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ORIGINAL RESEARCH

Design of 1,4-Dihydropyridine Hybrid Benzamide Derivatives: Synthesis and Evaluation of Analgesic Activity and Their Molecular Docking Studies

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Purpose: This study aims to determine the analgesic activity of 1,4-dihydropyridine hybrid benzamide derivatives. These hybrid derivatives were synthesized, and their analgesic activity was studied. The synthesis method applied was a one-step reaction involving a green chemistry approach.

Methods: The compounds were prepared via the amination method with a yield ranging between 82% and 93%. The title compounds were confirmed by means of IR, ^{1}H and ^{13}C NMR, and mass spectral analyses. The pharmacological activity of all the synthesized compounds was evaluated, and the analgesic activities were monitored in vivo (by tail immersion methods), with a digital analgesiometer. The drug response and damage of tail ata concentration of 10 mg/kg were measured by tail-flicking latency.

Results: The activity of compound 2c (81.35% activity at 5mg/kg) can be correlated with its salicylamidemoiety (13.99% activity at 5mg/kg), and diclofenac showed comparable activity (79.21% activity at 5mg/kg reference drugs). Compound 2c has a higher potential to inhibit COX proteins compared to diclofenac. The drug-like nature of the molecule 2c corresponds to its ADME properties.

Conclusion: In this study, all the synthesized compounds were found to possess significant analgesic activities; particularly, the performance of compound 2c is excellent. Thus, the preparative method described is an apt route for developing novel therapeutic formulations.

Keywords: amination method, 1,4-dihydropyridine, salicylamide, diclofenac, analgesic activity, molecular docking

Introduction

A number of calcium channel blockers have been investigated as model molecules in the drug discovery sector, but few novel analgesics have been introduced.[1,2] It is well recognized that T-type calcium channels play an important role in pain transmission among different calcium channel subfamilies.[3–6] A literature search on selective T-blockers identified some chemically diverse molecules, such as R(−) efonidipine[7] (Figure 1), as a dihydropyridine (DHPs) derivative belonging to this class. Many studies have shown that DHPs may also inhibit T-type calcium channels as well, although DHPs are the most commonly assumed L-type calcium channel inhibitors.[8,9] Subsequently, T-type calcium channels have emerged as suitable pharmacological targets for the therapeutic intervention into neurophysiological disorders such as pain and epilepsy and can also be targeted by DHPs.[10]

1,4-DHPs via a modified Hantzsch reaction under MW irradiation are reported. The pharmacological study showed good relaxant effects on the smooth muscles of the isolated rabbit gastric fundus, due to the blockade of Ca^{2+} channels, with a mechanism similar to that of NIF.

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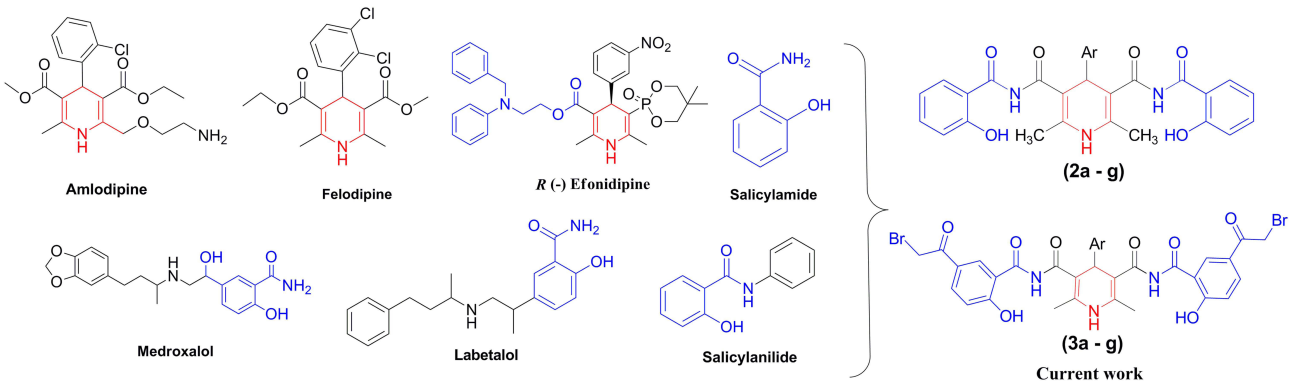


Figure 1 Pharmaceutically active compounds 1,4-dihydroyridine and other amide derivatives.

To learn more about the selectivity of T-types over L-types, some structure–activity relationship studies were conducted. Ester groups have been altered at almost every position in the typical DHP scaffold. These modulations at the C3- and C5-positions alter tissue selectivity.[11–13]

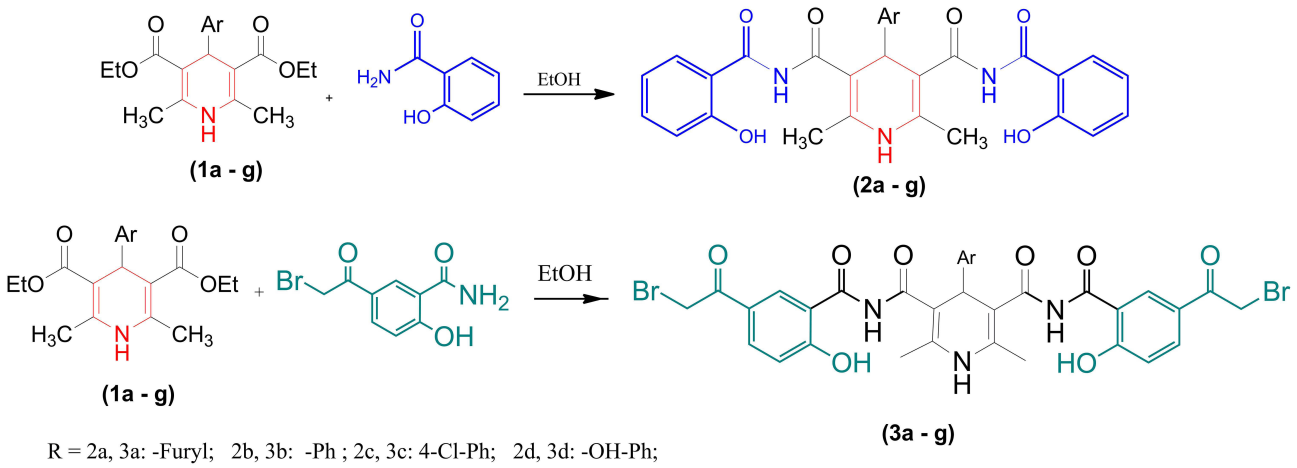
The new therapeutic utility of 1,4-dihydropyridine derivatives is a breakthrough in current clinical trials, encompassing their antimicrobial,[14,15] anticoagulant,[16] antihypertensive,[17] analgesic,[18,19] anticancer,[20] cytotoxic,[21] antitumor activities.[22,23] For instance, we previously reported the anticoagulant and antimicrobial activities of 1,4-dihydropyridine derivatives[24] and other pharmaceutical activities such as analgesic[25,26] and anti-inflammatory.[27–29] Figure 1 shows that various 1,4-dihydropyridine and other amide derivatives are well known for their pharmacological activities such as antimicrobial,[30] antifungal,[31] molluscicidal,[32] and anthelmintic.[33] The most commonly prescribed therapeutic agents for the management of pain and inflammation are nonsteroidal anti-inflammatory drugs (NSAIDs),[34] and many are used as analgesics too; These drugs relieve pain and edema by suppressing the production of prostaglandins and by inhibiting the enzyme cyclooxygenase (COX-1 and COX-2).

Understanding the mechanisms of pain reduction has led to new paradigms in pain management based on multimodal and pre-emptive strategies. Despite the fact that COX-2 selective inhibitors (coxibs) have been developed to treat chronic pain and post-surgical acute pain. NSAIDs are well-known anti-inflammatory medications. COX-1 and COX-2 are two iso-enzymes that exert different effects on body tissue repair and circulation.[35]

The COX-1 enzyme plays a role in the production of prostaglandins, thromboxane, and blood clotting as well as protecting the gastric mucosal. Inflammation is associated with an uptick in prostaglandin, as well as its regulation by the inducible enzyme COX-2. On the basis of these observations, COX-2-selective drugs such as celecoxib were rapidly developed and have become one of the most commercially successful classes of drugs.

Selective and non-selective NSAIDs investigated in this study could be good adjunctive options to general anesthetic agents in perioperative stages,[36] an outcome that needs further clinical investigations.[37] The addition of 1,4-dihydropyridine to selected or non-selective NSAIDs (celecoxib or diclofenac) causes an increase in analgesia with rapid onset and short duration.

Amides have been shown to possess an important pharmacophoric spectrum of wider biological activities.[38–40] Design and the synthesis of dihydropyridine derivatives with amide groups at the C3, C5 positions of DHP, which are important pharmacophores, have been planned. Many of the compounds previously reported exhibit both anti-inflammatory and analgesic activities and hence were selected as target molecules for this in vivo analgesic studies. Biological involvement of 1,4-DHP is strongly revealed in the published literature, especially the structure–activity relationship involving free −NH− group corresponding to prominent biological effects. The methyl groups at C-2 and C-6, and an aryl ring at C-4 are also important in this context.[41,42] On the basis of these observations, new dihydropyridine derivatives containing an amide group were designed, as shown in Scheme 1; DHP has been combined with different amide groups in order to investigate the analgesic properties of the derivatives.



Scheme 1 Method for synthesizing compounds (2a-g, 3a-g).

Materials and Methods

All chemicals of analytical grade were procured from Sigma-Aldrich. The IR spectra of all compounds were recorded (Thermo scientific Nicolet iS5 F) in the range of 4000–400 cm^{-1}. The ^{1}H NMR and ^{13}C NMR (Bruker DRX-300) spectra were taken on a spectrometer at 300MHz; GCMS model Clarus SQ8 (PerkinElmer) was used to record the mass spectra. Elemental analysis (C, H, N, and S) was performed using an elemental analyzer (VarioEL III).

A General Method for Preparing Compounds (2a-g)

A reaction mixture, diethyl 4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1a) (10mmol, 3.19 g), 2-hydroxybenzamide (20mmol, 2.74g), ethanol (5mL), was stirred and reflexed at 60°C for 30 min; TLC was used to monitor the reaction and confirm the product formation. Pure product was recrystallized from ethyl acetate. The synthesis route of compounds 2b-g was the same as described above.

4-(Furan-2-Yl)-N3,N5-Bis(2-Hydroxybenzoyl)-2,6-Dimethyl-1,4-Dihydropyridine-3,5-Dicarboxamide (2a)

White solid; yield 88%(187mg);mp 199–201°C; IR (cm^{-1}): 3174.54, 3074.57, 3034, 1652.67; ^{1}H NMR (300MHz): δ 11.19 (s, 2H, OH), 10. 60 (s, 2H, C_3-CONH and C_5-CO NH), 8.83 (s, 1H, NH), 7.81 (d, 2H, *J*=7.33Hz, Ph), 7.56 (d, *J*=6.19Hz, 1H, Furyl), 7.50 (dd, *J*=7.33Hz, *J*=7.37Hz, 2H, Ph), 7.19(dd, *J*=7.29Hz, *J*=7.34Hz, 2H, Ph), 6.90 (d, *J*=7.35Hz, 2H, Ph), 6.21 (d, *J*=6.21Hz, 1H, Furyl), 6.08 (dd, *J*=6.21Hz, *J*=6.29Hz, 1H, Furyl), 5.82 (s, 1H, 4-CH), 2.52 (s, 6H, 2,6- CH_3); ^{13}C NMR (300MHz):171.1 (2C), 164.2 (2C, C=O), 153.0, 142.6, 106.5 (4C, Furyl), 158.2, 133.0, 126.2, 121.1, 117.9, 117.1 (12C, Ar ring), 143.4 (2C, -C-CH_3), 112.8 (2C, -C-CO-), 32.4 (1C, CH), 18.0 (2C); EIMS: 501.52 (M^+,10%), 409.13(25%), 317 (16%), 261.11 (100%), 231.09 (74%), 175.10 (34%), 147.07 (16%), 81 (19%); Anal. Calcd. for $C_{27}H_{23}N_3O_7$: C, 64.67; H, 4.62; N, 8.38%; Found: C, 64.68; H, 4.61; N, 8.36%;

N3,N5-Bis(2-Hydroxybenzoyl)-2,6-Dimethyl-4-Phenyl-1,4-Dihydropyridine-3,5-Dicarboxamide (2b)

Light brown solid; yield 85% (183mg);mp 238–241°C; IR (KBr): 3162.51, 3045.32, 3034, 1612.47 $cm^{-1;}$ ^{1}H NMR (300MHz): δ 11.16 (s, 2H, OH), 10.62 (s, 2H), 8.83 (s, 1H, NH), 7.83 (2H, d, *J* =7.29Hz, Ph),7.48 (2H, dd, *J*=7.33Hz, *J*=7.35Hz Ph), 7.33 (d, *J*=7.18Hz, 2H, Ph), 7.26 (d, *J*=7.15Hz, 2H, Ph), 7.22 (d, *J*=7.16Hz, 1H, Ar), 7.16 (dd, *J*=7.30Hz, *J*=7.33Hz, 2H, Ph), 6.93 (d, *J*=7.36Hz, 2H, Ph), 4.35 (s, 1H, 4-CH), 2.53 (s, 6H); ^{13}C NMR (300MHz): 172.4 (2C), 163.7, 159.1, 132.9, 125.6, 120.2, 118.3, 117.2 (12C,Ar ring), 143.4 (2C, -C-CH_3), 126.5, 127.1, 124.1, 105.8 (6C, Ar ring), 112.1 (2C, -C-CO-), 32.1 (1C, CH), 18.6 (2C); EIMS (m/z) 512.02 (M^+,15%), 479.25 (12%), 327.12 (23%), 299.18

(16%), 271.10 (31%), 241.13 (19%), 213.18 (100%), 185.19 (18%), 157.19 (10%), 81.16 (24%),; Anal. Calcd. For ($C_{29}H_{25}N_3O_6$): C, 68.09; H, 4.93; N, 8.21%; Found: C, 68.07; H, 4.92; N, 8.20%.

4-(4-Chlorophenyl)-N3,N5-Bis(2-Hydroxybenzoyl)-2,6-Dimethyl-1,4-Dihydropyridine-3,5-Dicarboxamide (2c)

Light green solid; yield 82% (180mg);mp:141–145°C; IR (KBr): 3274.54, 3174.57, 3031.45, 1642.67, 827 $cm^{-1;}$ ^1H NMR (300MHz): δ 11.16 (s, 2H, OH), 10.68 (s, 2H), 8.81 (s, 1H, NH), 7.82 (d, *J*=7.24Hz, 2H, Ph), 7.71 (d, *J*=7.22Hz, 2H, Ph), 7.47 (dd, *J*=7.29Hz, *J*=7.31Hz 2H, Ph), 7.18 (dd, *J*=7.23Hz, *J*=7.30Hz, 2H, Ar), 7.12 (d, *J*=7.22Hz, 2H, Ar), 6.90 (d, *J*=7.32Hz, 2H, Ph), 4.82 (s, 1H, 4-CH), 2.54 (s, 6H); ^{13}C NMR (300MHz): δ172.5 (2C), 164.1 (2C), 131.1, 128.0, 130.7, 104.6 (6C,Ar ring), 159.5, 133.7, 126.9, 121.0, 118.4, 116.8 (12C, Ar ring),142.7 (2C, -C-CH_3), 111.9 (2C, -C-CO-), 44.2 (1C, CH), 18.1 (2C), EIMS (m/z) 545.12 (M^+,19%), 361.18 (23%), 333.29 (16%), 303.26 (21%), 275.13 (19%), 219.15 (12%), 109.16 (100%), 95.16 (12%), 81.21 (19%), 71.13 (09%); Anal. Calcd. for $C_{29}H_{24}ClN_3O_6$: C, 63.80; H, 4.43; N, 7.70%; Found: C, 63.82; H, 4.41; N, 7.69%.

N3,N5-Bis(2-Hydroxybenzoyl)-4-(4-Hydroxyphenyl)-2,6-Dimethyl-1,4-Dihydropyridine-3,5-Dicarboxamide (2d)

Light brown solid; yield 86% (189mg);mp:208–210°C; IR (KBr): 3164.54, 3174.57, 3028, 1662.67, 147 $cm^{-1;}$ ^1H NMR (300MHz): δ 11.17 (s, 2H, OH), 9.66 (s, 1H, OH), 10.62 (s, 2H), 8.81 (s, 1H, NH), 7.83 (d, *J*=7.10Hz, 2H, Ph), 7.17 (dd, *J*=7.11Hz, *J*=7.29Hz, 2H, Ph), 7.50 (dd, *J*=7.30Hz, J=7.27Hz, 2H, Ph), 6.61 (d, *J*=7.26Hz, 2H, Ph), 7.10 (d, *J*=7.09Hz, 2H, Ar), 6.92 (d, *J*=7.11Hz, 2H, Ph), 4.81 (s,1H, 4-CH), 2.54 (s, 6H, 2,6- CH_3); ^{13}C NMR (300MHz,): δ163.8 (2C), 172.0 (2C), 154.6, 116.1, 131.0, 138.2 (6C, Ar ring), 117.7, 126.0, 122.0, 132.7, 116.6, 157.8 (12C, Ar ring), 113.8 (2C, -C-CO-), 102.0 (2C, C-CH_3), 42.9 (1C, CH), 18.6 (2C, -CH_3); EIMS (m/z): 527.55 (M^+,31%); 495.21 (13%), 371.29 (19%), 343.18 (24%), 315.98 (38%), 288.19 (47%), 256.78 (31%), 229.65 (18%), 201.19 (24%), 185.34 (14%), 109.12 (21%), 81.26 (11%); Anal. Calcd. For ($C_{29}H_{25}N_3O_7$): C, 66.03; H, 4.78; N, 7.97%; Found: C, 66.01; H, 4.76; N, 7.95%.

N3,N5-Bis(2-Hydroxybenzoyl)-2,6-Dimethyl-4-(4-Nitrophenyl)-1,4-Dihydropyridine-3,5-Dicarboxamide (2e)

White solid; yield 79% (182mg);mp:193–195°C; IR (KBr): 3174.54, 3074.57, 3041.76, 1652.67, 1530 $cm^{-1;}$ ^1H NMR (300MHz): δ 11.12 (2H, s, OH), 10.61 (s, 2H), 8.81 (s, 1H, NH), 7.81 (d, *J*=7.19Hz, 2H, Ph), 8.03 (dd, *J*=6.21Hz,*J*=6.18Hz, 2H, Ph-NO_2), 7.46 (dd, *J*=7.31Hz, *J*=7.36Hz, 2H, Ph), 7.36 (dd, *J*=6.20Hz, *J*=6.16Hz, 2H, Ph-NO_2), 7.19 (dd, *J*=7.31Hz, *J*=7.35Hz, 2H, Ph), 6.90 (d, *J*=7.36Hz, 2H, Ph), 5.83 (s, 1H, 4-CH), 2.51 (s,6H, 2,6- CH_3); ^{13}C NMR (300MHz): 171.3 (2C), 164.1 (2C), 144.0, 122.1, 126.9, 127.2 (6C, Ar ring), 159.1, 132.6, 126.8, 121.6, 118.4, 117.0 (12C, Ar- ring), 143.8 (2C, -C-CH_3), 112.1 (2C, -C-CO-), 45.4 (1C, CH), 18.5 (2C, -CH_3); EIMS (m/z): 524.21.55 (M^+,20%), 372.28 (12%), 344.15 (19%), 316.28 (28%), 286.49 (19%), 258.48 (29%), 230.18 (17%), 181.98 (100%), 157.19 (36%), 81.12 (11%); Anal. Calcd. For $C_{29}H_{24}N_4O_8$: C, 62.59; H, 4.35; N, 10.07%; Found: C, 62.60; H, 4.33; N, 10.06%.

N3,N5-Bis(2-Hydroxybenzoyl)-4-(4-Methoxyphenyl)-2,6-Dimethyl-1,4-Dihydropyridine-3,5-Dicarboxamide (2f)

White solid; yield 87% (184mg);mp 231°C; IR (KBr): 3254.54, 3070.51, 1642.64, 808. 43 $cm^{-1;}$ ^1H NMR (300MHz): δ 11.10 (s, 2H, OH), 10.61 (s, 2H), 8.82 (s,1H, NH), 7.82 (d, *J*=7.31Hz, 2H, Ph), 7.51 (dd, *J*=7.32Hz, *J*=7.34Hz, 2H, Ph), 7.11 (dd, *J*=7.32Hz, *J*=7.34Hz, 2H, Ph), 7.02 (d, *J*=7.21Hz, 2H, Ph), 6.90 (d, *J*=7.35Hz, 2H, Ar), 6.61 (d, *J*=7.22Hz, 2H, Ar), 5.65 (s,1H, 4-CH), 2.52 (s, 6H, 2,6- CH_3), 3.73 (s, 3H, OCH_3); ^{13}C NMR (300MHz): 171.3 (2C), 164.1 (2C), 154.2, 138.1, 135.7,118.4, (6C, Ar ring), 159.1, 132.6, 126.5, 121.0, 118.2, 117.7, (12C, Ar ring), 143.9 (2C, -C-CH_3), 112.0 (2C, -C-CO-), 55.1 (1C, -OCH_3), 42.2 (1C, CH), 18.3 (2C, -CH_3); EIMS (m/z): 541.25 (M^+,28%), 509.56 (12%), 385.24 (18%), 357.54 (31%), 329.28 (19%), 299.25 (28%), 271.9 (21%), 215.19 (31%), 185.29 (100%), 157.19 (52%), 81.26 (21%); Anal. Calcd. For ($C_{30}H_{27}N_3O_7$): C,66.53; H, 5.03; N, 7.76%; Found: C, 66.51; H, 5.01; N, 7.78%.

4-(4-(Dimethylamino)phenyl)-N3,N5-Bis(2-Hydroxybenzoyl)-2,6-Dimethyl-1,4-Dihydro Pyridine-3,5-Dicarboxamide (2g)

Green yellow solid; yield 91% (188mg);mp:174–176 °C; IR (KBr): 3170.54, 3069.57, 1652.67, 810 $cm^{-1;}$ ^{1}H NMR (300MHz): δ 11.10 (s, 2H, OH), 10.21 (s, 2H, C_3-CONH and C_5-CO NH), 8.82 (s, 1H, NH), 7.81 (d, 2H, *J*=7.31Hz, Ph), 7.15 (dd, *J*=7.30Hz, *J*=7.39Hz, 2H, Ph), 7.50 (dd, *J*=7.39Hz, *J*=7.34Hz, 2H, Ph), 7.10 (d, *J*=7.54Hz, 2H, Ph), 6.90 (d, J=7.35Hz, 2H, Ar), 6.61 (d, *J*=7.56Hz, 2H, Ar), 5.79 (s, 1H, 4-CH), 3.11 (s, 6H), 2.55 (6H, s, 2,6- CH_3); ^{13}C NMR (300MHz): 171.2 (2C), 164.1 (2C), 148.2, 133.2, 128.1, 111.6, (6C, Ar ring), 143.4 (2C, -C-CH_3), 157.9, 132.0, 124.2,120.1, 117.1, 116.1 (12C, Ar ring), 112.8 (2C, -C-CO-), 103.3 (2C, $(CH_3)_2$, 32.1 (1C, CH), 18.2 (2C); EIMS (m/z): 555.23 (M^+,22%), 522.69 (29%), 398.20 (14%), 370.16 (21%), 342.59 (32%), 314.25 (16%), 314.21 (23%), 284.32 (100%), 288.21 (21%), 185.34 (41%), 157.25 (31%), 81.19 (19%); Anal. Calcd. For ($C_{31}H_{30}N_4O_6$): C, 67.14; H, 5.45; N, 10.10%; Found: C, 67.13; H, 5.46; N, 10.11%.

Synthesis of N3,N5-Bis(5-(2-Bromoacetyl)-2-Hydroxybenzoyl)-4-(Furan-2-Yl)-2,6-Dimethyl-1,4-Dihydropyridine-3,5-Dicarboxamide (3a)

A reaction mixture, compound 1a (10mmol, 3.19g), 5-(2-bromoacetyl)-2-hydroxybenzamide (20mmol, 5.16g), ethanol (5mL) stirred with reflexes by 30min at 60°C. TLC was used to monitor and establish the reaction. To get the pure product, it was recrystallized in ethyl acetate. Compounds were synthesized using the same experimental method 3b-g.

N3,N5-Bis(5-(2-Bromoacetyl)-2-Hydroxybenzoyl)-4-(Furan-2-Yl)-2,6-Dimethyl-1,4-Dihy Dropyridine-3,5-Dicarboxamide (3a)

Yellow solid; yield 81% (241mg);mp:192–195°C; IR (KBr): 3171.54, 3065.57, 1641.34 $cm^{-1;}$ ^{1}H NMR (300MHz) δ 11.19 (s, 2H, OH), 8.86 (s, 1H, NH), 7.80 (d, 2H, *J*=7.30Hz, Ph), 7.71 (s, 2H, Ph), 7.41 (d, *J*=6.19Hz, 1H, Furyl), 7.21 (d, *J*=7.31Hz, 2H, Ph), 7.15 (s, 2H, C_3-CONH and C_5-CO NH), 6.21 (1H, d, *J*=6.31Hz, Furyl), 6.02 (1H, dd, *J*=6.20Hz, *J*=6.30Hz, Furyl), 5.61 (s, 1H, CH), 4.11 (s, 4H, CH_2-Br), 2.56 (s, 6H); ^{13}C NMR (300MHz): 191.0 (2C), 174.1 (2C), 167.1 (2C), 152.1, 140.3, 109.1, 105.6 (4C, Furyl), 163.5, 133.1, 129.2, 128.6,119.6, 118.0, (12C, Ar ring), 149.8 (2C, C-CH_3), 102.1 (2C, -C-CO-NH-), 41.2 (1C, CH), 31.0 (2C, CH_2-Br), 18.2 (2C); EIMS (m/z): 743.16 (M^+, 24%), 585.23 (16%), 557.32 (31%), 525.36 (51%), 469.32 (41%), 317.51 (31%), 259.35 (23%), 231.28 (100%), 203.96 (16%), 175.69 (21%), 147.13 (18%), 81.12 (11%); Anal. Calcd. for $C_{31}H_{25}Br_2N_3O_9$: C, 50.09; H, 3.39; N, 5.65; %; Found: C, 50.10; H, 3.38; N, 5.66; %.

N3,N5-Bis(5-(2-Bromoacetyl)-2-Hydroxybenzoyl)-2,6-Dimethyl-4-Phenyl-1,4-Dihydro Pyridine-3,5-Dicarboxamide (3b)

Brown solid; yield 86% (234mg);mp:203–205°C; IR (KBr): 3175.59, 3068.60, 648.36 $cm^{-1;}$ ^{1}H NMR (300MHz,): δ 11.08 (s, 2H, OH), 8.78 (s, 1H, NH), 7.78 (d, 2H, *J*=7.32Hz, Ph), 7.68 (s, 2H, Ph), 7.32 (d, *J*=7.32Hz, 2H, Ph), 7.25 (d, *J*=7.30Hz, 2H, Ph), 7.22 (t, *J*=7.34Hz, 1H, Ar), 7.23 (d, *J*=7.33Hz, 2H, Ph), 7.13 (s, 2H), 4.61 (1H, s, CH), 4.10 (s, 4H, CH_2-Br), 2.41 (s, 6H); ^{13}C NMR (300MHz):191.2 (2C), 174.8 (2C), 167.0 (2C), 164.1, 133.6, 129.8, 128.0, 119.1, 118.3 (12C, Ar ring), 148.6 (2C, C-CH_3), 127.8, 127.6, 125.6, 105.6 (6C, Ar ring), 102.9 (2C, -C-CO-NH-), 41.7 (1C, CH), 31.8 (2C, CH_2-Br), 18.7 (2C); EIMS (m/z) 753.08 (M^+,28%), 595.39 (32%), 567.28 (16%), 535.21 (21%), 479.24 (17%), 327.19 (24%), 271.54 (19%), 241.98 (21%), 213.65 (100%), 185.65 (29%), 157.54 (12%), 81.12 (19%),; Anal. Calcd. for $C_{33}H_{27}Br_2N_3O_8$: C, 52.61; H, 3.61; N, 5.58; %. Found: C, 52.60; H, 3.62; N, 5.56; %.

N3,N5-Bis(5-(2-Bromoacetyl)-2-Hydroxybenzoyl)-4-(4-Chlorophenyl)-2,6-Dimethyl-1,4-Dihydropyridine-3,5-Dicarboxamide (3c)

White solid; yield 93% (265mg);mp:166–168°C; IR (KBr): 3178.55, 3070.59, 1645.35, 827.65 $cm^{-1;}$ ^{1}H NMR (300MHz): δ 11.09 (s, 2H, OH), 8.71 (s, 1H, NH), 7.76 (d, 2H, *J*=7.29Hz, Ph), 7.70 (d, *J*=7.30Hz, 2H, Ph), 7.60 (s, 2H, Ph), 7.23 (d, *J*=7.30Hz, 2H, Ph), 7.15 (d, *J*=7.31Hz, 2H, Ph), 7.11 (s, 2H), 4.62 (s, 1H, CH), 4.17 (s, 4H, CH_2-Br), 2.43 (s, 6H,); ^{13}C NMR (300MHz): 191.3 (2C), 174.3 (2C), 167.3 (2C), 131.6, 130.8, 128.6, 105.6 (6C, Ar ring), 164.2, 133.2, 128.2, 128.3, 119.0, 118.1, (12C, Ar ring), 148.4 (2C, C-CH_3), 102.2 (2C, -C-CO-NH-), 41.6 (1C, CH), 31.2 (2C, CH_2-Br), 18.3 (2C); EIMS (m/z):

787.10 (M^+,10%), 629.16 (15%), 601.38 (21%), 573.54 (19%), 545.95 (24%), 513.65 (19%), 361.19 (24%), 333.18 (16%), 303.21 (24%), 275.10 (22%), 247.25 (27%), 213.25 (100%), 185.25 (24%), 157.12 (13%), 81.12 (19%); Anal. Calcd. for $C_{33}H_{26}Br_2ClN_3O_8$: C, 50.31; H, 3.33; N, 5.33; %. Found: C, 50.30; H, 3.31; N, 5.32; %.

N3,N5-Bis(5-(2-Bromoacetyl)-2-Hydroxybenzoyl)-4-(4-Hydroxyphenyl)-2,6-Dimethyl-1,4-Dihydropyridine-3,5-Dicarboxamide (3d)

White powder; yield 84% (219mg);mp 150–152°C; IR (KBr): 3180.55, 3070.55, 1645.38, 1477 $cm^{-1;}$ ^1H NMR (300MHz) δ 11.12 (s, 2H, OH), 9.43 (s, 1H, OH), 8.80 (s, 1H, NH), 8.20 (d, 2H, *J*=7.31Hz, Ph), 7.85 (s, 2H, Ph), 7.23 (d, 2H, *J*=7.31Hz, Ph), 7.15 (s, 2H,), 7.10 (d, *J*=7.09Hz, 2H, Ar), 6.59 (d, *J*=7.01Hz, 2H, Ar), 5.68 (s,1H, CH), 4.11 (s, 4H, CH_2-Br), 2.55 (s, 6H, 2,6-CH_3); ^{13}C NMR (300MHz_6): 191.3 (2C), 174.9 (2C), 167.7 (2C), 155.2, 137.4, 130.2, 114.9 (6C, Ar ring), 162.7, 131.9, 128.9, 128.1, 118.2, 117.9 (12C, Ar ring), 149.1 (2C, C-CH_3), 102.5 (2C, -C-CO-NH-), 41.0 (1C, CH), 31.0 (2C, CH_2-Br), 18.2 (2C); EIMS (m/z): 769.16 (M^+, 27%), 611.24 (23%), 582.95 (21%), 555.36 (17%), 527.58 (32%), 495.21 (27%), 343.25 (22%), 287.13 (19%), 257.16 (27%), 229.23 (21%), 213.25 (100%), 185.65 (19%), 157.21 (21%), 81.25 (12%); Anal. Calcd. for $C_{33}H_{27}Br_2N_3O_9$: C, 51.52; H, 3.54; N, 5.46; %. Found: C, 51.53; H, 3.51; N, 5.45; %.

N3,N5-Bis(5-(2-Bromoacetyl)-2-Hydroxybenzoyl)-2,6-Dimethyl-4-(4-Nitrophenyl)-1,4-Dihydropyridine-3,5-Dicarboxamide (3e)

Brown solid; yield 83% (234mg);mp:195–197°C; IR (KBr): 3174.54, 3067.56, 1653.34, 1530.01 $cm^{-1;}$$^1H$ NMR (300MHz):δ 11.23 (s, 2H, OH), 8.74 (s, 1H, NH), 8.32 (d, *J*=7.23Hz, 2H, Ph), 7.62 (2H, s, Ph), 7.72 (d, *J*=7.41Hz, 2H, Ph), 7.45 (d, *J*=7.20Hz, 2H, Ph), 7.17 (d, *J*=7.40Hz, 2H, Ph), 7.15 (s, 2H,), 4.62(s, 1H, CH), 4.12 (s, 4H, CH_2-Br), 2.43 (s, 6H); ^{13}C NMR (300MHz,): 191.4 (2C), 175.3 (2C), 167.2 (2C), 144.2, 127.4, 126.1,123.1 (6C, Ar ring), 164.3, 134.1, 129.2, 128.4, 119.1, 118.2 (12C, Ar ring), 147.2 (2C, C-CH_3), 102.1 (2C, -C-CO-NH-), 41.2 (1C, CH), 31.2 (2C, CH_2-Br), 18.2 (2C, -CH_3); EIMs (m/z) 798.45 (M^+, 34%), 640.23 (19%), 556.21 (24%), 524.12 (21%), 372.26 (20%), 316.23 (13%), 286.21 (36%), 258.23 (13%), 213.25 (100%), 137.25 (19%), 109.23 (09%), 81.32 (05%); Anal. Calcd. for $C_{33}H_{26}Br_2N_4O_{10}$: C, 49.64; H, 3.28; N, 7.02; %. Found: C, 49.63; H, 3.26; N, 7.01; %.

N3,N5-Bis(5-(2-Bromoacetyl)-2-Hydroxybenzoyl)-4-(4-Methoxyphenyl)-2,6-Dimethyl-1,4-Di Hydropyridine-3,5-Dicarboxamide (3f)

Pale brown solid;yield 93% (251mg);mp:196–197°C; IR (KBr): 3175.04, 3064.16, 1650.11 (OCNH), 801.20 $cm^{-1;}$ ^1H NMR (300MHz)δ 11.16 (s, 2H, OH), 8.72 (s,1H, NH), 7.75 (d, *J*=7.30Hz, 2H,Ph), 7.61 (s, 2H, Ph), 7.23 (d, *J*=7.28Hz, 2H, Ph), 7.19 (s, 2H, C_3-CONH and C_5-CO NH), 7.02 (d, *J*=7.32Hz, 2H, Ph), 6.62 (d, *J*=7.31Hz, 2H, Ar), 4.68 (s, 1H, CH), 4.21 (4H, s, CH_2-Br), 3.32 (s, 3H), 2.23 (s, 6H); ^{13}C NMR (300MHz): 192.5 (2C), 173.1 (2C), 166.2 (2C), 155.1, 138.8, 136.4,118.1 (6C, Ar ring), 165.3, 134.1, 128.1, 127.9, 118.9, 118.2, (12C, Ar ring), 149.1 (2C, C-CH_3), 103.2 (2C, -C-CO-NH-), 55.1 (1C, OCH_3), 42.2 (1C, CH), 32.7 (2C, CH_2-Br), 19.1 (2C, -CH_3), 55.2; EIMS (m/z) 799.13 (M^+, 12%), 640.19 (22%), 612.32 (13%), 584.32 (24%), 556.32 (29%), 525.12 (13%), 372.12 (32%), 314.25 (27%), 258.78 (17%), 213.21 (29%), 137.21 (11%), 109.32 (14%), 81.10 (08%); Anal. Calcd. for $C_{34}H_{29}Br_2N_3O_9$: C, 52.13; H, 3.73; N, 5.36; %. Found: C, 52.11; H, 3.72; N, 5.34; %.

N3,N5-Bis(5-(2-Bromoacetyl)-2-Hydroxybenzoyl)-4-(4-(Dimethylamino)phenyl)-2,6-Dimethyl-1,4-Dihydropyridine-3,5-Dicarboxamide (3g)

White solid; yield 90% (231mg);mp:195–197°C; IR (KBr): 3174.54, 3067.56, 1653.34, 807.21 $cm^{-1;}$ ^1H NMR (300MHz) δ 11.11 (s, 2H, OH), 8.83 (s, 1H, NH), 7.77 (s, 2H, Ph), 7.71 (d, 2H, *J*=7.30Hz, Ph), 7.29 (d, 2H, *J*=7.31Hz, Ph), 7.18 (d, *J*=7.11Hz, 4H, Ar), 7.10 (s, 2H, C_3-CONH and C_5-CO NH), 4.71 (s, 1H, CH), 4.10 (s, 4H, CH_2-Br), 3.08 (s, 6H, -$N(CH_3)_2$), 2.45 (s, 6H, 2,6- CH_3); ^{13}C NMR (300MHz): 191.6 (2C), 167.9 (2C), 174.4 (2C), 148.9, 133.0,128.5, 111.9 (6C, Ar ring), 163.1, 133.3, 129.0, 128.1, 119.1,118.5 (12C, Ar ring), 149.3 (2C, C-CH_3), 102.9 (2C, -C-CO-NH-), 43.0 (1C, CH), 40.9 (2C, -$N(CH_3)_2$), 31.4 (2C, CH_2-Br), 18.2 (2C); EIMS (m/z): 791.23 (M^+, 20%), 638.25 (32%), 554.21 (18%), 522.28 (22%), 384.25 (17%), 370.23 (32%), 314.25 (30%), 284.12 (18%), 256.23 (21%),

213.25 (100%), 185.12 (29%), 157.32 (14%), 81.24 (17%); Anal. Calcd. for ($C_{35}H_{32}Br_2N_4O_8$): C, 52.78; H, 4.05; N, 7.03; %. Found: C, 52.75; H, 4.04; N, 7.02; %.

Pharmacological Activity

Analgesic Activity

Compounds 2a-g, and 3a-g were screened for their analgesic activity. In total, five albino mice weighing 25–30g were selected and included in each of the four groups. A 12-hour-long light–dark cycle was followed and the laboratory temperature was maintained at ambient conditions (each animal was allowed a 1 week adaption before being experimented). They were administered (5 or 10mg/kg) with the compound under study, dissolved in DMSO, volume being 0.1 mL and the control animals were orally administered volume of 0.1mL DMSO. The animal studies were carried out by the Karpagam College of Pharmacy, Department of Pharmacology, Pollachi Road, Othakkalmandapam, Coimbatore-641,032, approved by the Animal Ethics Committee of Karpagam Faculty of Medical Sciences & Research (IAEC No. –KFMSR/B.Pharm/05/2021). The experiments were conducted in compliance with the guidelines of the Institutional Animal Committee, governed by CPCSEA guidelines, Government of India.

Tail Flick Method

The tail flick method, previously reported was employed in this study,[43,44] on mice to determine the analgesia, to find the radiant heat. During this test, a high intensity beam of light of was absorbed on the tail. As the final outcome, a tail flick retort was taken in seconds. An analgesiometer with 5.4 amp conversion (tail flick method 1Nco) is used; 2–4 hours were set as the pre-drug response time, the damage occurred on tail corresponds to a time of 10–15 sec.

It is possible to attribute the analgesic effects produced by the synthesized test molecules to a central mechanism involving receptor systems or to a peripheral mechanism correlated to the inhibition of prostaglandins, leukotrienes, and other endogenous substances. Analgesia pre-emptively prevents central sensitization with anti-nociceptive treatment. Diclofenac has well-known anti-nociceptive effects. Diclofenac was administered intraperitoneally to rats before and after acute and inflammatory-induced pain to investigate the analgesic effects.[45] In general, NSAIDs are considered to be analgesic by inhibiting cyclo-oxygenase via a peripheral site of action. NSAIDs have been demonstrated to have central anti-nociceptive mechanisms.[46,47]

Statistical Analysis

The mean and standard deviations (SEM) are provided; ANOVA was used to analyze the statistics of all variables after which Tukey's post hoc comparison test was done.

Molecular Docking

The compound showed an interaction during molecular docking studies using Autodock vina 1.1.2,[48] and there exist a binding mode between compounds 2C and the COX proteins. Coordination geometry of the COX-2 protein complexed with SC-558 (PDB ID: 1CX2) inhibitor[49] and crystal structure of COX-1 protein bound with indomethacin-(*R*)-alpha-ethyl-ethanolamide[50] (PDB ID: 2OYE) were downloaded from http://www.rcsb.org. ChemDraw Ultra 12.0 and Chem3D Pro 12.0 were used to create the 3D structures of compounds 2c and diclofenac (www.cambridgesoft.com). Autodock Vina 1.5.6 program package was used (http://mgltools.scripps.edu). According to our findings, the 1CX2 protein search grid contains center_x: 51.794, center_y: 16.641, and center_z: 8.985 with size_x: 52, size_y: 24, and size_z: 51 having a spacing of 1.0 Å. According to the search grid, its center_x is 243.133, center_y is 98.798, and center_z is −36.556 of dimension (size) of _x 48, _y 42, and _z 48 (with 1.0 Å).

ADME and Molecular Property Predictions

By using Lipinski's "rule of five",[51] the molecular property of compound 2c was calculated and the approach was theoretical and related to ADME and toxicity. The parameters of Liminski were predicted by Swiss ADME, a web-based tool.[52] The measure of evaluation corresponds to the topological polar surface area (tPSA).[53] The bioavailability is driven by gastrointestinal absorption.[54]

Calculation of percentages: % ABS = 109 – (0.345 × tPSA).

Results and Discussion

Chemistry

A previously reported[55] method was used to synthesize 1,4-dihydropyridine 1a-h, and an amination method was used to prepare 2a-g, and 3a-g through reactions of compounds 1a-h with benzamide (Scheme 1). Compounds 2a-2g and 3a-3g of ^{1}H NMR and ^{13}C NMR spectra were given in the (Supplementary Material Figures 1–28). Based on the IR spectrum, 2a was found to have absorption bands at 3174.54, 1652.67, and 3074.57 cm^{-1}, which correlate well with groups −NH−, HNCO−, and aromatic −CH. Compound 2a shows ^{1}H NMR signals of 5.84, 10.60, and 8.84 ppm, consistent with 4CH−, −CONH, and −NH− protons respectively. The ^{13}C NMR spectrum of 2a displays signals at δ 171.1, 164.2, and 32. 4 ppm, consistent to the C=O, C=O and 4-C−, respectively. A molecular ion peak is detected at m/z 501.52 (M^{+}, 10%) in the mass spectrum of compound 2a, confirming its molecular weight. In the IR spectrum of 3a, absorption bands were observed at 3065.57, 3171.54, and 1641.34 cm^{-1}, consistent with the -CH, -NH, and -HNCO groups. The ^{1}H NMR signal of 3a is consistent with the 4CH− and −CONH proton, signals at δ 5.61 and 7.15 ppm, respectively. In the ^{13}C NMR spectrum of 3a, there were peaks at δ 41.2, 167.1, and 174.1 ppm, corresponding to the 4-C, -C-CO-NH, and -C=O carbon atoms, respectively. In the mass spectrum of compound 3a, there is a peak at m/z 743.16 (M^{+}, 24%), which corresponds to the conformed molecular weight.

Analgesic Activity

At a dose of 10 mg/kg, compound 2c was more effective (60 min) than diclofenac or salicylamide. The variations in analgesic activities are shown in Figure 2 and the results are summarized in Table 1. These are excellent target

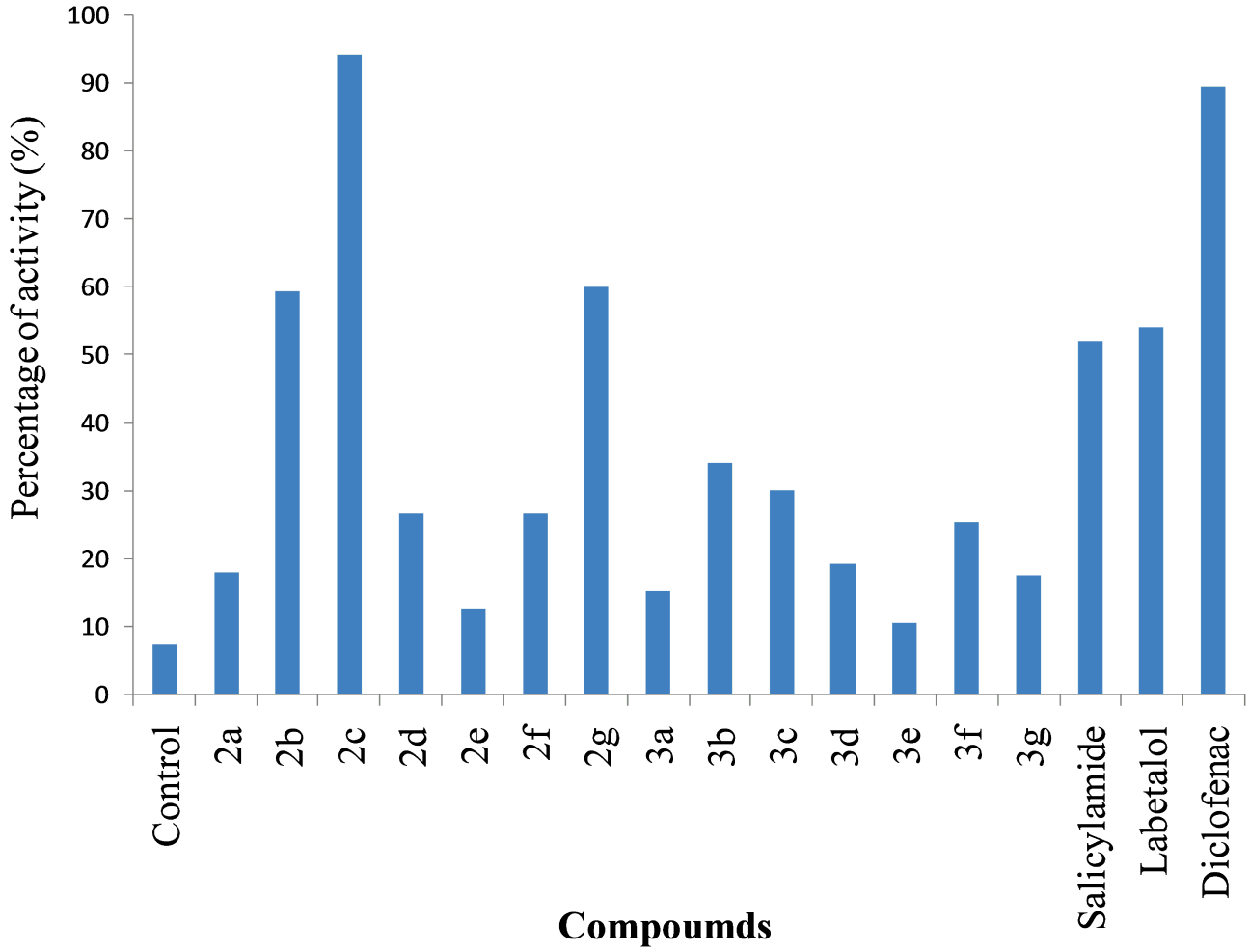


Figure 2 Analgesic activities of compounds 2a-g, and 3a-g at 10mg/kg (60min).

Table 1 The Analgesic Properties of Compounds 2a-g, and 3a-g

Compounds	Dose (mg/kg)	Tail-Flicking Latency[a]			
		30 Min	% of Activity	60 Min	% of Activity
Control	0	0.8 ± 0.01	05.31	01.1 ± 0.21	07.32
2a	5 10	1.8 ± 0.17 2.6 ± 0.87	12.01 17.32	01.8 ± 0.21 02.7 ± 0.02	12.01 18.03
2b	5 10	2.1 ± 0.16 4.0 ± 0.15	14.03 26.62	08.0 ± 0.01 08.9 ± 0.38	53.37 59.38
2c	5 10	2.1 ± 0.10 5.8 ± 0.00	14.01 38.66	12.2± 0.14 14.1 ± 0.01	81.35 94.07
2d	5 10	1.7 ± 0.14 3.1 ± 0.10	11.33 20.66	02.0 ± 0.01 04.0 ± 0.17	13.33 26.64
2e	5 10	1.2± 0.14 1.4 ± 0.32	08.01 09.33	01.6 ± 0.12 01.9 ± 0.19	10.65 12.61
2f	5 10	2.1 ± 0.14 2.4 ± 0.17	14.05 16.03	03.2 ± 0.01 04.0 ± 0.10	21.33 26.62
2g	5 10	2.1 ± 0.14 2.4 ± 0.17	14.05 16.03	08.1 ± 0.05 09.0 ± 0.04	54.04 60.01
3a	5 10	1.0 ± 0.26 1.2 ± 0.36	06.67 08.00	02.1 ± 0.12 02.3 ± 0.70	14.07 15.33
3b	5 10	1.9 ± 0.17 3.0 ± 0.15	12.66 20.04	03.2 ± 0.01 05.1 ± 0.16	21.31 34.02
3c	5 10	1.5 ± 0.01 1.8 ± 0.14	10.01 12.04	04.2 ± 0.01 04.5 ± 0.05	28.09 30.01
3d	5 10	1.2 ± 0.05 1.3 ± 0.01	08.04 08.62	02.6 ± 0.07 02.9 ± 0.05	17.32 19.35
3e	5 10	1.0 ± 0.11 1.3 ± 0.32	06.67 09.00	01.4 ± 0.11 01.6 ± 0.32	09.33 10.61
3f	5 10	2.2 ± 0.05 2.1 ± 0.01	14.62 14.03	03.2 ± 0.01 03.8 ± 0.04	21.35 25.34
3g	5 10	1.0 ± 0.11 1.2 ± 0.46	06.67 08.0	01.7 ± 0.45 02.6 ± 0.32	11.31 17.62
Salicylamide	5 10	2.7 ±0.20 5.1 ± 0.89	18.0 34.0	05.9 ± 0.75 07.8 ± 0.29	39.33 52.0
Labetalol	5 10	3.9 ± 0.16 5.9 ± 0.06	26.0 39.33	05.2 ± 0.11 08.1 ± 0.02	34.6 54.0
Diclofenac	5 10	5.2 ± 0.06 7.4 ± 0.02	34.66 49.34	10.2 ± 0.11 13.4 ± 0.02	79.21 89.32

Notes: [a]Significance levels p <0.001 vs control; The maximal tail flick response is 15 sec, it is considered as 100%.

compounds with sensible active properties, based on the results of in vivo tests. As a result of electron-donating groups present on the aryl ring, the activity development took place and also the presence of 4-chlorophenyl moeityin compound 2c is enhanced the activity compared to un-substituted derivative. Compound 2c has 2-hydroxybenzamide group and it shows the best activity compared to diclophenac.

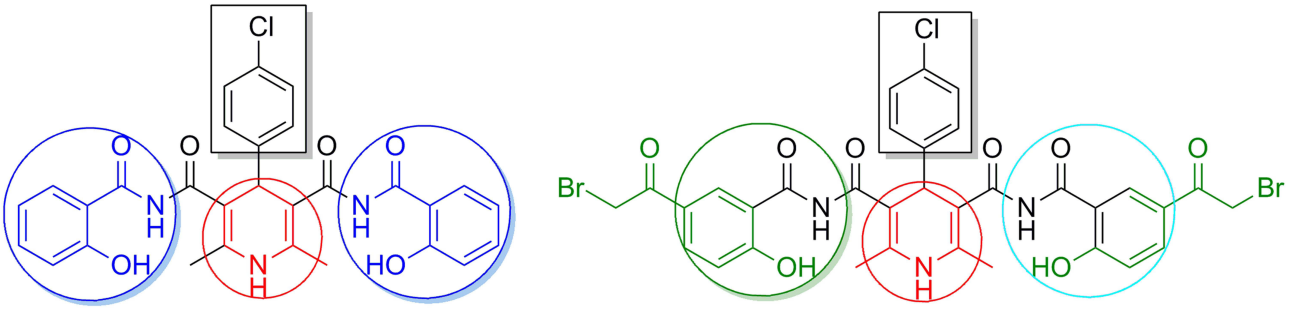


Figure 3 Structure–activity relationship of compounds 2c and 3c.

The relationship between the structure of a compound and its analgesic activity is illustrated by several rules. In Figure 3, we see that in the para position there is a phenyl group which acts as a lipophilic moiety, whereas the hydrogen bonding related to −C=O− and −NH groups act as hydrophilic centres. Hence, the 1,4-dihydropyridine derivatives with salicylamide are important pharmacophoric agents showing biological activity.

Compound 2c, which possesses a 1,4-dihydropyridine structure with 4-Cl-Ph, showed significant analgesic activity and it was more effective due to electron withdrawing groups (4'-Cl). Compound 3c which is having a 1,4-dihydropyridine (DHP) ring of 4'-substitution of hydroxybenzamide was less active.

However, compounds 3a-g were less active than other compounds due to two nearby halogen groups interfering with the amide functionality. Literature indicates that thiophenecarboxamide has the greatest pharmacological effects.[56] Similarly, compounds 2b and 2c showed moderate-to-good analgesic activities compared to the un-substituted 2-hydroxybenzamide derivative. Compounds 2a–g show an activity in the range 12.01–94.07% at 60 min duration, and these results are comparable with that of the reference drug.

A selective COX-2 inhibitor is a better choice for stopping the pain-stimulating action of cycloxygenase-2 enzyme, but COX-2 inhibitors are associated with a wider range of side effects.[57,58] The use of selective COX-2 inhibitors or coxib drugs can antagonize COX-2 enzyme selectively, which is essential. We found that compound 2c had good docking scores (−9.7 kcal/mol) against COX-2 enzyme, while our standard drug Diclofenac had docking scores of –7.5 kcal/mol. Therefore, compound 2c may be a promising candidate for selectively inhibiting COX-2.

The selective COX-2 inhibitor is more effective than the conventional NSAIDs and has low GI and high cardiovascular side effects compared to the latter. 1,4-dihydropyridine hybrid benzamide is a COX-2 inhibitor with a high degree of selectivity towards its target. It provides an alternative to other selective and traditional NSAIDs in treating patients with arthritis and other painful conditions.

Docking Studies

Compound 2c and the control diclofenac were studied for their docking performances with COX enzyme proteins 1CX2 and 2OYE using Autodock Vina program. The 2c displayed more binding affinity (−9.7 kcal/mol) to the 1CX2 protein than shown in the case of 2OYE protein (−8.7 kcal/mol). However, it has only a lower binding affinity towards proteins 1CX2 and 2OYE than the control, diclofenac. The binding energy values of these two proteins are −7.5 and −8.2 kcal/mol, respectively. Because of its amide moiety, compound 2c showed remarkable binding affinity towards the COX proteins, 1CX2 and 2OYE, compared to diclofenac. Protein–ligand bonds are stabilized by hydrogen bonding, and the favorable bond distance between the donor and acceptor is less than 3.5 Å.[59] A comparison of hydrogen bond distances existing in diclofenac and compound 2c showed a value less than 3.5 Å. A hydrogen bond is formed between compound 2c and the receptor 1CX2 (bond length: 2.18), Tyr122 (bond length: 2.84) and the hydrogen bonding interactions of Lys546 (bond length: 1.88) were found to be complex. Residues Arg44, Arg61, Lys79 and Pro542 have shown complex hydrophobic interactions. Diclofenac, used as a control forms only one hydrogen bond with 1CX2. A hydrogen bond was

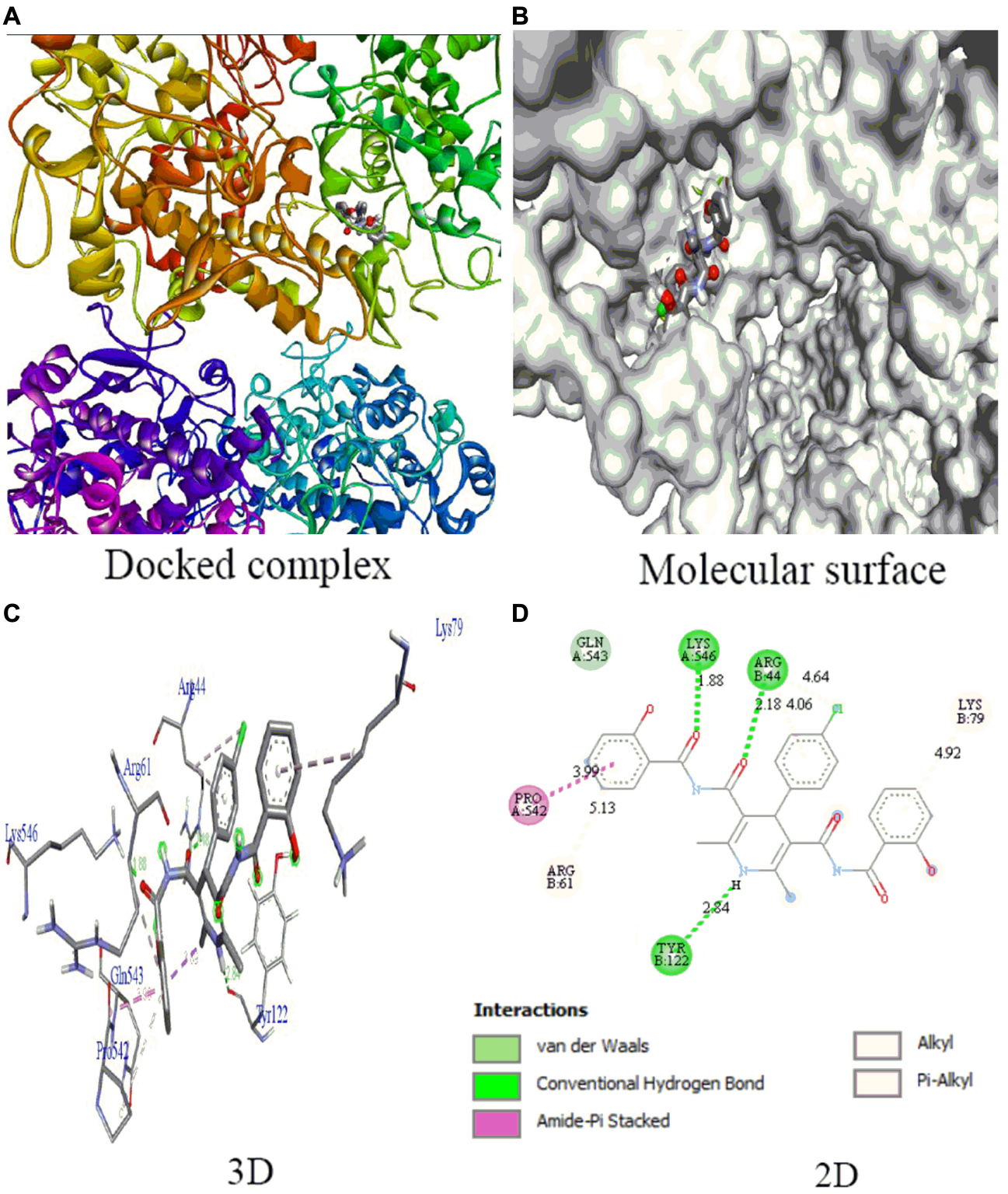


Figure 4 The binding of 2c with 1CX2 protein complex: (**A**) Docking complex; (**B**) Molecular surface; (**C**) 3D interaction; (**D**) 2D interaction.

formed in the residue Gln461 (bond length: 2.24). It was found that the hydrophobic interactions among Cys36, Pro40, Cys41, Cys47, Leu152 and Pro153 were complex. