Journal of Applied Pharmaceutical Science Vol. 7 (10), pp. 037-047, October, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.71006
ISSN 2231-3354

Synthesis, Pharmacological Evaluation, Molecular Docking and *in silico* ADMET Prediction of Nitric Oxide Releasing Biphenyls as Anti-Inflammatory Agents

Monika G. Shinde, Siddharth J. Modi, Vithal M. Kulkarni*

Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, Maharashtra 411038, India.

ARTICLE INFO

Article history:
Received on: 31/05/2017
Accepted on: 06/08/2017
Available online: 30/10/2017

Key words:
Biphenyl derivatives, Docking, ADMET, Anti-inflammatory activity.

ABSTRACT

Various 2-(4'-methyl-N-phenyl substituted)-1,1'-biphenyl-2-ylcarboxamido-2-oxoethyl nitrate analogues were synthesized and evaluated for analgesic, anti-inflammatory, ulcerogenic activity and nitric oxide release study. Compounds VM-4, VM-6, VM-9, VM-10, VM-11 and VM-12 exhibited good analgesic and anti-inflammatory activity compared to standard drug diclofenac. The compounds also showed decreased gastro-intestinal ulcerogenicity and gastro-protective activity in histopathological studies resulting in absence of mucosal injury. All the synthesized compounds were found to have significant *in vivo* nitric oxide releasing activity. The molecular docking was performed to understand the binding mode of these compounds. Results of this study indicated that these NO-NSAIDs may have affinity towards COX-2 active site which can further be explored for selective or non-selective inhibitors.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used medicines for treatment of various inflammatory conditions such as cancers, diabetes, Alzheimer's and Parkinson's disease. (Husain *et al.*, 2016, Mahdi *et al.*, 2015). The anti-inflammatory effect of NSAIDs arises from their ability to inhibit cyclooxygenase (COX) enzyme. Bis-dioxygenation and subsequent reduction of arachidonic acid (AA) to prostaglandin (PG) G2 and PGH2 formation was catalyzed by COX Enzyme (Rouzer and Marnett, 2003). This process leads to gastrointestinal (GI), renal and hepatic side effects in the patients who undergo chronic treatment. GI irritation, ulceration, dyspepsia and bleeding are common side effects of NSAIDS therapy. The treatment options which can be used for inflammatory diseases are unsatisfactory and complicated due to their compromised efficacy and adverse effect profile (Zayed *et al.*, 2014). As a result Morbidity and Mortality are major consequences of toxicity induced by NSAIDs (Bhandari *et al.*, 2010). Hence, the solution for this problem is to boost and reform safety profile of NSAIDs. One of the approaches is attaching the nitric oxide releasing group to the parent NSAID by a short-chain ester linkage called Nitric oxide (NO)-releasing NSAIDs (Miller *et al.*, 2007).

The advantage of NO-NSAIDs is that the anti-inflammatory and antipyretic activity of original NSAIDs will remain same.

* *Corresponding Author*
Dr. Vithal M. Kulkarni, Professor Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune – 411038 Maharashtra, India.
Tel : +91-9890802623
Fax : +91-20-25439383
E-mail : vmkulkarni75 @ gmail.com

Further, nitric oxide release leads increase in gastro-intestinal defense mechanism, over production of mucous and bicarbonate release (Bhandari *et al.,* 2009). So, NO-NSAIDS have emerged as reliable option. Among the NO-NSAIDs that have come into clinical trials are NO-naproxen, NO-aspirin, NO-ketoprofen, etc. Potency aggravated by 10 fold with minimizing the animal writhing in NO-naproxen as compared to naproxen after intra-peritoneal injection of acetic acid. *In vivo* and *in vitro* studies reveal that NO-aspirin shows more potent anti-thrombotic action as compare to aspirin. Further, NO-flurbiprofen increases renal function as well as reduces nephrotoxicity. NO-flurbiprofen and NO-aspirin debilitated the brain inflammatory response, in an animal model of chronic neurodegenerative disease.

The GI toxicity of NO-flurbiprofen and NO-naproxen is currently under clinical trials. The biphenyl moiety possess many pharmacological activities e.g. anti-tubercular (deSouzaa *et al.,* 1999; Palmer *et al.,* 2009), antimicrobial (Castellano *et al.,* 2003, Deep *et al.,* 2010), antifungal (Kokubun *et al.,* 1995; Johann *et al.,* 2010) anti-hypertensive (Sharma *et al.,* 2010; Challa *et al.,* 2014, Gentili *et al.,* 2005) and anti-inflammatory (Shah *et al.,* 2010, Bhansali *et al.,* 2015; Koster *et al.,* 2014) etc. In order to diverge from such a conventional strategy, a library of derivatives containing biphenyl 4-substituted 2-carboxamide covalently linked with NO via a linker have been attempted.

In this paper we report structure optimization, design, synthesis and pharmacological evaluation of some biphenyl derivatives coupled with NO donor moiety. We have performed docking studies using SYBYL-X 1.3 to establish the binding modes of synthesized compounds to understand mode of anti-inflammatory activity.

MATERIAL AND METHODS

Chemistry

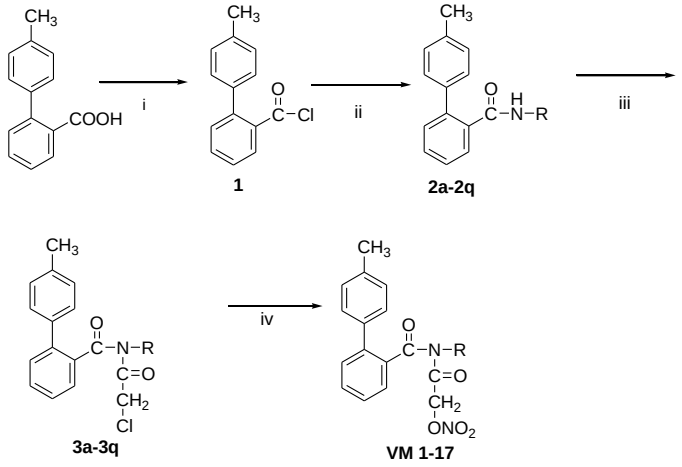
All the chemicals used were procured from commercial sources such as Sigma-Aldrich, Merck and Loba Chemie and were purified prior to use. The melting points of synthesized compounds were taken on Veego VMP-D digital melting point apparatus by open capillary. Merck pre-coated silica gel F_{254} TLC plates was used for monitoring of reaction.

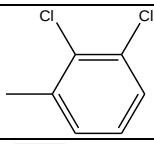
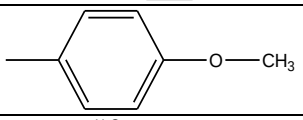
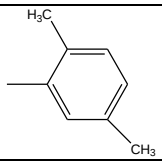
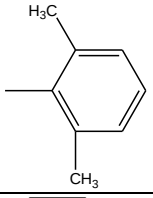
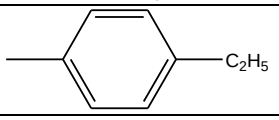
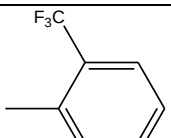
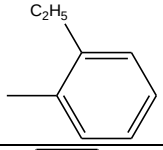
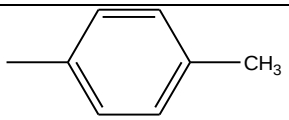
TLC plates were visualized using iodine in a chamber or observed under UV light. Fourier transform infrared (FT-IR) spectra were recorded in anhydrous potassium bromide (KBr) disk on "Jasco FTIR 4100" and are reported in cm^{-1}.

