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Synthesis and Docking studies of ethyl 4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-substituted-6-substitutedphenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate as anti-inflammatory, analgesic and nitric oxide releasing agents.

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ABSTRACT

In the present study, a series of pyrimidine derivatives (6a-6z) was synthesized and tested for its anti-inflammatory, analgesic and nitric oxide releasing activity. The main aim of this study was to develop new chemical entities as potential anti-inflammatory agents. In this article, synthesis of a series of molecules containing important pharmacophore substituted diaryl rings on 6-membered heterocycle similar to coxibs and nitric oxide releasing moiety are described. All the synthesized compounds were tested in vivo for their anti-inflammatory, analgesic studies and in vitro for their nitric oxide-releasing properties. Out of the twenty six synthesized compounds, four compounds showed significant anti-inflammatory and analgesic activity which was compared with standard. All the synthesized compounds exhibited significant nitric oxide-releasing activity.

Keywords: Pyrimidine, Docking, Anti-inflammatory, Analgesic, Nitric oxide.

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INTRODUCTION

Fever and inflammation is reduces due to the drug like painkiller. It is the most commonly taken drug today. Selective cyclo-oxygenase-2 (COX-2) inhibitors exhibited less advaerse effects compared with conventional Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), although the magnitude of this reduction continues to be debated in the literature [1]. As reported, the selective COX-2 inhibitors also cause significant adverse effects in the renal and cardiovascular systems, possibly more serious than those caused by conventional NSAIDs.

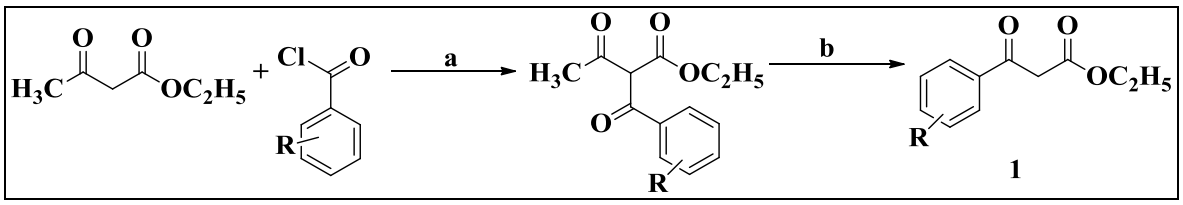
Recent strategies adopted to minimize the side effects of NSAIDs include the use of hybrid molecules, the dual LOX/COX inhibitors and COX inhibitors together with a nitric oxide-releasing functional group [2-4].

Synthetic approaches based on chemical modification of NSAIDs have been taken with the aim of improving safety profile. Our previous studies had described the synthesis of hybrid molecules with nitric oxide-releasing group that resulted in an increased anti-inflammatory activity with reduced GI-ulcerogenicity [1]. In our attempt to continue to discover new, safer, and potent agents for the treatment of inflammatory diseases, we have synthesized compounds containing pharmacophore of diaryl pyrimidine ring, the pharmacophore somewhat similar to coxibs and nitric oxide-releasing group to accentuate potency. The compounds designed so were found to possess much significant anti-inflammatory, analgesic with significant nitric oxide releasing activity.

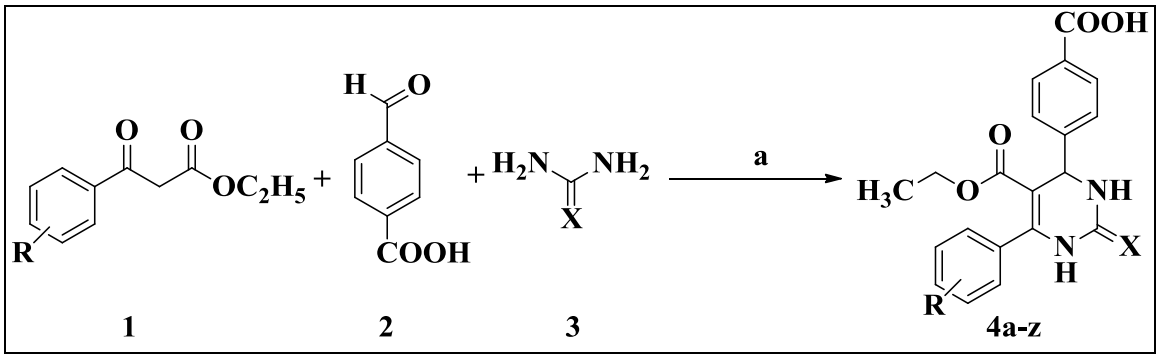
MATERIALS AND METHODS

Synthetic studies

All the chemicals & solvents were purchased from Avra chemicals & Sigma-Aldrich. Melting points were uncorrected & recorded on optimelt digital melting point apparatus. IR spectra were recorded on bruker alpha E FTIR spectrophotometer. ^{1}H NMR were recorded on varian 400 MHz spectrometer by using TMS as internal standard and DMSO as a solvent. Mass spectra were recorded on scinpor Q-TOF.



Scheme 1: Synthesis of ethyl-3-oxo-3-(susbstitued phenyl)-propanoate (a) aq NaOH (b) NH_4Cl



Scheme 2: Synthesis of 4-(5-(ethoxycarbonyl)-2-substituted-6-substituted phenyl-1,2,3,4-tetrahydropyrimidin-4-yl)benzoic acid (a) K_2CO_3, C_2H_5OH

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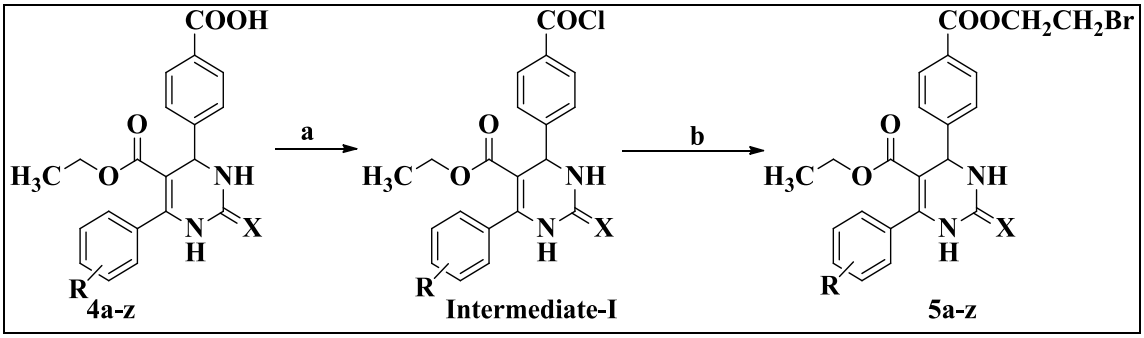
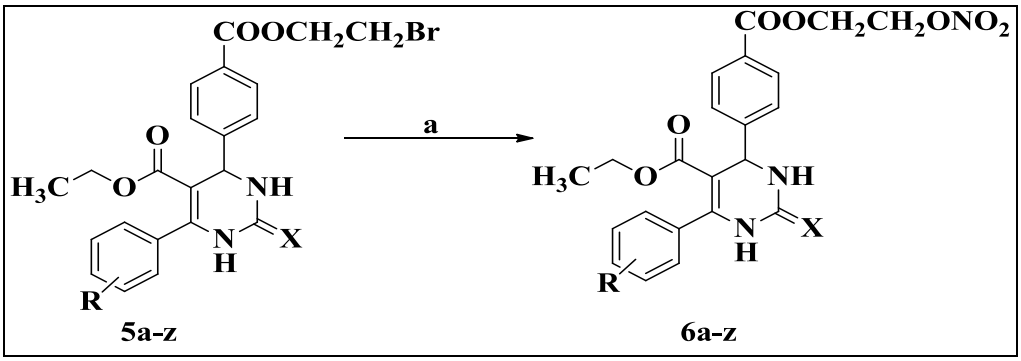


