): 3213 (NH), 1670 (C=O), 1618 (C=C). ^{1}HNMR (DMSO-d_6) δ/ppm:4.82(s,2H, CH_2), 6.52-7.78 (m,14H, Ar-H), 9.24 (s,1H, NH): ESI-MS: 388($M+H^+$), Anal. calcd. for $C_{22}H_{17}N_3O_2S$: C,68.20; H, 4.42; N, 10.85; S:8.28. Found: C, 68.12, H, 4.36, N, 10.78. S: 8.40.

2-(biphenyl-4-yloxy)-N-(5-(4-nitrophenyl)-[1,3,4] thiadiazol-2-yl)-acetamide: 3b

Yield:46.9%, MP:255-257°C, IR (KBr in cm^{-1}): 3186 (NH Stretching),1690(C=O Stretching),1615(C=C Stretching),1337&1547 (NO_2 group Stretching); ^{1}HNMR (DMSO-d_6, 400MHz) δ/ppm: 4.60(s, 2H, CH_2), 7.00-8.20 (m, 13H, Ar-H), 8.12 (s, 1H, NH) ; ESI Mass :433 ($M+H^+$), Anal. calcd. for $C_{22}H_{16}N_4O_4S$: C, 61.10; H, 3.73; N, 12.96; S, 7.41. Found: C, 60.96, H, 3.70, N, 13.79.S,7.34.

2-(biphenyl-4-yloxy)-N-(5-(3-nitrophenyl)-[1,3,4] thiadiazol-2-yl)-acetamide: 3c

Yield: 46.9%, MP:248-250°C, IR(KBr in cm^{-1}): 3264(NH Stretching),1672(C=O Stretching),1608(C=C Stretching),1358&1537 (NO_2 group Stretching); ^{1}HNMR (DMSO-d_6, 400MHz) δ/ppm: 4.80(s, 2H, CH_2), 7.15-7.90(m, 13H, Ar-H), 8.2 (s, 1H, NH); ESI Mass

:433 ($M+H^+$), Anal. Calcd. for $C_{22}H_{16}N_4O_4S$: C, 61.10; H, 3.73; N, 12.96; S, 7.41. Found: C, 61.05, H, 3.82, N, 12.85.S,7.48.

2-(biphenyl-4-yloxy)-N-(5-(4-chlorophenyl- [1,3,4] thiadiazol-2-yl)-acetamide: 3d

Yield:47.6%, MP:185-187°C, IR(KBr in cm^{-1}): 3193(NH Stretching), 1690(C=O Stretching),1615(C=C Stretching), 740 (C-Cl Stretching); ^{1}HNMR (DMSO-d_6, 400MHz) δ/ppm: 4.75(s, 2H, CH_2), 6.70-7.80(m, 13H, Ar-H), 8.70(s, 1H, NH); ESI Mass : 422($M+H^+$). 424(M+2), Anal.calcd.for $C_{22}H_{16}ClN_3O_2S$: C, 62.63; H, 3.82; N, 9.96, S:7.60. Found: C, 62.60, H, 3.76, N, 10.02. S:7.64.

2-(biphenyl-4-yloxy)-N-(5-(4-methoxy-3-nitrophenyl)- [1,3,4] thiadiazol-2-yl)-acetamide: 3e

Yield:48.2%, MP:189-192°C, IR (KBr in cm^{-1}): 3192(NH Stretching),1696(C=O Stretching),1615(C=C Stretching),1020 (C-O Stretching); ^{1}HNMR (DMSO-d_6, 400MHz) δ/ppm: 3.86 (s, 3H, OCH_3), 5.00(s, 2H, CH_2), 7.18-8.20(m, 12H, Ar-H), 8.2 (s, 1H, NH); ESI Mass : 463($M+H^+$), Anal. Calcd. for $C_{23}H_{18}N_4O_5S$: C, 59.73; H, 3.92, N, 12.11, S, 6.93. Found: C,59.68; H, 4.00; N,12.15; S,6.98.