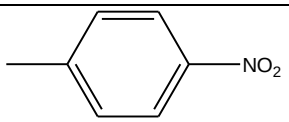
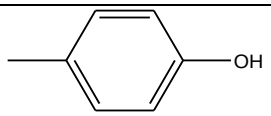
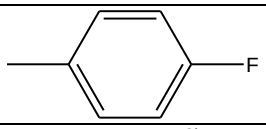
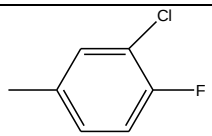
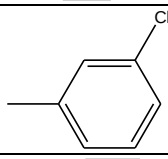
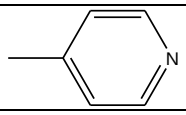
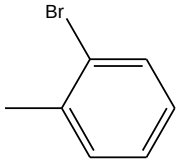
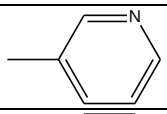
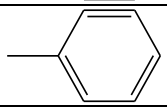
Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded in DMSO-D_6 using "BrukerAvance (400 MHz) with tetra methyl silane (TMS) as an internal standard. Elemental analysis of compounds was determined on vario MICRO V2.0.3, Elementar analysis system GmbH.

Scheme of Synthesis

Reagents and conditions: (i) thionyl chloride, 8hr, reflux (ii) various aromatic and heteroaromatic amine, triethyl amine, toluene, 16hr, reflux (iii) Chloroacetyl chloride, acetone, reflux, 4hr (iv) $AgNO_3$, Acetonitrile, stirring at room temperature, 4hr.



Compound Code	R
VM-1	
VM-2	
VM-3	
VM-4	
VM-5	
VM-6	
VM-7	
VM-8	

VM-9	
VM-10	
VM-11	
VM-12	
VM-13	
VM-14	
VM-15	
VM-16	
VM-17	

4’-Methylbiphenyl-2-carbonyl chloride (1)

4’-Methylbiphenyl-2-carboxylic acid (0.01mol) and thionyl chloride (0.05mol) were refluxed with stirring for 6-8 hr.(Shah *et al.,* 2010). The progress of the reaction was monitored by TLC using solvent system n-hexane and ethyl acetate (8:2). After the completion of reaction, remaining thionyl chloride was removed in vacuum and the residue was taken in dry toluene which was removed by rotary evaporation. The crude acid chloride was used directly without purification in the next step of reaction.

General procedure of 4’-methylbiphenyl-2-carboxamide derivatives (2a-2q)

A mixture of 4’-methyl biphenyl-2-carbonyl chloride (0.01mol), aromatic or heterocyclic amine (0.01mol) and triethylamine (0.01mol) in dry toluene (20 ml) were refluxed with stirring for 14-16 hr. (Shah *et al.,* 2010).The progress of reaction was monitored by TLC. The reaction mixture was filtered and washed with 25 ml of water to remove traces of triethylamine. The toluene layer was dried over anhydrous sodium sulphate and solvent was removed off on rotary evaporator to obtain the product. The residue was crystallized from methanol.

General procedure of N-(2-chloroacetyl)-4'-methyl-N-phenyl substituted-biphenyl]-2-carboxamide (3a-3q)

Chloroacetyl chloride (0.04 mol) was added to a solution of carboxamide (0.04 mol) in acetone (50 ml) and the reaction mixture heated under reflux on water bath for 4 hr (Bhandari *et al.,* 2010). The solvent was removed under vacuum and residue was purified over column of silica gel and eluted with chloroform. The elute was concentrated and product was crystallized with ethanol to obtain the product.

General procedure of 2-(4'-methyl-N-phenyl substituted-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM 1-17)

A solution of the appropriate chloroalkyl derivative (0.0018mol) was extracted in dry acetonitrile (2 ml) and reacted portion wise with a solution of $AgNO_3$ (0.002 mol) in dry acetonitrile (5 ml) and the mixture was stirred at room temperature for 4 hr (Bhandari *et al.,* 2010). The mixture was then filtered, evaporated to dryness, and the residue was crystallized from absolute ethanol.

Pharmacology

The 2-(4'-methyl-N-phenyl substituted)-1,1'-biphenyl-2-ylcarboxamido-2-oxoethyl nitrate derivatives (VM 1-17) were tested for anti-inflammatory, analgesic and for acute ulcerogenicity. Diclofenac was taken as a reference standard. Albino rats of Wistar strain of either sex (120–140 g) were used to execute the experiment. Animal were fasted for 24 hr prior to the experiments and water provided *ad libitum* the relative humidity was maintained at 25 ± 2ºC, 50 ± 5% and 12 hr light/dark cycle. The suspension of test compound in 1% aqueous carboxy methyl cellulose (CMC) solution was administered orally to animals under experiment.

Acute oral toxicity study

As per OECD (Organization for Economic Cooperation and Development) (AOT No. 423) guidelines adult albino mice of either sex were subjected to acute toxicity studies. Doses of 550, 1750, 2000 mg/kg of test compounds were administered orally to mice and control group received normal saline 10 ml/kg, p.o. The mice were kept in polypropylene metabolic cages and observed continuously 2hr. for behavioral, neurological and autonomic profiles and for any lethality during next 48 hr. Use of AOT 423 software was made to obtain higher doses for LD_{50} determination as per OECD guidelines.

Acetic acid-induced abdominal writhing

Irritant (acetic acid) was injected intraperitoneally to induce pain in mice (Koster *et al.,* 1959). The animals show writhing i.e. contractions of abdomen, turning of trunk and extension of hind limb, which was observed in treated groups of

animals. Mice were treated according to the reported method. The animals were divided into nineteen groups (n=6) viz;
Group 1- Acetic acid control,
Group 2- Diclofenac (10 mg/kg, p.o.),
Group 3 to Group 19 - (Compound VM 1 to 17 (10 mg/kg, p.o)

The animals were pre-treated orally with test compounds and diclofenac, 60 min before administration of acetic acid (0.9%, intra-peritoneal). Period of 15 min was allocated for cumulative counting of the number of abdominal constrictions (full extension of both hind paws). The analgesic activity was as mean number of writhes and percent inhibition, which was calculated by following formula:

$$\% \text{ Inhibition} = [Wc - Wt / Wc] * 100$$

where, Wc and Wt are mean number of writhes observed in vehicle (control) group and treatment group respectively (Table 1).

Carrageenan-induced rat hind paw edema

Literature procedure (Winter *et al.,* 1962) was followed to test the effect of synthesized compounds on acute inflammation. Female Wistar rats (175-200gm) were used for the experiment. The rats were separated into nineteen groups (n=6) viz;
Group 1- Carrageenan control,
Group 2- Diclofenac (10mg/kg, p.o.),
Group 3 to Group 19- Compound VM 1 to 17 (10mg/kg, p.o.).

The hind paw edema was produced by injecting 0.1 ml of 1% carrageenan in the right hind paw of each rat under the sub-plantar region. Rats were pre-treated with orally administered test compounds and diclofenac 1hr before carrageenan injection. The rat pedal volume up to the ankle joint was measured using plethysmometer (UgoBasile, Italy) at 1, 3, and 5 hr. after the carrageenan injection (time 0 considered). Increase in the paw edema volume was considered as the difference between 1, 3 and 5 hr and expressed as the mean difference in paw volume (ml). Percent inhibition of edema volume between treated and a control group was calculated as following formula and results were presented in Table 2.

$$\% \text{ Inhibition} = [Vc\ Vt / Vc] * 100$$

Where, Vc and Vt represent mean increase in paw volume in control and treated groups, respectively.

Acute ulcerogenicity studies

Literature procedure (Cioli *et al.,* 1979) was followed to evaluate the acute ulcers in rats. Electron microscope was used for examination of mucosal damage. Scoring system was used to assess mucosal damage. Score description 0.0 Normal (no injury). 0.5 Latent injury or widespread bleeding. 1.0 Slight injury. 2.0 Severe injury. 3.0 Very severe injury. 4.0 Widespread lined injury or widened injury/erosion.

Severity index of gastric mucosal damage was depicted by the mean score of each treated group minus the mean score of control group. Data are expressed as mean ulcer score. One-way ANOVA by Dunnett's test was used to determine the significance of the difference between the standard group and rats treated with the test compounds. The differences in results were considered significant when P was found to be <0.01.

Histopathology study

Rats were sacrificed 4 hr after the cold stress and stomach specimens were collected and put into 10% formalin solution for histopathology study (Sánchez-Fidalgo *et al.,* 2004). Greater curvature of stomach which included the ulcer base and both sides of the ulcer margin was taken and fixed in 10% formalin for 24 hr at 4º C and embedded in white solid paraffin. Eosin staining was carried out to analyze morphological changes. Changes in GI epithelial morphology were analyzed and recorded in the form of images. The results are shown in Fig. 3.

***In vitro* Nitric Oxide release assay**

Compound (20 ml) was taken in dimethyl sulfoxide (DMSO) and added to 2 ml of 1:1 v/v mixture of either 50 mM phosphate buffer (pH 7.4) or of an HCl solution (pH 1) with methanol, containing 5 × 10^{-}4 M L-cysteine. The final concentration of drug was 10^{-4} M. After 1 hr at 37 ºC, 1 ml of the reaction mixture was treated with 250 ml of Griess reagent [Sulfanilamide(4 g), N-naphthyl ethylene diaminedihydrochloride (0.2 g), 85% phosphoric acid (10 ml) in distilled water (final volume: 100 ml)]. The absorbance was measured at 540 nm. Calibration curve was prepared using Sodium nitrite standard solutions (10–80 mmol/ml). The results were expressed as the percentage of NO released (n = 2) relative to a theoretical maximum release of 1 mol NO/mol of test compound (Abadi *et al*., 2005)

Statistical analysis

Two-way ANOVA followed by post hoc Dunnett's test was used for data analysis. Data from the acetic acid induced writhing model are expressed as the mean number of writhes ± SEM and were analyzed by one-way ANOVA followed by Dunnett's test. The level of significance was set at $p < 0.05$. GraphPad Prism 5.2 (USA) statistical software was used to perform statistical calculation.

Docking

Docking was carried out using Sybyl-X 1.3. The crystal structure of Cyclooxygenase enzyme (COX-2) complexed with ibuprofen retrieved from Protein Data Bank (4PH9). Hydrogen was added and energy minimization was carried out using Tripos Force Field. All the compounds were designed using Sketch module in Sybyl and energy minimization was carried out using Tripos Force Field. The amino acid residues in a radius 5.0 Å around Ibuprofen were selected as the active site. Other docking parameters were maintained as default.

Prediction of ADMET properties for the designed derivatives

In-silico prediction of ADMET (absorption, distribution, metabolism, excretion and toxicity) properties is very important for lead identification and optimizations. AdmetSAR is a free

online server which is used to predict ADMET properties. The ADMET properties of 2-(4'-methyl-N-phenyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate derivatives were estimated using admetSAR online database (www.lmmd.ecust.edu.cn, accessed on 5thMarch 2017). It provides inclusive data for different entities linked with known ADMET profiles.

Bioactivity score prediction

Bioactivity of different 2-(4'-methyl-N-phenyl substituted)-1,1'-biphenyl-2-ylcarboxamido-2-oxoethyl nitrate derivatives is evaluated by calculating the activity score of glycoprotein coupled receptor ligand, protease inhibitor, ion channel modulator, kinase inhibitor, nuclear receptor ligand, enzyme inhibitor (Variya *et al.*, 2017) All these parameters were obtained using an online server database, molinspiration drug likeliness (www.molinspiration.com) and calculated drug likeliness scores of all derivatives were compared with the standard drug.

RESULTS AND DISCUSSION

Characterization Studies

Various 2-(4'-methyl-N-phenyl substituted)-1,1'-biphenyl-2-yl carboxamido-2-oxoethyl nitrate derivatives VM 1-17 were synthesized. Yields of final compounds were in the range of 48.00% to 74.04 % after crystallization from ethanol. Structure confirmation of synthesized compounds was done by IR, ^{1}H-NMR and mass spectroscopy.

2-(N-(2,3-dichlorophenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM -1)

Molecular formula: $C_{22}H_{16}Cl_2N_2O_5$(459.28); m.p.: 118-120^{o}C; yield 74.04%; Elemental analysis: Calcd. C, 57.53; H, 3.51; Cl, 15.44; N, 6.10; Found C, 56.50; H, 3.49; Cl, 15.20; N, 5.99;IR ν_{max} (cm^{-1}) (KBr): 1297,1665,3069,3305; ^{1}H-NMR (δ ppm; $CDCl_3$): 2.30 (s, 3H, -CH_3), 4.27 (s, 2H, -CH_2), 6.87-7.31 (m, 8H, biphenyl), 7.31-7.69 (m, 3H, -Ar).

2-(N-(4-methoxyphenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM- 2)

Molecular formula:$C_{23}H_{20}N_2O_6$ (420.41); m.p.:112-115^{o}C; yield 66.89%; Elemental analysis: Calcd. C, 65.71; H, 4.79; N, 6.66; Found C, 64.64; H, 3.99; N, 5.72; IR ν_{max} (cm^{-1}) (KBr): 1249,1647,3061,3325; ^{1}H-NMR (δ ppm; $CDCl_3$): 2.32 (s, 3H, -CH_3), 3.61 (s, 2H, -CH_2), 3.7 (s, 3H, -OCH_3), 6.80-7.37 (m, 8H, biphenyl), 7.37-7.73 (m, 4H, -Ar).

2-(N-(2,5-dimethylphenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM – 3)

Molecular formula: $C_{24}H_{22}N_2O_5$ (418.15); m.p.: 135-137^{o}C; yield 51.08%; Elemental analysis: Calcd. C, 68.89; H, 5.30; N, 6.69; Found C, 68.81; H, 5.12; N, 6.52;IR ν_{max} (cm^{-1}) (KBr): 1247,1671,3100,3321; ^{1}H-NMR (δ ppm; $CDCl_3$): 2.39 (s, 6H, -CH_3), 2.51 (s, 3H, CH_3), 4.28 (s, 2H, -CH_2), 6.84-7.40 (m, 8H, biphenyl), 7.40-8.21 (m, 4H, -Ar).

2-(N-(2,6-dimethylphenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM- 4)

Molecular formula: $C_{24}H_{22}N_2O_5$ (418.15); m.p.: 131-134^{o}C; yield 57.15%; Elemental analysis: Calcd. C, 68.89; H, 5.30; N, 6.69; Found C, 68.10; H, 5.21; N, 6.27;IR ν_{max} (cm^{-1}) (KBr): 1240,1698,3124,3320; ^{1}H-NMR (δ ppm; $CDCl_3$):2.10(s, 6H, -CH_3), 2.36 (s, 3H, -CH_3), 4.21 (s, 2H, -CH_2), 6.98-7.57 (m, 8H, biphenyl), 7.57-8.11 (m, 3H, -Ar).

2-(N-(4-ethylphenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM-5)

Molecular formula: $C_{24}H_{22}N_2O_5$ (418.15); m.p.: 146-148 ^{o}C; yield 63.83%; Elemental analysis: Calcd. C, 68.89; H, 5.30; N, 6.69; Found C, 68.46; H, 5.25; N, 6.59;IR ν_{max} (cm^{-1}) (KBr): 1247,1671,3100,3321;^{1}H-NMR (δ ppm; $CDCl_3$): 1.30 (s, 3H, -CH_3), 2.60 (s, 2H, -CH_2), 2.36 (s, 3H, -CH_3), 4.21 (s, 2H, -CH_2), 6.98-7.57 (m, 8H, biphenyl), 7.57-8.11 (m, 4H, -Ar).