Scheme 3: Synthesis of ethyl 4-(4-((2-bromoethoxy)carbonyl)phenyl)-2-substituted-6-substituted phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate(a)$SOCl_2$(b)HO-CH_2-CH_2-Br



Scheme 4: Synthesis of ethyl 4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-substituted-6-substitutedphenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate(a)$AgNO_3$,CH_3CN,12h

General procedure for the synthesis of ethyl-3-oxo-3-(susbstitued phenyl)-propanoate (1)

In a 250 ml round bottom flask 50 ml of water and 1.5 moles of freshly distilled ethyl acetoacetate was boiled at 95-1100 C. The mixture was cooled to 50 C in ice water bath and 6.5 ml 33% sodium hydroxide solution was added in the mixture. The mixture was stirred vigorously and 1.62 mole of benzoyl chloride was added simultaneously for about 2 h. The mixture was allowed to come to the room temperature and aqueous layer was separated. To the mixture 8 g of ammonium chloride was added and stirred slowly for overnight. The mixture was transferred to separatory funnel and aqueous layer was withdrawn. The oil layer was washed with cold water. An additional benzene was added and the product was distilled under reduced pressure to obtain desired product.

General procedure for the synthesis of 4-(5-(ethoxycarbonyl)-2-substituted-6-substitutedphenyl-1,2,3,4-tetrahydropyrimidin-4-yl)benzoic acid (4a-4z)

A mixture of ethyl-3-oxo-3-(phenyl)-propanoate (0.06 mol), urea (0.06 mol), aldehyde (0.06 mol) and K_2CO_3 (0.06 mol) in 100 ml ethanol (95%) was refluxed in oil bath. It was cooled, the crystalline solid thus obtained was filtered off, dried and dissolved in hot water. It was filtered again and neutralised with acetic acid. The solid thus obtained was filtered off, washed with cold water, dried and recrystallised from a suitable solvent [5].

General procedure for the synthesis of ethyl 4-(4-((2-bromoethoxy)carbonyl)phenyl)-2-substituted-6-substituted phenyl-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (5a-5z)

Thionyl chloride (0.2 mol) was added to 4-(5-(ethoxycarbonyl)-2-substituted-6-substituted phenyl-1,2,3,4-tetrahydropyrimidin-4-yl) benzoic acid **(4a-4z)** (0.1 mol), drop wise with stirring. The solution was refluxed for 2.5 h until no further fumes were evolved in fume hood chamber. The reaction mixture was cooled to room temperature and excess of thionyl chloride was removed under reduced pressure [6]. The product **(intermediate-I)** obtained was used immediately for further step.

1.3 mmol of 2-bromo ethanol and triethyl amine (2.6 mmol) were dissolved in 30 ml of anhydrous CH_2Cl_2 and the solution was cooled to 0^0C under an argon atmosphere. Then a solution of intermediate-I (2.0 mmol) in anhydrous CH_2Cl_2 (10 ml) was added drop wise. The resulting mixture was stirred for two hours, then poured into 30 ml of cold water, and then extracted with CH_2Cl_2 (3 x 30 ml). The combined extracts were dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was separated on a silica gel column to afford the desired product [7].

General procedure for the synthesis of ethyl 4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-substituted-6-substituted phenyl-1,2,3,4-tetrahy dropyrimidine-5-carboxylate (6a-6z)

A solution of the ethyl 4-(4-((2-bromoethoxy)carbonyl) phenyl)-2-substituted-6-substituted phenyl-1,2,3,4-tetrahy dropyrimidine-5-carboxylate **(5a-5z,** 0.85g) in dry acetonitrile (2 ml) was treated portion wise with a solution of silver nitrate (0.34 g) in dry acetonitrile (5 ml) and the whole mixture was stirred at room temperature for 3 h. The mixture was then filtered, evaporated to dryness, and the residue was recrystallized from absolute ethanol [1].

Analytical data:

Ethyl-6-(3-bromophenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6a):

Yellow solid; IR: 3008 (Aryl C-H stretch), 2850 (Aliphatic C-H stretch), 1720 (C=O stretch), 1610(C=C stretch), 1250(C-N stretch), 3330(N-H stretch), 1610 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.8 (t, 3H, -CH_3), 4.2 (m, 2H, CH_2), 4.5 (t, 2H, CH_2), 4.62 (t, 2H, CH_2), 5.4 (s, 1H, CH), 6.9 (s, 2H, NH), 7.1 (d, 2H, CH), 7.3 (m, 3H, CH), 7.4 (s, 1H, CH), 7.7 (d, 2H, CH). MS: *m/z* 535 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-6-(3-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6b):

White solid; IR: 3052 (Aryl C-H stretch), 2822 (Aliphatic C-H stretch), 1708 (C=O stretch), 1628 (C=C stretch), 1310 (C-N stretch), 3312 (N-H stretch), 1625 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.6 (t, 3H, CH_3), 4.1 (m, 2H, CH_2), 5.0 (t, 2H, CH_2), 5.15 (t, 2H, CH_2), 5.2 (s, 1H, CH), 6.9 (s, 2H, NH), 7.0 (s, 1H, CH), 7.2 (d, 2H, CH), 7.4 (s, 3H, CH), 7.6 (d, 2H, CH). MS: *m/z* 524 $[M+H]^+$.

Ethyl-6-(4-(methylthio) phenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-1,2, 3,4-tetrahydro pyrimidine-5-carboxylate (6c):

Off white solid; IR: 3090 (Aryl C-H stretch), 2852 (Aliphatic C-H stretch), 1690 (C=O stretch), 1670(C=C stretch), 1348(C-N stretch), 3352(N-H stretch), 1602 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.31 (t, 3H, CH_3), 2.42 (s, 3H, CH_3), 4.11 (m, 2H, CH_2), 4.82 (t, 2H, CH_2), 4.95 (t, 2H, CH_2), 5.4 (s, 1H, CH), 6.82 (s, 2H, NH), 7.13 (d, 2H, CH), 7.29 (d, 2H, CH), 7.4 (d, 2H, CH), 7.85 (d, 2H, CH). MS: *m/z* 502 $[M+H]^+$.

Ethyl-6-(3,5-dimethoxyphenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-1,2, 3,4-tetrahydro pyrimidine-5-carboxylate (6d):

Pale yellow solid; IR: 3022 (Aryl C-H stretch), 2907 (Aliphatic C-H stretch), 1682 (C=O stretch), 1607 (C=C stretch), 1340 (C-N stretch), 3392 (N-H stretch), 1210 (C-O stretch), 1555 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.25 (t, 3H, CH_3), 3.90 (s, 6H, CH_3), 4.18 (m, 2H, CH_2), 4.74 (t, 2H, CH_2), 4.89 (t, 2H, CH_2), 5.61 (s, 1H, CH), 6.35 (s, 2H, NH), 7.13 (s, 1H, CH), 7.29 (d, 2H, CH), 7.4 (d, 2H, CH), 7.85 (d, 2H, CH). MS: *m/z* 516 $[M+H]^+$.

Ethyl-6-(4-fluorophenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6e):

White solid; IR: 3022 (Aryl C-H stretch), 2907 (Aliphatic C-H stretch), 1682 (C=O stretch), 1607(C=C stretch), 1340(C-N stretch), 3392(N-H stretch), 1225(C-O stretch), 1555 (NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.32 (t, 3H, CH_3), 4.25 (m, 2H, CH_2), 4.69 (t, 2H, CH_2), 4.81 (t, 2H, CH_2), 5.35 (s, 1H, CH), 6.80 (s, 2H, NH), 7.19 (d, 2H, CH), 7.25 (d, 2H, CH), 7.49 (d, 2H, CH), 7.79 (d, 2H, CH). MS: *m/z* 474 $[M+H]^+$.

Ethyl-6-(4-methoxyphenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6f):

Off white solid; IR: 3030 (Aryl C-H stretch), 2863 (Aliphatic C-H stretch), 1690 (C=O stretch), 1612 (C=C stretch), 1341 (C-N stretch), 3321(N-H stretch), 1536(NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.25 (t, 3H, CH_3), 3.75 (s, 3H, CH_3), 4.39 (m, 2H, CH_2), 4.75 (t, 2H, CH_2), 4.96 (t, 2H, CH_2), 5.25 (s, 1H, CH), 6.92 (s, 2H, NH), 7.15 (d, 2H, CH), 7.30 (d, 2H, CH), 7.42 (d, 2H, CH), 7.72 (d, 2H, CH). MS: *m/z* 486 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-6-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6g):

Buff solid; IR: 3041 (Aryl C-H stretch), 2952 (Aliphatic C-H stretch), 1683 (C=O stretch), 1604 (C=C stretch), 1213 (C-N stretch), 3363 (N-H stretch), 1625 (NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.19 (t, 3H, CH_3), 4.21 (m, 2H, CH_2), 4.70 (t, 2H, CH_2), 4.99 (t, 2H, CH_2), 5.40 (s, 1H, CH), 6.75 (s, 2H, NH), 7.18 (d, 2H, CH), 7.39 (d, 2H, CH), 7.69 (d, 2H, CH), 7.80 (d, 2H, CH). MS: *m/z* 501 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-6-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydro pyrimidine-5-carboxylate(6h):

White solid; IR: 3055 (Aryl C-H stretch), 2923 (Aliphatic C-H stretch), 1711 (C=O stretch), 1620 (C=C stretch), 1299 (C-N stretch), 3372 (N-H stretch), 1573 (NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.25 (t, 3H, CH_3), 4.27 (m, 2H, CH_2), 4.68 (t, 2H, CH_2), 4.85 (t, 2H, CH_2), 5.27 (s, 1H, CH), 6.60 (s, 2H, NH), 7.11 (d, 2H, CH), 7.30 (d, 2H, CH), 7.59 (d, 2H, CH), 7.89 (d, 2H, CH). MS: *m/z* 524 $[M+H]^+$.

Ethyl-6-(4-bromophenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6i):

Gray solid; IR: 3033 (Aryl C-H stretch), 2859 (Aliphatic C-H stretch), 1698 (C=O stretch), 1670 (C=C stretch), 1311 (C-N stretch), 3313 (N-H stretch), 1622 (NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.15 (t, 3H, CH_3), 4.33 (m, 2H, CH_2), 4.72 (t, 2H, CH_2), 4.89 (t, 2H, CH_2), 5.11 (s, 1H, CH), 6.50 (s, 2H, NH), 7.19 (d, 2H, CH), 7.25 (d, 2H, CH), 7.66 (d, 2H, CH), 7.84 (d, 2H, CH). MS: *m/z* 535 $[M+H]^+$.

Ethyl-6-(4-(dimethylamino)phenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6j):

Off white solid; IR: 3051 (Aryl C-H stretch), 2944 (Aliphatic C-H stretch), 1722 (C=O stretch), 1608(C=C stretch), 1222(C-N stretch), 3355 (N-H stretch), 1555 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.20 (t, 3H, CH_3), 3.10 (s, 6H, CH_3), 4.25 (m, 2H, CH_2), 4.55 (t, 2H, CH_2), 4.94 (t, 2H, CH_2), 5.29 (s, 1H, CH), 6.44 (s, 2H, NH), 7.21 (d, 2H, CH), 7.32 (d, 2H, CH), 7.47 (d, 2H, CH), 7.80 (d, 2H, CH). MS: *m/z* 499 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-6-(2-(trifluoromethoxy) phenyl)-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6k):

White solid; IR: 3065 (Aryl C-H stretch), 2867 (Aliphatic C-H stretch), 1699 (C=O stretch), 1633 (C=C stretch), 1223 (C-N stretch), 3373 (N-H stretch), 1235 (C-O stretch),1621(NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.22 (t, 3H, CH_3), 4.30 (m, 2H, CH_2), 4.49 (t, 2H, CH_2), 4.89 (t, 2H, CH_2), 5.30 (s, 1H, CH), 6.27 (s, 2H, NH), 7.39 (d, 2H, CH), 7.90 (d, 2H, CH), 7.00 (s, 1H, CH), 7.15 (s, 1H, CH), 7.25 (s, 1H, CH), 7.31 (s, 1H, CH). MS: *m/z* 540 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-6-(4-(trifluoromethoxy) phenyl)-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6l):

White solid; IR: 3085 (Aryl C-H stretch), 2933 (Aliphatic C-H stretch), 1717 (C=O stretch), 1671 (C=C stretch), 1344 (C-N stretch), 3352 (N-H stretch), 1199 (C-O stretch), 1587 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.22 (t, 3H, CH_3), 4.22 (m, 2H, CH_2), 4.65 (t, 2H, CH_2), 4.87 (t, 2H, CH_2), 5.23 (s, 1H, CH), 6.77 (s, 2H, NH), 7.10 (d, 2H, CH), 7.35 (d, 2H, CH), 7.56 (d, 2H, CH), 7.79 (d, 2H, CH). MS: *m/z* 540 $[M+H]^+$.