2-(biphenyl-4-yloxy)-N-(5-(2,6-dichlorophenyl)- [1,3,4] thiadiazol-2-yl)-acetamide: 3f

Yield: 45.4%, MP:210-212°C, IR(KBr in cm^{-1}): 3200(NH Stretching),1690(C=O Stretching),1630(C=C Stretching); ^{1}HNMR(DMSO-d_6, 400MHz) δ/ppm: 4.86(s, 2H, CH_2),6.90-7.84(m, 12H, Ar-H), 9.00(s, 1H, NH); ESI Mass : 456($M+H^+$), 457(M+1), 458(M+2), Anal. Calcd. for $C_{22}H_{15}Cl_2N_3O_2S$: C, 57.90; H, 3.31; N, 9.21; S, 7.03. Found: C, 57.86, H, 3.28; N,9.32. S, 7.10.

2-(biphenyl-4-yloxy)-N-(5-(4-fluorophenyl)- [1,3,4] thiadiazol-2-yl)-acetamide: 3g

Yield: 35.6%, MP:195-197°C, IR(KBr in cm^{-1}): 3234(NH Stretching), 1678(C=O Stretching),1606(C=C Stretching), 1000 (C-F Stretching); ^{1}HNMR (DMSO-d_6, 400MHz) δ/ppm: 4.90(s, 2H, CH_2), 6.93-8.10(m, 14H, Ar-H), 9.62(s, 1H, NH); ESI Mass : 406($M+H^+$). Anal.calcd.for $C_{22}H_{16}FN_3O_2S$: C, 65.17; H, 3.98; N, 10.36, S:7.91. Found: C, 65.10, H, 4.00, N, 10.34. S:7.95.

2-(biphenyl-4-yloxy)-N-(5-(4-methylphenyl)- [1,3,4] thiadiazol-2-yl)-acetamide: 3h

Yield: 49.6%, MP:203-205°C, IR(KBr in cm^{-1}): 3190(NH Stretching), 1690(C=O Stretching),1615(C=C Stretching) ; ^{1}HNMR (DMSO-d_6, 400MHz) δ/ppm: 2.43(s,3H, CH_3),5.42 (s, 2H, CH_2), 6.50-7.52(m,14H,Ar-H), 9.36(s, 1H, NH); ESI Mass: 402.1 ($M+H^+$).Anal. Calcd. for $C_{23}H_{19}N_3O_2S$: C, 68.81; H, 4.77; N, 10.47;

S, 7.99. Found: C, 68.78, H, 4.80, N, 10.43; S, 7.96.

2-(biphenyl-4-yloxy)-N-(5-(4-cyanophenyl)- [1,3,4] thiadiazol-2-yl)-acetamide: 3i

Yield:48.3% MP:220-222°C, IR(KBr in cm^{-1}): 3195(NH Stretching), 2245(CN Stretching) 1660(C=O Stretching), 1613(C=C Stretching) ; ^{1}HNMR(DMSO-d_6, 400MHz) δ/ppm: 4.82(s, 2H, CH_2), 6.86-7.89(m, 14H, Ar-H), 9.38 (s, 1H, NH); ESI Mass : 413.4($M+H^+$).Anal. Calcd. for $C_{23}H_{16}N_4O_2S$: C, 66.97; H, 3.91; N,13.58, S,7.77. Found: C, 66.86; H, 3.95; N, 13.62, S:7.80.

2-(biphenyl-4-yloxy)-N-(5-(4-bromophenyl) - [1,3,4] thiadiazol-2-yl)-acetamide: 3j

Yield: 35.6%, MP:195-197°C, IR(KBr in cm^{-1}): 3184(NH Stretching), 1692(C=O Stretching),1610(C=C Stretching), 800 (C-Br Stretching); ^{1}HNMR(DMSO-d_6, 400MHz) δ/ppm: 5.38(s, 2H, CH_2), 7.20-8.24(m, 14H, Ar-H), 9.50(s, 1H, NH); ESI Mass : 466($M+H^+$), 468(M+2). Anal. calcd. for $C_{22}H_{16}BrN_3O_2S$: C, 56.16; H, 3.46; N, 9.01, S:6.88. Found: C, 55.98, H, 3.62, N, 9.34. S: 6.75.

2-(biphenyl-4-yloxy)-N-(5-(4-methoxyphenyl)- [1,3,4] thiadiazol-2-yl)-acetamide: 3k

Yield: 42.3%, MP:218-220°C, IR(KBr in cm^{-1}): 3188(NH Stretching), 1686(C=O Stretching),1620(C=C Stretching); ^{1}HNMR (DMSO-d_6, 400MHz) δ/ppm: 3.75(s, 3H, OCH_3), 5.28(s, 2H, CH_2), 6.60-7.80(m, 14H, Ar-H), 9.30(s, 1H, NH); ESI Mass : 418($M+H^+$); Anal.calcd.for $C_{23}H_{19}N_3O_3S$: C, 66.17; H, 4.59; N, 10.07, S:7.68. Found: C, 66.01, H, 4.62, N, 9.95. S:7.60.

2-(biphenyl-4-yloxy)-N-(5-(3-methylphenyl)- [1,3,4] thiadiazol-2-yl)-acetamide:3l

Yield: 42.4%, MP:199-201°C, IR(KBr in cm^{-1}): 3255(NH Stretching), 1684(C=O Stretching),1604(C=C Stretching) ; ^{1}HNMR(DMSO-d_6, 400MHz) δ/ppm: 2.40(s, 3H, CH_3),5.30(s, 2H), 6.62-7.72(m, 14H, Ar-H), 9.42(s, 1H,NH); ESI Mass: 402.1 ($M+H^+$).Anal.calcd.for $C_{23}H_{19}N_3O_2S$: C, 68.81; H, 4.77; N, 10.47; S, 7.99. Found: C, 68.76, H, 4.73, N, 10.53; S, 7.92.

2-(biphenyl-4-yloxy)-N-(5-(4-hydroxyphenyl)- [1,3,4] thiadiazol-2-yl)-acetamide:3m

Yield: 42.4%, MP:204-209°C, IR(KBr in cm^{-1}): 3198(NH Stretching), 1690(C=O Stretching),1614(C=C Stretching) ; ^{1}HNMR(DMSO-d_6, 400MHz) δ/ppm: 5.38(s, 2H, CH_2), 7.30-8.44(m, 14H, Ar-H), 9.40(s, 1H, NH); ESI Mass : 403.45 ($M+H^+$). Anal.calcd.for $C_{22}H_{17}N_3O_3S$: C, 56.16; H, 3.46; N, 9.01, S: 6.88. Found: C, 55.98, H, 3.62, N, 9.34. S: 6.75.

2-(biphenyl-4-yloxy)-N-(5-(3-hydroxyphenyl)- [1,3,4] thiadiazol-2-yl)-acetamide:3n

Yield: 42.4%, MP:206-212°C, IR(KBr in cm^{-1}): 3200(NH Stretching), 1686(C=O Stretching),1612(C=C Stretching) ; ^{1}HNMR(DMSO-d_6, 400MHz) δ/ppm: 5.36(s, 2H, CH_2), 7.28-8.40 (m, 14H, Ar-H), 9.42 (s, 1H, NH); ESI Mass: 403.45 ($M+H^+$).Anal.calcd.for $C_{22}H_{17}N_3O_3S$: C, 68.81; H, 4.77; N, 10.47; S, 7.99. Found: C, 68.76, H, 4.73, N, 10.53; S, 7.92.

2.3. Pharmacological Evaluation:

2.3.1. General:

The chemicals used in the experimental studies were procured from Merck, Himedia, Mumbai. The usage of the animals for pharmacological studies was carried in compliance with WHO ethical guidelines. Swiss albino rats of 180-200g were procured from an inbred colony from Sri Venkateswara Enterprises, Bangalore. The animals were provided with standard feed & water and were maintained under control conditions of temperature and light. Paw edema of the experimental Albino rats was measured by BASILE 7140 Plethysmometer. The experimental protocols were approved by the institutional ethical committee. Acute toxicity studies for the novel compounds were carried out in mice (500mg/kg, 1000mg/kg, 1500mg/kg p.o) **[36]**. Prior to the biological evaluation of the compounds, the animals were kept starved of food before 4h but fed with water.