2-(4'-methyl-N-(2-(trifluoromethyl)phenyl)-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM- 6)

Molecular formula: $C_{23}H_{17}F_3N_2O_5$ (458.11);m.p.: 127-129^{o}C; yield 48.02%; Elemental analysis: Calcd. C, 60.26; H, 3.74; F, 12.43; N, 6.11;Found C, 60.34; H, 3.71; F, 12.39; N,6.19; IR ν_{max} (cm^{-1}) (KBr): 1220, 1700, 3110, 3249; ^{1}H-NMR (δ ppm; $CDCl_3$): 2.39 (s, 3H, -CH_3), 4.10 (s, 2H, -CH_2), 6.18-.7.53 (m, 8H, biphenyl), 7.53-7.95 (m, 4H, -Ar).

2-(N-(2-ethylphenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM- 7)

Molecular formula: $C_{24}H_{22}N_2O_5$ (418.15); m.p.:128-130 ^{o}C; yield 65.24%; Elemental analysis: Calcd. C, 68.89; H, 5.30; N, 6.69; Found C, 68.27; H, 5.21; N, 6.52;IR ν_{max} (cm^{-1}) (KBr): 1220, 1700,3110,3249;^{1}H-NMR (δ ppm; $CDCl_3$): 1.30 (s, 3H, -CH_3), 2.39 (s, 3H, -CH_3), 2.60 (s, 2H, -CH_2), 4.10 (s, 2H, -CH_2), 6.18-.7.53 (m, 8H, biphenyl), 7.53-7.95 (m, 4H, -Ar).

2-(4'-methyl-N-(p-tolyl)-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM- 8)

Molecular formula: $C_{23}H_{20}N_2O_5$ (404.42);m.p.: 133-135^{o}C; yield 66.89%; Elemental analysis: Calcd. C, 68.31; H, 4.98; N, 6.93; Found C, 68.31; H, 4.98; N, 6.93; IR ν_{max} (cm^{-1}) (KBr): 1240, 1710,3105, 3254;^{1}H-NMR (δ ppm; $CDCl_3$): 2.28 (s, 2H, -CH_2), 2.34 (s, 3H, -CH_3), 4.27 (S, 2H, -CH_2), 7.00-.7.27 (m, 8H, biphenyl), 7.57-7.69 (m, 4H, -Ar).

2-(4'-methyl-N-(4-nitrophenyl)-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM- 9)

Molecular formula: $C_{22}H_{17}N_3O_7$ (435.39); m.p.: 160-162^{o}C; yield 71.08%; Elemental analysis: Calcd. C, 60.69; H, 3.94; N, 9.65; Found C, 60.61; H, 3.75; N, 9.60;IR ν_{max} (cm^{-1}) (KBr): 1205,1680,3065,3253; ^{1}H-NMR (δ ppm; $CDCl_3$):2.42 (s,

3H, -CH_3), 4.14 (s, 2H, -CH_2), 7.21-7.54 (m, 8H, biphenyl), 7.81-8.14 (m, 4H, -Ar).

2-(N-(4-hydroxyphenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM -10)

Molecular formula: $C_{22}H_{18}N_2O_6$ (406.39); m.p.: 138-140°C; yield 55.15%; Elemental analysis: Calcd. C, 65.02; H, 4.46; N, 6.89; Found C, 65.12; H, 4.81; N, 6.96;IR ν_{max} (cm^{-1}) (KBr): 1241,1610,3100,3302; ^{1}H-NMR (δ ppm; $CDCl_3$): 2.35 (s, 3H, -CH_3), 3.38 (s, 2H, -CH_2),5.21 (s, ^{1}H, -OH), 7.32-7.60 (m, 8H, biphenyl), 7.60-7.75 (m, 4H, -Ar).

2-(N-(4-fluorophenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM- 11)

Molecular formula: $C_{22}H_{17}N_2O_7F$ (408.38);m.p.: 118-120°C; yield 59.83%; Elemental analysis: Calcd. C, 64.70; H, 4.20; F, 4.65; N, 6.86; Found C, 64.85; H, 4.29; F, 4.56; N, 6.88;IR ν_{max} (cm^{-1}) (KBr): 1210,1631,3110,3321;^{1}H-NMR (δ ppm; $CDCl_3$): 2.5 (s, 3H, -CH_3), 4.20 (S, 2H, -CH_2), 7.10-7.41 (m, 8H, biphenyl), 7.42-7.55 (m, 4H, -Ar).

2-(N-(3-chloro-4-fluorophenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM -12)

Molecular formula: $C_{22}H_{16}ClFN_2O_5$ (442.82); m.p.: 157-159°C; yield 61.02%; Elemental analysis: Calcd. C, 59.67; H, 3.64; Cl, 8.01; F, 4.29; N, 6.33; Found C, 59.92; H, 3.41; Cl, 8.72; F, 4.34; N, 6.39;IR ν_{max} (cm^{-1}) (KBr): 1221,1621,3051,3254; ^{1}H-NMR (δ ppm; $CDCl_3$): 2.30 (s, 3H, -CH_3), 4.31 (s, 2H, -CH_2), 6.91-7.40 (m, 8H, biphenyl), 8.18-8.71 (m, 3H, -Ar).

2-(N-(3-chlorophenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM -13)

Molecular formula: $C_{22}H_{17}ClN_2O_5$ (424.83); m.p.: 118-120°C; yield 60.89%; Elemental analysis: Calcd. C, 62.20; H, 4.03; Cl, 8.35; N, 6.59; Found C, 62.92; H, 4.12; Cl, 8.92; N, 6.50; IR ν_{max} (cm^{-1}) (KBr): 1210,1608,3154,3351; ^{1}H-NMR (δ ppm; $CDCl_3$): 2.31 (s, 3H, -CH_3), 4.13 (s, 2H, -CH_2), 6.80-7.21 (m, 8H, biphenyl), 8.21-8.71 (m, 4H, -Ar).

2-(4'-methyl-N-(pyridin-4-yl)-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM -14)

Molecular formula: $C_{21}H_{17}N_3O_5$ (391.83); m.p.: 141-143°C; yield 70.08%; Elemental analysis: Calcd. C, 64.45; H, 4.38; N, 10.74; Found C, 64.40; H, 4.31; N, 10.27;IR ν_{max} (cm^{-1}) (KBr): 1210,1608,3154,3351;^{1}H-NMR (δ ppm; $CDCl_3$): 2.31 (s, 3H, -CH_3), 4.13 (s, 2H, -CH_2), 6.80-7.21 (m, 8H, biphenyl), 8.21-8.71 (m, 4H, -Ar).

2-(N-(2-bromophenyl)-4'-methyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM -15)

Molecular formula: $C_{22}H_{17}BrN_2O_5$ (469.28); m.p.: 126-129°C; yield 55.15%; Elemental analysis: Calcd. C, 56.31; H, 3.65; Br, 17.03; N, 5.97; Found C, 56.20; H, 3.61; Br, 17.10; N, 5.82; IR ν_{max} (cm^{-1}) (KBr): 1205,1613,3121, 3251;^{1}H-NMR (δ ppm; $CDCl_3$): 2.50 (s, 3H, -CH_3), 4.28 (s, 2H, -CH_2), 7.05-7.23 (m, 8H, biphenyl), 8.42-8.55 (m, 4H, -Ar).

2-(4'-methyl-N-(pyridin-3-yl)-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM – 16)

Molecular formula: $C_{21}H_{17}N_3O_5$ (391.38); m.p.: 118-120°C; yield 59.83%; Elemental analysis: Calcd. C, 64.45; H, 4.38; N, 10.74; Found C, 64.32; H, 4.31; N, 10.70;IR ν_{max} (cm^{-1}) (KBr): 1158,1664,3158,3254;^{1}H-NMR (δ ppm; $CDCl_3$): 2.31 (s, 3H, -CH_3), 4.73 (s, 2H, -CH_2), 7.10-7.41 (m, 8H, biphenyl), 8.43-8.96 (m, 4H, –Ar).