Ethyl-6-(4-bromo-3-methylphenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6m):

Yellow solid; IR: 3069 (Aryl C-H stretch), 2888 (Aliphatic C-H stretch), 1687 (C=O stretch), 1614(C=C stretch), 1252(C-N stretch), 3343 (N-H stretch), 1653 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.29 (t, 3H, CH_3), 2.29 (s, 3H, CH_3), 4.10 (m, 2H, CH_2), 4.50 (t, 2H, CH_2), 4.77 (t, 2H, CH_2), 5.15 (s, 1H, CH), 6.70 (s, 2H, NH), 7.90 (d, 2H, CH), 7.30 (d, 2H, CH), 7.01 (s, 1H, CH), 7.12 (s, 1H, CH), 7.50 (s, 1H, CH). MS: *m/z* 549 $[M+H]^+$.

Ethyl-6-(3-bromophenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6n):

Yellow solid; IR: 3082 (Aryl C-H stretch), 2910 (Aliphatic C-H stretch), 1690 (C=O stretch), 1653 (C=C stretch), 1322 (C-N stretch), 3361 (N-H stretch), 1163 (C=S stretch), 1501 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.83 (t, 3H, -CH_3), 4.25 (m, 2H, CH_2), 4.59 (t, 2H, CH_2), 4.65 (t, 2H, CH_2), 5.34 (s, 1H, CH), 6.93 (s, 2H, NH), 7.17 (d, 2H, CH), 7.32 (m, 3H, CH), 7.46 (s, 1H, CH), 7.74 (d, 2H, CH). MS: *m/z* 551 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-6-(3-(trifluoromethyl) phenyl)-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6o):

Brown solid; IR: 3055 (Aryl C-H stretch), 2888 (Aliphatic C-H stretch), 1702 (C=O stretch), 1610 (C=C stretch), 1253 (C-N stretch), 3350 (N-H stretch), 1145 (C=S stretch), 1656 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 M Hz, DMSO): δ= 1.68 (t, 3H, CH_3), 4.19 (m, 2H, CH_2), 5.09 (t, 2H, CH_2), 5.25 (t, 2H, CH_2), 5.31 (s, 1H, CH), 6.96 (s, 2H, NH), 7.08 (s, 1H, CH), 7.28 (d, 2H, CH), 7.43 (s, 3H, CH), 7.66 (d, 2H, CH). MS: *m/z* 540 $[M+H]^+$.

Ethyl-6-(4-(methylthio)phenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6p):

White solid; IR: 3088 (Aryl C-H stretch), 2946 (Aliphatic C-H stretch), 1715 (C=O stretch), 1625 (C=C stretch), 1249 (C-N stretch), 3389 (N-H stretch),1086 (C=S stretch),1557 (NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.27 (t, 3H, CH_3), 2.49 (s, 3H, CH_3), 4.15 (m, 2H, CH_2), 4.85 (t, 2H, CH_2), 4.90 (t, 2H, CH_2), 5.44 (s, 1H, CH), 6.88 (s, 2H, NH), 7.19 (d, 2H, CH), 7.32 (d, 2H, CH), 7.45 (d, 2H, CH), 7.89 (d, 2H, CH). MS: *m/z* 518 $[M+H]^+$.

Ethyl-6-(3,5-dimethoxyphenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6q):

Pale yellow solid; IR: 3045 (Aryl C-H stretch), 2865 (Aliphatic C-H stretch), 1717 (C=O stretch), 1609 (C=C stretch), 1325 (C-N stretch), 3323 (N-H stretch), 1236 (C-O stretch), 1155 (C=S stretch), 1630 (NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.20 (t, 3H, CH_3), 3.88 (s, 6H, CH_3), 4.10 (m, 2H, CH_2), 4.79 (t, 2H, CH_2), 4.85 (t, 2H, CH_2), 5.69 (s, 1H, CH), 6.40 (s, 2H, NH), 7.19 (s, 1H, CH), 7.33 (d, 2H, CH), 7.45 (d, 2H, CH), 7.91 (d, 2H, CH). MS: *m/z* 532 $[M+H]^+$.

Ethyl-6-(4-fluorophenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6r):

White solid; IR: 3072 (Aryl C-H stretch), 2941 (Aliphatic C-H stretch), 1683 (C=O stretch), 1667 (C=C stretch), 1263 (C-N stretch), 3363 (N-H stretch), 1093(C=S stretch), 1569 (NO_2 Stretch) cm^{-1}

^1H NMR (400 M Hz, DMSO): δ= 1.27 (t, 3H, CH_3), 4.20 (m, 2H, CH_2), 4.60 (t, 2H, CH_2), 4.88 (t, 2H, CH_2), 5.39 (s, 1H, CH), 6.88 (s, 2H, NH), 7.11 (d, 2H, CH), 7.26 (d, 2H, CH), 7.52 (d, 2H, CH), 7.88 (d, 2H, CH). MS: *m/z* 490 $[M+H]^+$.

Ethyl-6-(4-methoxyphenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6s):

White solid; IR: 3086 (Aryl C-H stretch), 2893 (Aliphatic C-H stretch), 1693 (C=O stretch), 1619 (C=C stretch), 1324 (C-N stretch), 3316 (N-H stretch), 1226 (C-O stretch), 1121 (C=S stretch), 1636 (NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.19 (t, 3H, CH_3), 3.69 (s, 3H, CH_3), 4.44 (m, 2H, CH_2), 4.70 (t, 2H, CH_2), 4.90 (t, 2H, CH_2), 5.29 (s, 1H, CH), 6.95 (s, 2H, NH), 7.21 (d, 2H, CH), 7.39 (d, 2H, CH), 7.49 (d, 2H, CH), 7.77 (d, 2H, CH). MS: *m/z* 502 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-6-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6t):

Yellow solid; IR: 3033 (Aryl C-H stretch), 2941 (Aliphatic C-H stretch), 1715 (C=O stretch), 1656 (C=C stretch), 1269 (C-N stretch), 3389 (N-H stretch), 1093 (C=S stretch), 1555 (NO_2 Stretch) cm^{-1}

^1H NMR (400 MHz, DMSO): δ= 1.22 (t, 3H, CH_3), 4.28 (m, 2H, CH_2), 4.74 (t, 2H, CH_2), 4.90 (t, 2H, CH_2), 5.45 (s, 1H, CH), 6.77 (s, 2H, NH), 7.28 (d, 2H, CH), 7.40 (d, 2H, CH), 7.72 (d, 2H, CH), 7.89 (d, 2H, CH). MS: *m/z* 517 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-6-(4-(trifluoromethyl) phenyl)-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6u):

White solid; IR: 3069 (Aryl C-H stretch), 2922 (Aliphatic C-H stretch), 1719 (C=O stretch), 1608 (C=C stretch), 1289 (C-N stretch), 3330 (N-H stretch), 1107 (C=S stretch), 1635 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.21 (t, 3H, CH_3), 4.35 (m, 2H, CH_2), 4.59 (t, 2H, CH_2), 4.79 (t, 2H, CH_2), 5.20 (s, 1H, CH), 6.77 (s, 2H, NH), 7.19 (d, 2H, CH), 7.39 (d, 2H, CH), 7.50 (d, 2H, CH), 7.98 (d, 2H, CH). MS: *m/z* 540 $[M+H]^+$.