2.3.2. Antiinflammatory activity:

The synthesized compounds were screened for their antiinflammatory activity using carrageenan-induced paw edema method **[37-39]**. The experiment was performed on Albino rats, randomly divided into groups of six, one group was kept as control and received only 0.5% carboxymethyl cellulose (CMC) solution. The other groups received the test compounds and the standard drug. The rats were administered orally with the test compounds (100mg/kg), 100mg/kg Diclofenac sodium 1h before injection of 0.05mL of 1% suspension of Carrageenan into the sub plantar region of the rat hind paw. The volume of the injected paw was measured by water displacement in a plethysmometer before and after 3h of carrageenan treatment. Average edema volumes of the rats for test group and positive control group were compared statistically with the vehicle control group and expressed as percent edema inhibition, calculated by using the formula,

Percentage edema inhibition = 100 ($1-V_t/V_c$)

where, V_t = volume of edema in treated group and V_c= volume of the edema in the control group. Statistical significance of the results was tested by Anova and Dunnett's t-test.

2.3.3. Analgesic activity:

Analgesic activity was evaluated by acetic acid-induced writhing method in mice **[40, 41]**. For this experiment, Swiss albino

mice were divided into groups consisting of six animals each, weighing about 20-25 g, of either sex were used. Mice were kept individually in the test cage before the administration of the acetic acid injection and habituated for 30 min. The test groups were dosed with the test compounds and the standard group with ibuprofen, at a dose of 100 mg/kg po. All the compounds were dissolved in 1% CMC solution. One group was kept as control and received p.o 1%CMC. After 1 hr of drug administration, 0.1 ml of 1% acetic acid was given to mice intraperitoneally. In this test, the mice showed stretching movements involving arching of the back, elongation of the body and extension of hind limbs which were counted for 5-15 min after acetic acid injection.

Percent inhibition %= 100(1-W_t/W_c)

Where, W_c no. of writhes for the control group, and W_t no. of writhes for the treated group. Statistical significance of the results was tested by Tukey-Kramer multiple comparisons test.

2.3.4. In Silico Evaluation/ Molecular docking:

The molecular docking study plays a pivotal role in drug design process in predicting and establishing the predominant binding modes of the ligands or the target compounds with a specific protein of known three-dimensional structure **[42, 43]**. This also assists us in the interpretation of the structural features of the binding site, binding mode of analogues and in turn the key fragments in the title compound responsible for the interactions. The crystal structure of COX-2 (PDB ID:5F19) of Homo sapiens was retrieved from protein data bank (PDB). The protein preparation wizard to upgrade and underrate the protein structure by executing the OPLS force field until the RMSD constraint reached to 0.2 A^0. The bound water and ligands were excluded from the protein and the receptor grid for COX-2 active site was generated using XP- GLIDE. The 3D structure of the aforesaid compounds was drawn by employing chem3D ultra 12.0 software. The drug likeness properties of the designed ligands were evaluated by SwissADME software with the help of Lipinski rule of five. The ligands and the standard drugs into the human COX-2 grid created to attain their binding affinities. The best pose per each ligand was selected and rest of the poses with RMS deviation less than $0.5A^0$ and atomic displacement less than $1.3A^0$ were rejected. The docking studies reveal the orientation and interactions of the ligand within the environment of target.

3. RESULTS AND DISCUSSION

3.1. Chemistry:

Synthesis of 2-(biphenyl-4-yloxy)-N-(5-substituted phenyl- [1,3,4] thiadiazole-2-yl)-acetamides was achieved with an

efficient synthetic route outlined in **Scheme 1**. The present work involves the condensation of 2-(4-phenylphenoxy) acetyl chloride with substituted thiadiazole derivatives to obtain the newer effective compounds. The intermediate 5-substituted phenyl – 1,3,4-thiadiazole -2- amines **(1)** were obtained on treatment of various substituted carboxylic acids with thiosemicarbazide. The 2-(4-phenylphenoxy) acetic acid **(2)** was prepared from 4- phenyl phenol and chloroacetic acid, which on further reaction with thionyl chloride in presence of catalytic amount of DMF yields 2-(4-phenylphenoxy) acetyl chloride. The reaction of compounds (**1)** and (**2)** in presence of triethylamine and DCM afforded target compounds, 2-(biphenyl-4-yloxy)-N-(5-subtituted phenyl-[1,3,4] thiadiazole-2-yl)-acetamides **(3).**