2-(4'-methyl-N-phenyl-[1,1'-biphenyl]-2-ylcarboxamido)-2-oxoethyl nitrate (VM – 17)

Molecular formula: $C_{22}H_{18}N_2O_5$ (390.39); m.p.: 120-123°C; yield 71.02%; Elemental analysis: Calcd. C, 67.69; H, 4.65; N, 7.18; Found C, 67.60; H, 4.60; N, 7.11;IR ν_{max} (cm^{-1}) (KBr): 1158, 1664, 3158, 3254;^{1}H-NMR (δ ppm; $CDCl_3$): 2.31 (s, 3H, -CH_3), 4.73 (s, 2H, -CH_2), 7.10-7.41 (m, 8H, biphenyl), 8.21-8.62 (m, 4H, –Ar).

Acute oral toxicity

The acute toxicity of compounds VM-4, VM-6, VM-9, VM-10 and VM-12 in mice was found to be greater than 2000 mg/kg. No lethal or toxic reactions were reported up to the anti-inflammatory dose range. All the compounds were having LD_{50} greater than 2000 mg/kg.

Analgesic activity

Analgesic activity was carried out using acetic acid induced writhing model. Writhing test is based on tissue injury increase the sensitivity to pain. Compounds VM-4, VM-6, VM-9, VM-12 were active compounds in the present series. The results of analgesic activity are present in Table 1. The graph of analgesic activity is present in Figure 1.

Table 1: Effect of VM1-16 series on writhing induced by acetic acid in mice and *in vitro* release of nitric oxide.

Group	No. of writhing ± SEM	% inhibition	% NO release
Acetic acid control	56 ± 0.9	-	-
Diclofenac	27 ± 0.8	51.79***	-
VM-1	49 ± 0.7	12.50**	3.79
VM-2	50 ± 0.9	10.72*	4.23
VM-3	42 ± 0.8	25***	2.88
VM-4	31 ± 0.8	44.65***	5.21
VM-5	47 ± 1.1	16.08***	8.12
VM-6	34 ± 0.8	39.29***	8.56
VM-7	49 ± 0.6	12.50***	5.81
VM-8	48 ± 0.7	14.29**	7.45
VM-9	32 ± 0.7	42.86***	9.96
VM-10	38 ± 0.6	32.15***	10.39
VM-11	47 ± 0.9	16.08***	2.47
VM-12	31 ± 0.8	44.65***	9.97
VM-13	46 ± 1.4	17.86**	3.42
VM-14	45 ± 1.3	19.65***	3.68
VM-15	50 ± 1.0	10.72***	7.81
VM-16	52 ± 1.3	7.15*	4.03
VM-17	39± 0.9	30.36***	4.23

Values are mean ± SEM., n=6 in each group; Statistical analysis by One-way ANOVA followed by Dunnett's test using Graphpad 5.2 software; P value *<0.05; **<0.01; ***<0.001 compared to vehicle treated group.

Fig. 1: Analgesic activity of VM 1-17 compared with standard drug determined by acetic acid induced writhing in mice.

Table 2: Effect of VM 1-17 series on carrageenan induced rat hind paw edema.

Group	Difference in paw volume at hrs (Mean +SEM)		
	1 h	3h	5h
Carrageenan control	1.31 ± 0.01	1.49 ± 0.02	1.78 ± 0.02
Diclofenac	1.20 ± 0.02*(8.40)	1.03 ± 0.03***(12.76)	0.96 ± 0.01***(46.07)
VM-1	1.27 ± 0.01^{ns}(3.06)	1.32 ± 0.01***(11.41)	1.37 ± 0.01***(23.04)
VM-2	1.27 ± 0.02 ^{ns}(3.06)	1.23 ± 0.02***(17.45)	1.20 ± 0.02***(32.59)
VM-3	1.17 ± 0.03*(10.69)	1.28 ±0.01***(14.10)	1.35 ±0.02***(44.39)
VM-4	1.24 ± 0.02 ^{ns}(5.35)	1.16 ± 0.01***(22.15)	0.99 ± 0.02***(24.15)
VM-5	1.28 ± 0.03 ^{ns}(2.30)	1.47 ± 0.01 ^{ns}(1.35)	1.59 ± 0.01***(10.68)
VM-6	1.21 ± 0.02 ^{ns}(7.64)	1.09 ± 0.02***(26.85)	0.98 ± 0.03***(44.95)
VM-7	1.23 ± 0.02 ^{ns}(6.11)	1.34 ± 0.02***(10.07)	1.40 ± 0.02***(21.35)
VM-8	1.26 ± 0.01 ^{ns}(3.82)	1.30 ± 0.02***(12.76)	1.28 ± 0.01***(28.81)
VM-9	1.24 ± 0.01 ^{ns}(5.35)	1.22 ± 0.01***(18.13)	1.10 ± 0.01***(38.21)
VM-10	1.26 ± 0.01 ^{ns}(3.82)	1.18 ± 0.01***(20.81)	1.07 ± 0.03***(39.89)
VM-11	1.29 ± 0.02 ^{ns}(1.53)	1.44 ± 0.02^{ns}(3.34)	1.63 ± 0.01***(8.43)
VM-12	1.27 ± 0.02 ^{ns}(3.03)	1.07 ± 0.01***(28.19)	0.99 ± 0.01***(44.39)
VM-13	1.28 ± 0.01 ^{ns}(2.30)	1.38 ± 0.01*(7.39)	1.52 ± 0.02***(14.61)
VM-14	1.23 ± 0.02 ^{ns}(6.11)	1.28 ± 0.02***(14.04)	0.99 ± 0.01***(44.39)
VM-15	1.29 ± 0.01 ^{ns}(1.55)	1.41 ± 0.01 ^{ns}(5.37)	1.37 ± 0.01***(23.04)
VM-16	1.26 ± 0.02 ^{ns}(3.82)	1.32 ± 0.01***(20.81)	1.39 ± 0.01***(21.92)
VM-17	1.27 ± 0.01 ^{ns}(3.03)	1.35 ± 0.02***(9.4)	1.48 ± 0.01***(16.86)

Values are mean ± SEM., n=6 in each group; Statistical analysis by Two-way ANOVA followed by bonferronipost hoc Dunnett's test using Graphpad5.2 software; ns- non significant P value *<0.05; **<0.01; ***<0.001 compared to vehicle (carboxymethyl cellulose, 10 ml/kg) treated group. The figures in parentheses indicatethe percent inhibition.

Anti-inflammatory activity

Carrageenan-induced rat paw edema assay method was used to compare anti-inflammatory activity. The obtained results are given in Table 2. The pharmacological experiments of this study showed that among the tested compounds VM-4, VM-6, VM-9, VM-10, VM-11, VM-12 exhibited significant anti-inflammatory activities as compared to standard NSAIDs. For comparing the anti-inflammatory activity diclofenac sodium was used as a standard. The biphasic edema induced by carrageenan mediates its first phase by the release of histamine and 5- hydroxytryptamine followed by kinin release and then prostaglandin in the later phase. In most clinically effective anti-inflammatory agents the second phase (3 hour) of edema is reported to be most sensitive. Anti-inflammatory effects of these series in third hour of edema suggest involvement of inhibition of prostaglandin.

Compound VM-11 and VM-12 shows highest anti-inflammatory activity in the series. Compound VM-6 shows similar activity as compared to standard drug. Comparison of anti-inflammatory activity with standard drug is shown in Figure 2.