Ethyl-6-(4-bromophenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6v):

White solid; IR: 3061 (Aryl C-H stretch), 2869 (Aliphatic C-H stretch), 1722 (C=O stretch), 1655 (C=C stretch), 1298 (C-N stretch), 3389 (N-H stretch), 1169 (C=S stretch), 1598 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.10 (t, 3H, CH_3), 4.39 (m, 2H, CH_2), 4.68 (t, 2H, CH_2), 4.80 (t, 2H, CH_2), 5.20 (s, 1H, CH), 6.66 (s, 2H, NH), 7.14 (d, 2H, CH), 7.33 (d, 2H, CH), 7.59 (d, 2H, CH), 7.89 (d, 2H, CH). MS: *m/z* 551 $[M+H]^+$.

Ethyl-6-(4-(dimethylamino)phenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6w):

Off white solid; IR: 3087 (Aryl C-H stretch), 2896 (Aliphatic C-H stretch), 1698 (C=O stretch), 1651 (C=C stretch), 1285 (C-N stretch), 3360 (N-H stretch), 1124 (C=S stretch), 1626 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.29 (t, 3H, CH_3), 3.15 (s, 6H, CH_3), 4.29 (m, 2H, CH_2), 4.49 (t, 2H, CH_2), 4.85 (t, 2H, CH_2), 5.19 (s, 1H, CH), 6.49 (s, 2H, NH), 7.27 (d, 2H, CH), 7.38 (d, 2H, CH), 7.52 (d, 2H, CH), 7.87 (d, 2H, CH). MS: *m/z* 515 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-6-(2-(trifluoromethoxy) phenyl)-1,2,3,4-tetra hydropyrimidine-5-carboxylate (6x):

White solid; IR: 3031 (Aryl C-H stretch), 2945 (Aliphatic C-H stretch), 1712 (C=O stretch), 1621 (C=C stretch), 1256 (C-N stretch), 3378 (N-H stretch), 1132 (C=S stretch), 1563 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.29 (t, 3H, CH_3), 4.21 (m, 2H, CH_2), 4.55 (t, 2H, CH_2), 4.93 (t, 2H, CH_2), 5.26 (s, 1H, CH), 6.37 (s, 2H, NH), 7.30 (d, 2H, CH), 7.81 (d, 2H, CH), 7.09 (s, 1H, CH), 7.19 (s, 1H, CH), 7.29 (s, 1H, CH), 7.41 (s, 1H, CH). MS: *m/z* 556 $[M+H]^+$.

Ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-6-(4-(trifluoromethoxy) phenyl)-1,2,3,4-tetra hydropyrimidine-5-carboxylate (6y):

White solid; IR: 3057 (Aryl C-H stretch), 2859 (Aliphatic C-H stretch), 1719 (C=O stretch), 1641 (C=C stretch), 1321 (C-N stretch), 3339 (N-H stretch),1089 (C=S stretch), 1598 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.17 (t, 3H, CH_3), 4.29 (m, 2H, CH_2), 4.59 (t, 2H, CH_2), 4.80 (t, 2H, CH_2), 5.29 (s, 1H, CH), 6.70 (s, 2H, NH), 7.19 (d, 2H, CH), 7.30 (d, 2H, CH), 7.50 (d, 2H, CH), 7.89 (d, 2H, CH). MS: *m/z* 556 $[M+H]^+$.

Ethyl-6-(4-bromo-3-methylphenyl)-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (6z):

Reddish brown solid; IR: 3069 (Aryl C-H stretch), 2887 (Aliphatic C-H stretch), 1722 (C=O stretch), 1647 (C=C stretch), 1256 (C-N stretch), 3328 (N-H stretch),1146 (C=S stretch), 1645 (NO_2 Stretch) cm^{-1}

^{1}H NMR (400 MHz, DMSO): δ= 1.20 (t, 3H, CH_3), 2.20 (s, 3H, CH_3), 4.19 (m, 2H, CH_2), 4.59 (t, 2H, CH_2), 4.67 (t, 2H, CH_2), 5.25 (s, 1H, CH), 6.77 (s, 2H, NH), 7.95 (d, 2H, CH), 7.35 (d, 2H, CH), 7.09 (s, 1H, CH), 7.19 (s, 1H, CH), 7.59 (s, 1H, CH). MS: *m/z* 565 $[M+H]^+$.

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Pharmacology:

All the method for pharmacological work have been performed as per our previously published work [1].

Docking Methodology:

Molecular Docking Studies were performed using Glide v6.2 (Schrödinger, LLC).The coordinates for COX-2 enzyme were taken from RCSB Protein Data Bank (PDB Id. 1CX2) and prepared for docking using protein preparation wizard. Water molecules in the structure were removed and termini were capped by adding ACE and NMA residue. The bond orders and formal charges were added for hetero groups and hydrogens were added to all atoms in the structure. Side chains that were not close to the binding cavity and do not participate in salt bridges were neutralized. After preparation, the structures were refined to optimize the hydrogen bond network using OPLS_2005 force field. This helps in reorientation of the side chain hydroxyl group. The minimization was terminated when the energy converged or the RMSD reached a maximum cut off of 0.30 Å. Grids were then defined around refined structure by centering on ligand using default box size. The extra precision (XP) docking mode for compounds, optimized by Ligprep, was performed on generated grid of protein structure [8].

RESULTS AND DISCUSSION

Chemistry:

The synthesis of target compounds 6a-6z is shown in scheme 1-4. In the presence of sodium hydroxide substituted benzoyl chloride was reacted with ethyl acetoacetate to form ethyl 2-(substituted benzoyl)-3-oxobutanoate which later reacted with ammonium chloride to form ethyl 3-oxo-3-(substituted phenyl)propanoate (1). 4-(5-(ethoxycarbonyl)-2-substituted-6-substituted phenyl-1,2,3,4-tetrahydropyrimidin-4-yl)benzoic acid (4a-4z) have been synthesized by base catalysed condensation of ethyl 3-oxo-3-(substituted phenyl)propanoate(1), urea/thiourea(2) and aromatic aldehyde (3). 4-(5-(ethoxycarbonyl)-2-substituted-6-substituted phenyl-1,2,3,4-tetrahydropyrimidin-4-yl)benzoic acid (4a-z) further reacted with thionyl chloride to afford ethyl 4-(4-(chlorocarbonyl)phenyl)-2-substituted-6-substitutedphenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate which on reaction with 2-bromo ethanol gives ethyl 4-(4-((2-bromoethoxy)carbonyl)phenyl)-2-substituted-6-substitutedphenyl-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (5a-5z).