The chemical structures of the title compounds **(3a - n)** were established using spectroscopic evidences. The IR spectrum of synthesized compounds showed–C=O stretching of amide bond in the region of 1660-1692 cm^{-1},N-H stretching at 3186 - 3264 cm^{-1}, a band due to C=C stretching at 1604 - 1620 cm^{-1}, C=N band around 1610 cm^{-1} and 1253 cm^{-1} band for C-O-C linkage. ^{1}H NMR spectra of title compounds **(3a –n)** showed a singlet at δ 9.0 to 9.6 group due to NH proton a sharp singlet is observed at δ 4.5 to 5.45due to – CH_2 proton and aromatic protons were observed as multiplet at δ 7.29 to 7.83. In mass spectra of all the synthesized compounds **(3a - n)**, the molecular ion peaks are all in accordance with their molecular weights. Detailed spectral data is given in experimental protocols.

3.2. *In vivo* anti-inflammatory activity:

The acute antiinflammatory activity of the synthesized compounds **(3a - n)** was determined following the carrageenan induced paw edema method in rats **(Table 1)**. The test compounds and the standard drug, Indomethacin was evaluated at 100mg/kg p.o and the edema were measured after 3h. Among the synthesized compounds, most of the derivatives showed significant activity. It has been found that the derivative **3f** (71%) with 2,6-dichlorophenyl substitution elicited exceptional anti-inflammatory activity, which is equipotent with the standard drug Indomethacin (69%). The derivatives **3c** (69%), **3j** (67%) and **3d** (64%) with 3-nitrophenyl, 4-bromophenyl and 4-chlorophenyl respectively as substituents exhibited significant activity. After close examination of various chemical structures of COX-2 enzyme inhibitors, revealed that almost all drugs possess differently substituted diaryl portion on the heterocyclic nucleus. Further these compounds have substituent's like SO_2,

NH_2, Cl, F etc. in their basic structures. Hence our results obtained coincide exactly with earlier reports on the anti-inflammatory drugs.

3.3. Molecular docking:

Based on the molecular docking studies, the binding free energies obtained clearly outlines the affinity of the designed ligands with the COX-2 protein. Based on the score obtained, the compounds were graded, and this information can be used for further structural optimization. The results are tabulated in **Table 1**. When docked to COX-2 protein, the compounds **3h**,**3e**, **3l, 3a, 3i** found to exhibit the highest fitness score correlating with the findings of the *in vivo* studies. The title compounds, standard drugs indomethacin, celecoxib, and valdecoxib showed at least one association with ASN382 amino acid through hydrogen bonds. The amino acids GLN203, LEU391, ALA199, THR206, ASN222, LYS211, THR212, TYR148, ILE274, VAL447, VAL444, ALA443, PHE200, LEU391, PHE210, HIS207, GLN454, HIS214, VAL295, HIE388, LEU294 and THR206 at the active site of the target are responsible for the interactions of the ligand. It was found that ASN382, THR212, GLN289 and CYS797 amino acids of COX-2 are important for strong hydrogen bonding interaction with the inhibitors. The title compounds were proven to possess best binding affinities when compared with the standard drugs Indomethacin, Celecoxib and Valdecoxib. The binding energy score for the designed compounds was in the range of -67.26 to -77.84 Kcal/mole, whereas for standard drugs it was from -30.39 to -66.60 Kcal/mole. In the light of these observations, the title compounds have greater affinity and interactions than the standard drugs. These findings give an idea not only of the level of anti-inflammatory activity but also of the structural components responsible for the interactions with COX-2's active site.

3.4. Analgesic Activity:

The compounds with proven anti-inflammatory activity were selected and screened for analgesic activity. All the compounds examined **3b, 3c, 3d, 3f, 3i, 3j** significantly inhibited writhing compared to the control group treated with acetic acid alone. In acetic acid induced writhing study, the compounds 3f (73%), 3c (64%), 3j (67%) are equipotent with the standard drug aspirin which also exhibited substantial anti-inflammatory activity. Thus, the compound **3f** was explicit to be the best pharmacophore among the developed ligands with proven anti-inflammatory and analgesic action, and the results have been tabled in **Table 2**.