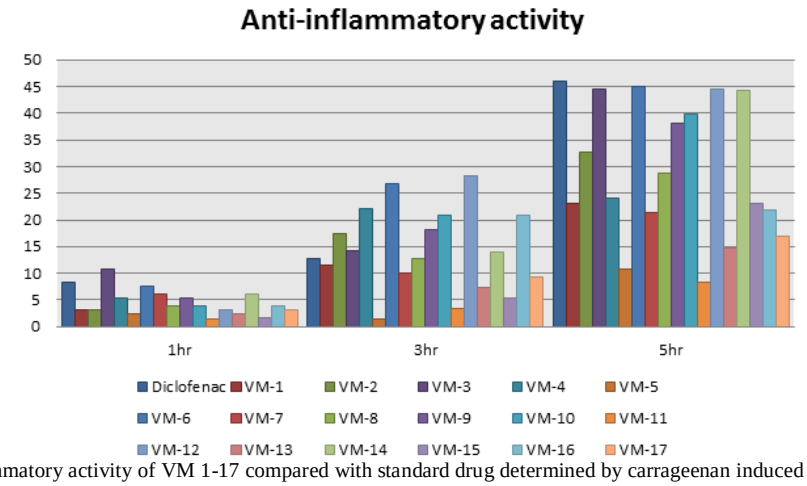


Fig. 2: Anti-inflammatory activity of VM 1-17 compared with standard drug determined by carrageenan induced rat hind paw edema.

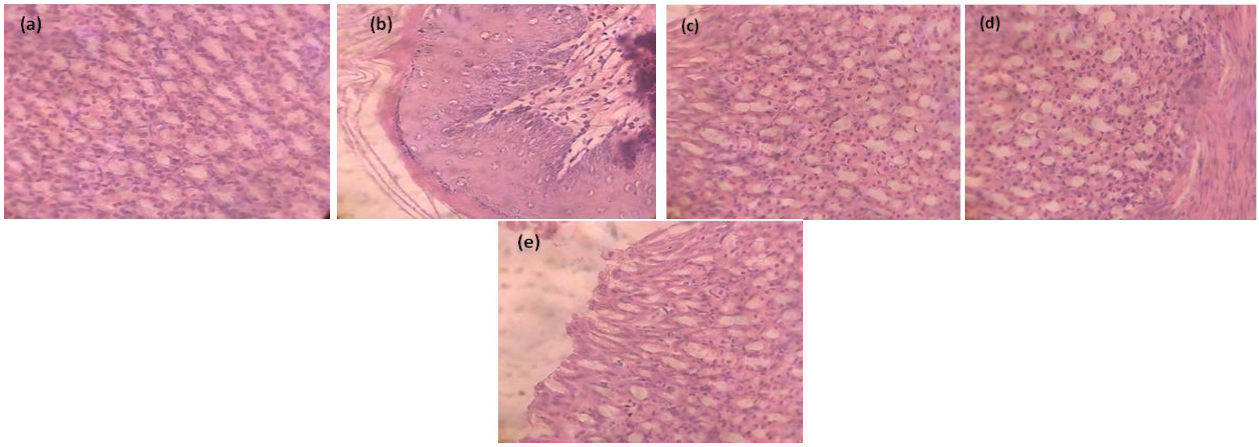


Fig. 3: Haematoxylin and eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats. As illustrated in figure specimen: (a) showed intact mucous membrane in control treated rat showing granular tissues composed of macrophages, fibroblasts and endothelial cells forming microvessels. (b) Congestion of mucosal blood vessels was observed in diclofenac treated group. (c) Less gastric damage was observed to mucosa of rat treated with test compound, VM 16. (d) Disruption of gastric epithelial layer was observed for VM 6 specimen but less than diclofenac. (e) Disruption of gastric epithelial layer was observed for VM 4 specimen but less than diclofenac.

Acute ulcerogenicity studies

The compounds VM-4, VM-6, VM-9, VM-10 and VM-12 which possessed promising analgesic activity and anti-inflammatory activity were further screened for ulcerogenicity activity of stomach. Compounds VM-6, VM-9, VM-10 and VM-12 showed less signs of gastric ulceration compared to standard drug. The ulcer index is shown in Table 3. A less incidence of gastric erosion was observed for the highest active compound VM-12.

Table 3: Ulcer index of most active compounds.

Group	Ulcer index
Diclofenac	1.84
VM-6	1.03
VM-9	1.18
VM-10	1.43
VM-12	0.92

Histopathology study

The protective mucous layer of stomach specimen showed complete disruption along with severe ulceration in diclofenac treated rat (Fig. 3 specimen b). The gastric epithelial layer showed complete disruption by proliferation and migration of some epithelial cells from ulcer margin into ulcer crater in the tissue of diclofenac treated rat.

Electron microscope was used for scanning of stomach specimen which unfolded that the rat treated with 2-(4'-methyl-N-phenyl-[1,1'-biphenyl]-2-ylcarboxamido)- 2-oxoethyl nitrate derivatives (Fig. 3 specimen c and d) showed less gastric mucosal injury compared with diclofenac treated group, while disruption of gastric epithelial layer was observed in Fig. 3 specimen e but less than diclofenac.

Molecular Docking

To investigate interaction between Cyclooxygenase enzyme and synthesized derivatives molecular docking was carried out.

Active compounds VM-4 binds with ARG amino acid. While VM-6 binds with ARG and ASN amino acid with docking score of 4.5 and 4.9 respectively.

Prediction of ADMET properties for the synthesized compounds

Solubility of the drug is one of the crucial parameter which affects the pharmacokinetic and pharmacodynamics of the drug, starting from the site of drug administration, absorption into systemic circulation, movement in the blood and excretion from the body. ADMET of final biphenyl derivatives were calculated using online software admetSAR. Various permeability parameters such as Blood Brain Barrier (BBB) penetration, $Caco_2$ cell permeability Human Intestinal Absorption (HIA), renal organic cation transport and AMES toxicity test were calculated by using software. P-glycoprotein is one of the membrane efflux transporter having vital role determining the absorption, distribution, metabolism, excretion, and toxicology behaviors of some drugs and molecules in development and also plays a major role in the multidrug resistance (MDR) phenomenon. P-gp substrates recognition at the early stages of the drug discovery process is very important. (Li D *et al.*, 2014, Raub, 2006) Cytochrome enzymes (CYPs) are involved in the metabolisms of the majority of therapeutic drugs. CYPs are the most important drug-metabolizing enzymes and play important roles in the detoxification of xenobiotic and the biosynthesis of endogenous molecules (He *et al.*, 2015) Carcinogenic potential of the drug have direct or indirect correlation with the molecular properties of the compounds. Carcinogenicity, oral toxicity and acute dose toxicity in rat (LD_{50}) were summarized in Table 4 Based on the obtained data from admet SAR, all the biphenyl derivatives may be able to pass through the human intestine barrier and can be absorbed from the intestine. Most of synthesized derivatives does not show any toxicity and mutagenicity.

Table 4.1: Predicted biological permeability of standards and synthesized biphenyl derivatives.

Molecules	Blood Brain Barrier	Human Intestinal Absorption	$CACO_2$ Permeability	Renal Organic Cation Transporter	AMES Toxicity	Biodegradation
	Probability	Probability	Probability	Probability	Probability	Probability
Diclofenac	0.9541	0.9548	0.8867	0.9086	0.9133	0.9719
VM-1	0.9199	0.9827	0.5278	0.8566	0.6303	0.9491
VM-2	0.7985	0.9665	0.5384	0.8342	0.7553	0.8592
VM-3	0.8409	0.9713	0.5184	0.8917	0.6690	0.8954
VM-4	0.8409	0.9713	0.5184	0.8917	0.6690	0.8954
VM-5	0.8497	0.9894	0.5211	0.8990	0.7357	0.7935
VM-6	0.9313	0.9842	0.5317	0.8833	0.6264	0.9922
VM-7	0.8497	0.9894	0.5211	0.8990	0.7357	0.7935
VM-8	0.8753	0.9734	0.5187	0.8779	0.7448	0.8414
VM-9	0.8867	0.9923	0.5139	0.8663	0.7981	0.8848
VM-10	0.7001	0.9503	0.5653	0.8587	0.7706	0.8145
VM-11	0.9384	0.9825	0.5317	0.8717	0.6470	0.9831
VM-12	0.9381	0.9917	0.5324	0.8658	0.6015	0.9847
VM-13	0.9199	09827	0.5278	0.8566	0.6303	0.9491
VM-14	0.8484	0.9652	0.5362	0.8441	0.7784	0.8734
VM-15	0.9135	0.9751	0.5342	0.8548	0.6455	0.9600
VM-16	0.7985	0.9538	0.5387	0.8472	0.7868	0.9449
VM-17	0.9019	0.9795	0.5199	0.8652	0.7801	0.7395
	BBB+	HIA+	#$CACO_2^-$	Non Inhibitor	$AMES Non Toxic	Non Readily Biodegradable

Table 4.2: Predicted biological permeability of standards and synthesized biphenyl derivatives.

Molecules	P-Glycoprotein Substrate	CYP-3A4 Inhibitors	CYP-2D6 Inhibitors	CYP Inhibitory Promiscuity	HERG Inhibition
	Probability	Probability	Probability	Probability	Probability
Diclofenac	0.7976	0.6724	0.8480	0.6607	0.9514
VM-1	0.8028	0.6459	0.8113	0.8834	0.9380
VM-2	0.7776	0.7124	0.8744	0.7336	0.9232
VM-3	0.8033	0.6593	0.8929	0.6940	0.9615
VM-4	0.8033	0.6593	0.8929	0.6940	0.9615
VM-5	0.7693	0.6196	0.8908	0.7452	0.9704
VM-6	0.7953	0.6187	0.8699	0.8320	0.9918
VM-7	0.7693	0.6196	0.8908	0.7452	0.9704
VM-8	0.7997	0.6378	0.8925	0.7375	0.9646
VM-9	0.7937	0.6091	0.8655	0.8445	0.9388
VM-10	0.7637	0.6413	0.8322	0.6443	0.9173
VM-11	0.7893	0.6150	0.8472	0.8323	0.9617
VM-12	0.8015	0.6426	0.8259	0.8592	0.9408
VM-13	0.8028	0.6459	0.8113	0.8834	0.9380
VM-14	0.7733	0.6260	0.8542	0.7589	0.9543
VM-15	0.7962	0.6143	0.8369	0.8944	0.9579
VM-16	0.6983	0.6100	0.8815	0.6945	0.9466
VM-17	0.8009	0.6041	0.8764	0.7759	0.9531
	Non Substrate	Non Inhibitor	Non Inhibitor	*High CYP Inhibitory Promiscuity	Weak Inhibitors

Table 4.3: Predicted biological permeability of standards and synthesized biphenyl derivatives.

Molecules	Carcinogens	Acute Oral Toxicity	Carcinogenicity (Three Class)	Rat Acute Toxicity LD50, Mol/Kg	*Tetrahymena pyriformis* Toxicity pIGC50 (Ug/L)
	Probability	Probability	Probability	Probability	Probability
Diclofenac	0.6706	0.7602	0.7245	3.6447	0.8854
VM-1	0.6538	0.6468	0.5430≠	2.4925	1.3391
VM-2	0.5072	0.6087	0.4766≠	2.7651	0.8336
VM-3	0.6695	0.6368	0.4617	2.6603	0.9858
VM-4	0.6695	0.6368	0.4617	2.6603	0.9858
VM-5	0.6697	0.6325	0.4842	2.7120	0.8857
VM-6	0.6327	0.6244	0.5353	2.6677	1.1815
VM-7	0.6697	0.6325	0.4842	2.7120	0.8857
VM-8	0.6363	0.6376	0.4868	2.6546	0.8948
VM-9	0.5587	0.6630	0.5166	2.6167	0.9305
VM-10	0.5053	0.6683	0.4869	2.6306	0.9432
VM-11	0.6215	0.6206	0.5273	2.5975	1.1642
VM-12	0.6571	0.6349	0.5456	2.5347	1.5169
VM-13	0.6538	0.6468	0.5430	2.4925	0.3391
VM-14	0.5865	0.6263	0.4631	2.7180	0.8757
VM-15	0.6039	0.6351	0.5084	2.5986	1.4188
VM-16	0.5737	0.6273	0.4591	2.6680	0.9650
VM-17	0.5815	0.6535	0.4843	2.6242	0.8890
	Non Carcinogens	Class-III	≠Danger		

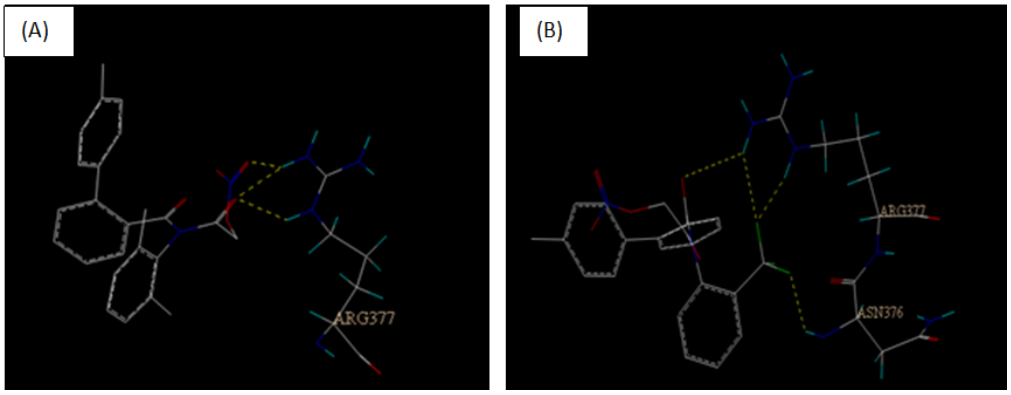


Fig. 4: Docking interaction of active compound (A) VM 4 , (B) VM 6.

CONCLUSION

A novel series of biphenyl derivatives possessing a variety of hetero aryl amines attached to nitric oxide releasing group were synthesized. Structure of all final compounds were characterized by IR, ^{1}H-NMR, mass and elemental analysis and were subjected for analgesic and anti-inflammatory activity by *in vivo* models and were subjected to nitric oxide releasing studies. The compound VM-12 was found as the most active. The synthesized compounds VM-4, VM-6, VM-9, VM-10 VM-11 exhibited significant analgesic and anti-inflammatory activity compared to standard drug. Furthermore, these compounds showed little ulcerogenic activity as that of diclofenac. Histopathological studies showed that synthesized derivatives not just maintained but showed aggravated anti-inflammatory activity and are without gastro intestinal toxicities and also exhibit good nitric oxide releasing property. Structure activity relationship study indicates that VM-11 having fluorine atom in the structure increases the activity. VM-12 having fluorine and chlorine atoms and VM-6 having trifluoro group results in good activity. VM-9 having NO_2 in the structure also increases the activity. The synthesized compounds along with their anti-inflammatory activity were further subjected for docking and ADMET studies. Molecular docking study revealed that these compounds fit into the cavity of COX-2 receptor via interaction with ARG amino acid. Also, the pharmacokinetic parameters for the entire 17 synthesized compounds were obtained within the acceptable range defined for human use revealing their potential as possible drug-like compounds. Hence these compounds can serve as good leads for further modification and optimization to obtain better compounds.

ACKNOWLEDGEMENT

One of the authors (Monika G. shinde) is grateful to Dr. Babasaheb Ambedkar Research and Training Institute (BARTI) for award Dr. Babasaheb Ambedkar National Research Fellowship (BANRF). We are also thankful to Department of Pharmacology

for their support. The authors are thankful to Dr. S.S. Kadam, Vice Chancellor and Dr. K. R. Mahadik, Principal, Poona College of Pharmacy, Pune for their help.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

REFERENCES

Abadi AH, Hegazy GH, El-Zaher AA. Synthesis of novel 4-substituted-7-trifluoromethylquinoline derivatives with nitric oxide releasing properties and their evaluation as analgesic and anti-inflammatory agents. Bioorg Med Chem. 2005;13(20):5759-5765.

Bhandari S V, Dangre SC, Bothara KG, *et al*. Design, synthesis and pharmacological screening of novel nitric oxide donors containing 1, 5-diarylpyrazolin-3-one as nontoxic NSAIDs. Eur J Med Chem. 2009; 44(11):4622-4636.

Bhandari S V, Parikh JK, Bothara KG, *et al*. Design, synthesis, and evaluation of anti-inflammatory, analgesic, ulcerogenicity, and nitric oxide releasing studies of novel indomethacin analogs as non-ulcerogenic derivatives. J Enzyme Inhib Med Chem. 2010;25(4):520-530.

Bhansali SG, Kulkarni VM. Design, synthesis, docking, QSAR, ADME studies and pharmacological evaluation of biphenyl-2-oxadiazoles as anti-inflammatory agents. Der Pharma Chemica. 7(1):156-173.

Castellano S, Stefancich G, Chillotti A, Poni G. Synthesis and antimicrobial properties of 3-aryl-1-(1, 1′-biphenyl-4-yl)-2-(1H-imidazol-1-yl) propanes as "carba-analogues" of the N-arylmethyl-N-[(1, 1′-biphenyl)-4-ylmethyl])-1H-imidazol-1-amines, a new class of antifungal agents. Farm. 2003; 58(8): 563-568.

Challa NR, Mamidisetty B, Ghanta MR, Padi PR. Synthesis and pharmacological evaluation of 5-[2′-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-4, 5, 6, 7-tetrahydro-thieno [3, 2-c] pyridine derivatives as platelet aggregation inhibitors. J Saudi Chem Soc. 2014;18(5):513-519.

Cioli V, Putzolu S, Rossi V, Barcellona PS, Corradino C. The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats.ToxicolApplPharmacol. 1979;50(2):283-289.

de Souzaa AO, Alderete JB, Schimidt F, Sato DN, Durána N. Structure-Activity Relationship Analysis of 4'-Bromo-[1, 1'-biphenyl]-4-yl 4-X-Phenyl Methanone Derivatives and Activity against Mycobacterium tuberculosis. Arzneimittel-forschung. 1999;49(12):1025-1029.

Deep A, Jain S, Sharma PC, Verma P, Kumar M, Dora CP. Design and biological evaluation of biphenyl-4-carboxylic acid hydrazide-hydrazone for antimicrobial activity. Synthesis (Stuttg). 2010;182:183OC.

Fiorucci S, Antonelli E, Burgaud J-L, Morelli A. Nitric Oxide—Releasing NSAIDs. Drug Saf. 2001;24(11):801-811.

Gentili F, Bousquet P, Carrieri A, *et al*. Rational design of the new antihypertensive I1-receptor ligand 2-(2-biphenyl-2-yl-1-methyl-ethyl)-4, 5-dihydro-1H-imidazole. Lett Drug Des Discov. 2005;2(8):571-578.

He W, Wu J-J, Ning J, *et al*. Inhibition of human cytochrome P450 enzymes by licochalcone A, a naturally occurring constituent of licorice. ToxicolVitr. 2015;29(7):1569-1576.

Husain A, Ahmad A, Khan SA, Asif M, Bhutani R, Al-Abbasi FA.Synthesis, molecular properties, toxicity and biological evaluation of some new substituted imidazolidine derivatives in search of potent anti-inflammatory agents. Saudi Pharm J. 2016;24(1):104-114.

Johann S, Sá NP, Lima LARS, *et al*. Antifungal activity of schinol and a new biphenyl compound isolated from *Schinus terebinthifolius* against the pathogenic fungus *Paracoccidioides brasiliensis*. Ann Clin Microbiol Antimicrob. 2010; 9(1):30.

Kokubun T, Harborne JB, Eagles J, Waterman PG. Antifungal biphenyl compounds are the phytoalexins of the sapwood of Sorbusaucuparia. Phytochemistry. 1995;40(1):57-59.

Koster R, Anderson M, De Beer EJ.Acetic acid-induced analgesic screening. In: FEDERATION PROCEEDINGS; 1959.

Li D, Chen L, Li Y, Tian S, Sun H, Hou T. ADMET evaluation in drug discovery. 13. Development of in silico prediction models for p-glycoprotein substrates. Mol Pharm. 2014;11(3):716-726.

Mahdi MF, Raauf AM, Kadhim FA. Design, Synthesis and Acute Anti-Inflammatory Evaluation of New Non-Steroidal Anti-Inflammatory Agents Having 4-Thiazolidinone Pharmacophore. J Nat Sci Res. 2015;5(6):21-28.

Miller MR, Megson IL. Recent developments in nitric oxide donor drugs.Br J Pharmacol. 2007;151(3):305-321.

Palmer BD, Thompson AM, Sutherland HS, *et al*. Synthesis and Structure– Activity Studies of Biphenyl Analogues of the Tuberculosis Drug (6 S)-2-Nitro-6-{[4-(trifluoromethoxy) benzyl] oxy}-6, 7-dihydro-5 H-imidazo [2, 1-b][1, 3] oxazine (PA-824). J Med Chem. 2009;53(1):282-294.

Raub TJ. P-glycoprotein recognition of substrates and circumvention through rational drug design. Mol Pharm. 2006;3(1):3-25.

Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. J Lipid Res. 2009;50(Supplement):S29-S34.

Sánchez-Fidalgo S, Martín-Lacave I, Illanes M, Motilva V. Angiogenesis, cell proliferation and apoptosis in gastric ulcer healing. Effect of a selective cox-2 inhibitor.Eur J Pharmacol. 2004;505(1):187-194.

Shah, U.A., Wagh, N.K., Deokar, H.S., Kadam, S.S., Kulkarni, V.M. Design, synthesis, pharmacological screening and molecular docking of biphenyl analogues as anti-inflammatory agents. Int. J. Pharm. Biosci., 2010; 1(4): 501-511.

Sharma MC, Kohli D V, Sharma S, Sharma AD. Synthesis and antihypertensive activity of some new benzimidazole derivatives of 4′-(6-methoxy-2-substituted-benzimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid in the presences of BF3· OEt2.Pel Res Lib. 2010; 1(1):104-115.

Variya, B., Modi, S., Savjani, J., & Patel, S., In silico molecular docking and pharmacokinetic prediction of gallic acid derivatives as PPAR-γ Agonists. International Journal of Pharmacy and Pharmaceutical Sciences. 2017; 9(1):102-107.

Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. ExpBiol Med. 1962;111(3):544-547.

Zayed MF, Hassan MH. Synthesis and biological evaluation studies of novel quinazolinone derivatives as antibacterial and anti-inflammatory agents. Saudi Pharm J. 2014; 22(2): 157-162.

How to cite this article:

Shinde MG, Modi SJ, Kulkarni VM. Synthesis, Pharmacological Evaluation, Molecular Docking and *in silico* ADMET Prediction of Nitric Oxide Releasing Biphenyls as Anti-Inflammatory Agents. J App Pharm Sci, 2017; 7 (10): 037-047.