The target compounds were obtained by reactingethyl 4-(4-((2-bromoethoxy)carbonyl)phenyl)-2-substituted-6-substitutedphenyl-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (5a-5z) with silver nitrate in the presence of acetonitrile to give ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-substituted-6-substitutedphenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6a-6z, Table 1).

Table 1: Characterization data for synthesized compounds (6a-6z)

Sr. No.	Entry	R	X	MF	MW	% yield	M.P.(^{0}C)
1	6a	3-Br	O	$C_{22}H_{20}BrN_3O_8$	534	79	175-176
2	6b	3-CF_3	O	$C_{23}H_{20}F_3N_3O_8$	523	73	218-220
3	6c	4-SCH_3	O	$C_{23}H_{23}N_3O_8S$	501	87	187-189
4	6d	3,5-OCH_3	O	$C_{24}H_{25}N_3O_{10}$	515	76	155-157
5	6e	4-F	O	$C_{22}H_{20}FN_3O_8$	473	93	169-170
6	6f	4-OCH_3	O	$C_{23}H_{23}N_3O_9$	485	84	222-223
7	6g	4-NO_2	O	$C_{22}H_{20}N_4O_{10}$	500	80	138-140
8	6h	4-CF_3	O	$C_{23}H_{20}F_3N_3O_8$	523	90	208-210
9	6i	4-Br	O	$C_{22}H_{20}BrN_3O_8$	534	88	219-220
10	6j	4-N(CH_3)$_2$	O	$C_{24}H_{26}N_4O_8$	498	77	134-136
11	6k	2-OCF_3	O	$C_{23}H_{20}F_3N_3O_9$	539	73	126-127
12	6l	4-OCF_3	O	$C_{23}H_{20}F_3N_3O_9$	539	81	203-205
13	6m	3-CH_3,4-Br	O	$C_{23}H_{22}BrN_3O_8$	548	69	234-235
14	6n	3-Br	S	$C_{22}H_{20}BrN_3O_7S$	550	65	180-181
15	6o	3-CF_3	S	$C_{23}H_{20}F_3N_3O_7S$	539	72	224-225
16	6p	4-SCH_3	S	$C_{23}H_{23}N_3O_7S_2$	517	80	193-194

17	6q	3,5-OCH_3	S	$C_{24}H_{25}N_3O_9S$	531	76	161-162
18	6r	4-F	S	$C_{22}H_{20}FN_3O_7S$	489	88	176-177
19	6s	4-OCH_3	S	$C_{23}H_{23}N_3O_8S$	501	85	229-230
20	6t	4-NO_2	S	$C_{22}H_{20}N_4O_9S$	516	82	144-145
21	6u	4-CF_3	S	$C_{23}H_{20}F_3N_3O_7S$	539	87	213-214
22	6v	4-Br	S	$C_{22}H_{20}BrN_3O_7S$	550	78	225-226
23	6w	4-$N(CH_3)_2$	S	$C_{24}H_{26}N_4O_7S$	514	80	140-141
24	6x	2-OCF_3	S	$C_{23}H_{20}F_3N_3O_8S$	555	63	211-212
25	6y	4-OCF_3	S	$C_{23}H_{20}F_3N_3O_8S$	555	69	208-209
26	6z	3-CH_3,4-Br	S	$C_{23}H_{22}BrN_3O_7S$	564	66	229-230

Pharmacology:

The synthesized compounds were subjected to the evaluation of anti-inflammatory, analgesic and nitric oxide-releasing properties. Celecoxib was used as reference standard.

Many of the newly synthesized compounds were found to show good anti-inflammatory, analgesic and nitric oxide releasing activity. From the anti-inflammatory and analgesic activity data (Table 2 and Table 3), it is observed that compound 6m, 6n, 6o and 6z are the most active among all tested compounds. Substitution of bromo, dimethoxy and trifluoromethoxy groups on phenyl ring at R position and substitution of oxo group at X position (6a, 6d, 6l) shows equipotent activity with celecoxib.

Table 2: Results of anti-inflammatory activity of synthesized compounds against carrageenan-induced rat paw edema model in rats (6a-6z)

Comp Code	Change in paw volume in (ml) after drug treatment(±SEM)			Anti-inflammatory activity (% Inhibition)		
	1h	2h	3h	1h	2h	3h
Control	1.68±0.022**	1.90±0.036**	2.01±0.058**	-	-	-
Celecoxib	0.65±0.052**	0.62±0.058**	0.59±0.074**	61.30	67.36	70.64
6a	0.75±0.032**	0.71±0.062**	0.68±0.028**	55.35	62.63	66.16
6b	1.48±0.014**	1.45±0.039**	1.42±0.061**	11.90	23.68	29.35
6c	1.27±0.032**	1.29±0.047**	1.31±0.077**	24.40	32.10	34.82
6d	0.81±0.063**	0.79±0.033**	0.77±0.082**	51.78	58.42	61.69
6e	0.99±0.035**	0.96±0.064**	0.94±0.019**	41.07	49.47	53.23
6f	1.11±0.032**	1.13±0.029**	1.16±0.045**	33.92	40.52	42.28
6g	1.51±0.028**	1.54±0.054**	1.56±0.081**	10.11	18.94	22.23
6h	0.91±0.065**	0.89±0.084**	0.86±0.04**	45.83	46.84	57.21
6i	0.90±0.022**	0.88±0.063**	0.86±0.018**	46.42	53.68	57.21
6j	1.21±0.095**	1.23±0.055**	1.25±0.073**	27.79	35.26	37.81
6k	0.88±0.052**	0.85±0.066**	0.83±0.064**	47.61	55.26	58.70
6l	0.79±0.027**	0.77±0.093**	0.75±0.014**	52.97	59.47	62.68
6m	0.73±0.021**	0.70±0.065**	0.68±0.088**	56.54	63.15	66.16
6n	0.64±0.08**	0.62±0.078**	0.60±0.064**	61.90	67.36	70.14
6o	0.66±0.056**	0.63±0.040**	0.60±0.093**	60.71	66.84	70.14
6p	0.69±0.022**	0.72±0.078**	0.75±0.060**	58.92	62.10	62.68
6q	0.81±0.036**	0.83±0.044**	0.85±0.033**	51.78	56.31	57.71
6r	1.01±0.095**	1.04±0.023**	1.07±0.059**	39.88	45.26	46.70
6s	1.17±0.044**	1.19±0.054**	1.21±0.068**	30.35	37.36	44.27
6t	0.71±0.074**	0.73±0.059**	0.75±0.047**	57.73	61.57	62.68
6u	0.85±0.017**	0.87±0.069**	0.88±0.044**	49.40	54.21	56.21
6v	1.20±0.065**	1.22±0.037**	1.25±0.033**	28.57	35.78	37.81
6w	1.15±0.024**	1.17±0.078**	1.19±0.062**	31.54	38.42	40.79
6x	0.76±0.007**	0.74±0.069**	0.72±0.093**	54.76	61.05	64.17
6y	0.85±0.011**	0.83±0.067**	0.81±0.046**	49.40	56.31	59.70
6z	0.74±0.085**	0.71±0.087**	0.69±0.054**	55.95	62.63	65.67

Data analyzed by one way ANOVA followed by Dunnett's 't' test, (n = 6), * P<0.05, ** P<0.01 significant from control*ns* not significant

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Table 3: Results of analgesic activity of synthesized compounds against acetic acid-induced writhing test in mice (6a-6z)

Compound code	No of Writhes in 5-15 min. after treatment (Mean ± S.E)	% Inhibition
Control	25.89±0.21**	-
Celecoxib	8.52±0.57**	67.09
6a	9.55±0.65**	63.11
6b	15.09±0.60**	41.71
6c	14.23±0.29**	45.03
6d	10.10±0.32**	60.98
6e	12.39±0.31**	52.14
6f	11.54±0.72**	55.42
6g	13.41±0.10**	48.20
6h	11.72±0.47**	54.73
6i	10.65±0.22**	58.86
6j	13.99±0.62**	45.96
6k	12.82±0.50**	50.48
6l	10.01±0.37**	61.33
6m	8.96±0.19**	65.39
6n	8.70±0.60**	66.39
6o	9.23±0.24**	64.34
6p	9.98±0.69**	61.45
6q	10.78±0.86**	58.36
6r	11.11±0.80**	57.08
6s	12.11±0.33**	53.22
6t	9.90±0.51**	61.76
6u	11.53±0.42**	55.46
6v	13.28±0.91**	48.70
6w	10.80±0.63**	58.28
6x	10.05±0.86**	61.18
6y	11.69±0.55**	54.84
6z	8.80±0.84**	66.01

Data analyzed by one way ANOVA followed by Dunnett's 't' test,(n = 6), ** P<0.01 significant from control

Substitution of fluoro, methoxy, trifluoromethyl, bromo, trifluoromethoxy groups on phenyl ring at R position and substitution of oxo group at X position (6e, 6f, 6h, 6i, 6k) shows moderate activity. Substitution of trifluoromethyl, methylthio, nitro, dimethylamino groups on phenyl ring at R position and substitution of oxo group at X position (6b, 6c, 6g, 6j) shows low activity. Substitution of bromo, trifluoromethyl, methylthio, nitro, trifluoromethoxy, methyl-bromo groups on phenyl ring at R position and substitution of thio group at X position (6n, 6o, 6p, 6t, 6x, 6z) shows equipotent activity with celecoxib. Substitution of dimethoxy, fluoro, methoxy, trifluoromethyl, dimethylamino, trifluoromethoxy groups on phenyl ring at R position and substitution of thio group at X position (6q, 6r, 6s, 6u, 6w, 6y) shows moderate activity. Substitution of bromo group on phenyl ring at R position and substitution of thio group at X position (6v) shows low activity.

From in vitro nitric oxide releasing data (Table 4) it is observed that compound 6c, 6f, 6k, 6m, 6o, 6s, 6w and 6y shows less nitric oxide releasing properties. Compound 6a, 6d, 6e, 6g, 6i, 6n, 6p, 6q, 6t, 6v, 6x and 6z shows moderate nitric oxide releasing properties. Compound 6b, 6h, 6j, 6l, 6r and 6u shows potent nitric oxide releasing properties.

From nitric oxide releasing activity on rat aortic muscle (Table 4) it is observed that compound 6e shows potent EC_{50} values. Compound 6a, 6c, 6g, 6l, 6m, 6o, 6u, 6w and 6y shows less EC_{50} values. Compound 6b, 6d, 6i, 6n, 6p, 6r, 6s, 6v, 6x and 6z shows moderate EC_{50} values. Compound 6f, 6h, 6j, 6k, 6q and 6t shows high EC_{50} values.

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Table 4: EC_{50} values and nitric oxide-releasing properties of the compounds (6a–6z)

Sr. No.	Compound Code	EC_{50}	% NO release
1	6a	52.45	0.41
2	6b	47.55	0.79
3	6c	53.43	0.30
4	6d	49.52	0.54
5	6e	30.33	0.60
6	6f	33.78	0.33
7	6g	56.84	0.47
8	6h	33.10	0.67
9	6i	48.22	0.45
10	6j	35.65	0.72
11	6k	37.89	0.35
12	6l	54.12	0.63
13	6m	50.58	0.37
14	6n	47.57	0.58
15	6o	51.21	0.36
16	6p	45.07	0.42
17	6q	39.60	0.50
18	6r	42.90	0.61
19	6s	40.57	0.38
20	6t	37.53	0.60
21	6u	53.87	0.69
22	6v	44.60	0.57
23	6w	51.01	0.35
24	6x	48.12	0.51
25	6y	56.11	0.37
26	6z	47.31	0.59

Docking Study:

In all series, the docking poses of compounds showing higher docking score (G-score) were compared with that of standard celecoxib in the active site of COX-2 enzyme. Similarly the docking pose of all other compounds were compared with that of compound showing higher G-score within series for comparing their pharmacophoric features desired for good binding affinity toward COX-2 enzyme.

Docking study showed that most of pyrimidine derivatives docked in the active site of COX-2 enzyme and showed moderate binding affinity toward it as compared to that of celecoxib (Fig 1).This may due to replacement of 5-membered hetero ring as scaffold by 6-membered pyrimidine ring.

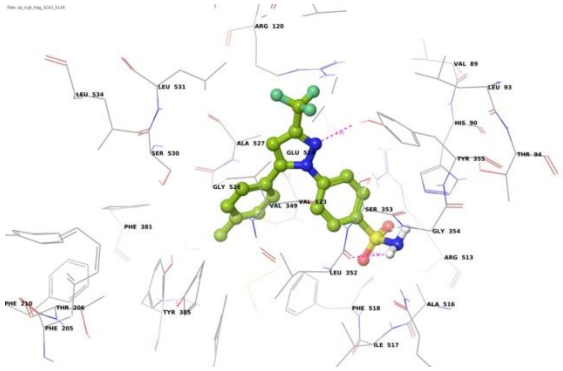

Figure 1: Docking pose of celecoxib in active site of COX-2 enzyme

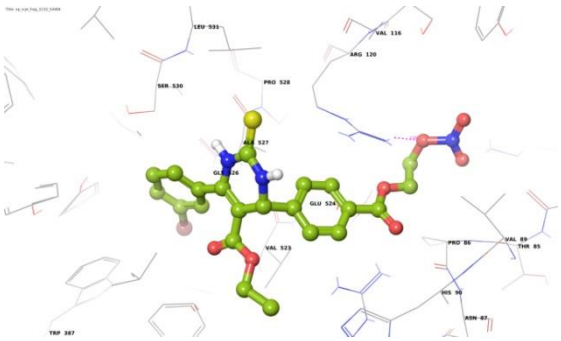

Figure 2: Docking pose of Compound 6n in active site of COX-2 enzyme

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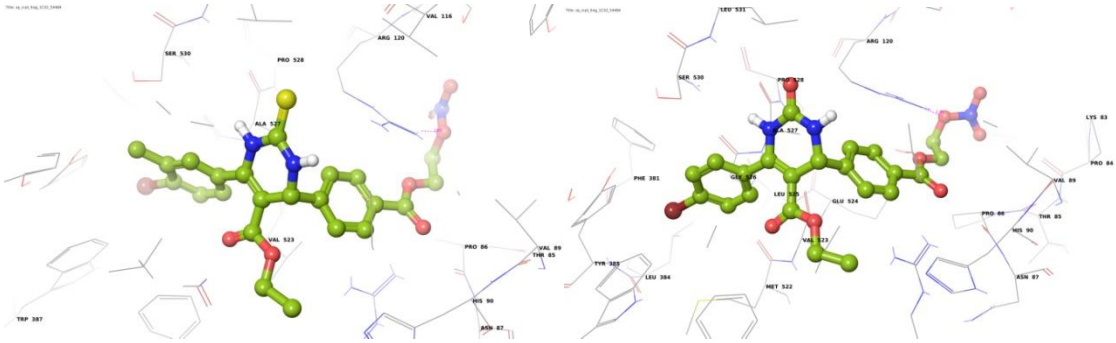

Figure 3: Docking pose of Compound 6z in active site of COX-2 enzyme. **Figure 4 Docking pose of Compound 6i in active site of COX-2 enzyme**

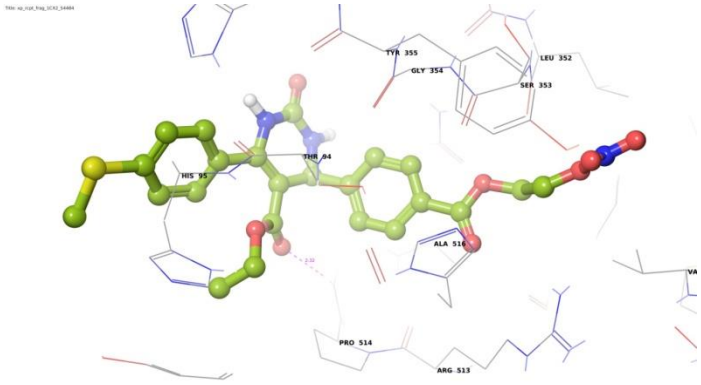

Figure 5: Docking pose of Compound 6c in active site of COX-2 enzyme

The Compounds 6n, 6o and 6z showed good G-score toward COX-2 enzyme among the pyrimidine derivatives. This indicate that thepresence of phenyl ring with hydrophobic substituents like Br, CF_3, F, CH_3in compounds at position R showed higher binding affinity than compound substituted phenyl ring with electron withdrawing substituents like OCH_3, $N(CH_3)_2$ etc (Fig 2, 3, 4 and 5).The presence of two side chain containing electron withdrawing group may be one of the probable reason for moderate binding affinity of pyrimidine derivatives and it was clearly indicated by low hydrophobic reward and Vander Waal interaction score in Table 5. Thus the six membered central ring along with diaryl system and two side chain chains along with electron withdrawing groups is unable to acquired desired "V" shaped structure (as by standard) for binding into active site of COX-2 enzyme. Similar to other series, the most of compounds in the series stabilize ligand-enzyme complex by forming H-bond with Arg120.

Table 5: Docking score of ethyl-4-(4-((2-(nitrooxy)ethoxy)carbonyl)phenyl)-2-substituted-6-substitutedphenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6a-6z)

Sr. No.	Entry	G-Score	H Bond	Lipophilic EvdW	Phob En
1	Celecoxib	-10.5	-1.3	-6.1	-1.5
2	6a	-6.23	-0.99	-1.08	-3.95
3	6b	-6.66	-0.34	-2.21	-3.44
4	6c	-0.37	-0.66	0	-3.72
5	6d	-5.18	-0.99	0	-4.04
6	6e	-5.9	-0.33	-1.77	-3.12
7	6f	ND*	ND*	ND*	ND*
8	6g	-4.62	-0.99	-0.46	-3.06
9	6h	-0.3	-1.27	0	-3.23
10	6i	-4.57	-0.33	-1.59	-4.03
11	6j	-3.83	-0.33	-1.08	-3.76
12	6k	-6.55	-1.32	-1.1	-3.5

13	6l	-3.56	-0.33	-1.11	-3.5
14	6m	-6.98	-0.34	-1.9	-4.21
15	**6n**	**-7.27**	**-0.33**	**-1.77**	**-4.32**
16	6o	-7.21	-0.33	-2.42	-4.04
17	6p	-5.9	-0.33	-1.63	-3.02
18	6q	-5.24	-0.99	-0.14	-3.77
19	6r	-5.76	-0.33	-1.09	-3.87
20	6s	-4.98	-0.33	-0.81	-3.95
21	6t	-4.45	-0.33	-0.82	-3.6
22	6u	-1.98	-0.66	0	-2.98
23	6v	-0.56	-0.66	0	-3.86
24	6w	-5.68	-0.33	-1.12	-3.22
25	6x	-4.94	-0.33	-0.91	-3.65
26	6y	-3.66	-0.65	0	-2.89
27	**6z**	**-7.23**	**-0.34**	**-1.88**	**-4.24**

ND* Not Docked
H Bond: Chem score hydrogen bond pair term.
Lipophilic EvdW: Chem score lipophilic pair term and fraction of total protein ligand vaderwall energy.
Phob En: Hydrophobic enclosure reward.

CONCLUSIONS

Twenty six compounds were synthesized and screened for analgesic, anti-inflammatory and nitric oxide-releasing studies. Docking study for these synthesized compounds was also performed. Most of the compounds exhibited significant anti-inflammatory, analgesic and nitric oxide releasing activity. Compounds 6m, 6n, 6o and 6z exhibited most prominent and constituent anti-inflammatory and analgesic activity. From the detailed analysis of the results of pharmacological studies, we conclude that the synthesized compounds have not only retained but showed enhanced anti-inflammatory profile. Also all the synthesized derivatives exhibited significant vasorelaxant activity. Therefore, it can be concluded that the rational, based on which these NCEs were designed, has been proven to be superior compared to the currently used NSAIDs.

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