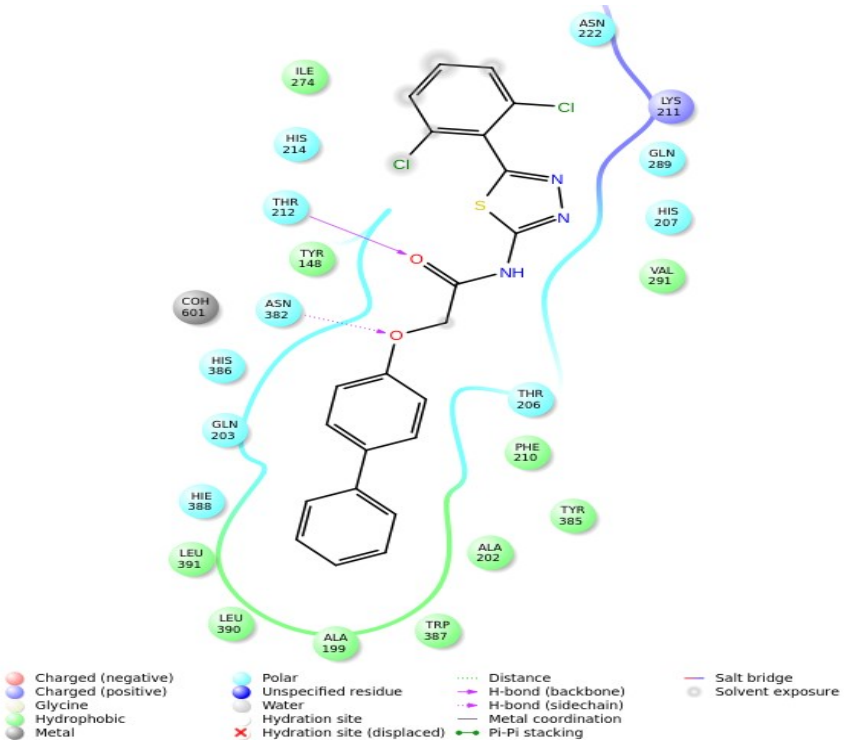

Figure 3: Docking interaction of 3f with COX-2

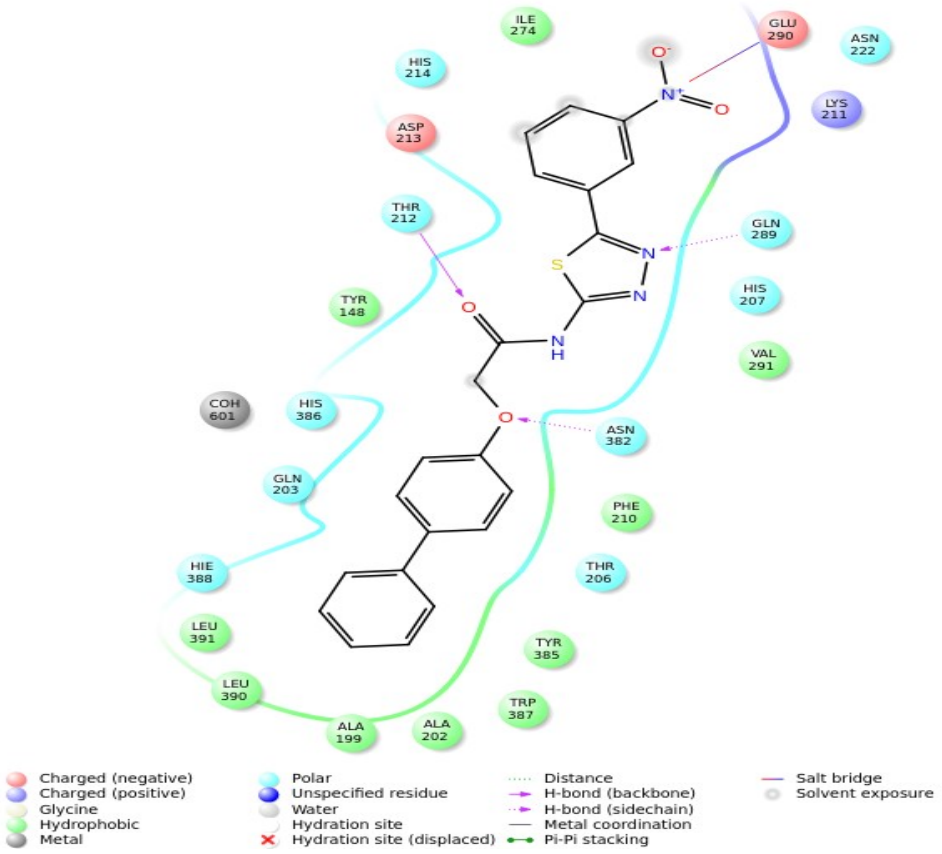

Figure 4: Docking interactions of compound 3c with the COX-2

Table 1: Anti -inflammatory activity of the title compounds (3a-3n)

S. No	Compound	*In vivo*	Docking	
		% Inhibition of Inflammation After 3 h	Docking free Energy (Kcal/mol)	XP G Score (kcal/mol)
1.	3 a	50.81**	-67.26	-7.671
2.	3 b	62.24**	-71.93	-8.932
3.	3 c	69.09**	-72.96	-9.188
4.	3 d	64.01***	-72.01	-9.08
5.	3 e	56.93*	-70.684	-8.792
6.	3 f	71.65**	-77.84	-9.611
7.	3 g	54.68**	-68.97	-8.932
8.	3 h	52.00**	-68.523	-8.637
9.	3 i	61.02***	-70.60	-8.904
10.	3 j	67.22***	-71.95	-9.041
11.	3 k	51.01**	-67.34	-8.11
12.	3 l	51.95**	-68.20	-8.202
13.	3m	54.89**	-69.182	-8.779
14.	3n	51.09**	-67.40	-8.131
15.	Control	-----		
16.	Indomethacin	68.14***	-66.60	-7.97
17.	Celecoxib	NT	71.04	-8.98
18.	Valdecoxib	NT	-72.46	-9.22

Significance Levels $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ by Dunnet test. At 100 mg/ kg (p.o) edema volume was measured 3h after carrageenan and activity is presented as % inhibition of inflammation. NT – Not tested

Table 2: Analgesic activity of the title compounds: (3b, 3c, 3d, 3e, 3f, 3i, 3j,3m)

S.NO	COMPOUND	% Writhing Inhibition
1.	3b	61.27*
2.	3c	64.74**
3.	3d	62.43*
5.	3f	73.0**
6.	3i	57.23*
7.	3j	67.04**
8.	Standard: Aspirin	78.0**

Significance Levels $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ by Tukeys multiple comparison test when compared with acetic acid treated control group; No. of writhes of control group -58

4. CONCLUSION

The research study reports the successful synthesis of series of 2-(biphenyl-4-yloxy)-N-(5-subtituted phenyl- [1,3,4] thiadiazole-2-yl)-acetamides and established the pharmacological significance of title compound as anti-inflammatory and analgesic agents. All the compounds tested demonstrated moderate to good anti-inflammatory and analgesic activities. In addition to these studies docking of the target ligands was done to depict the important structural components contributing to the activity. It was interesting to note that the compound **3f** with 4-dicholorophenyl substituent exhibited potent anti-inflammatory activity in comparison to the standard drug Indomethacin. The compounds **3c, 3j & 3d** were proven to be quite good as anti-inflammatory compounds. The designed ligands **3f, 3c, 3j** also showed highest analgesic activity. Hence among the compounds designed, synthesized and screened, the derivative 2-(biphenyl-4-yloxy)-N-(5-(2,6-dichlorophenyl)- [1,3,4] thiadiazol-2-yl)-acetamide (**3f)** is betrayed to be the best pharmacophore with drug

like properties. Thus, the present attempt to synthesize derivatives with biphenyl substitution on various thiadiazole derivatives proved to be promising class of compounds with an interesting pharmacological profile.

Consent for publication:

Not applicable.

Conflict of interest:

The authors declare no conflict of interest.

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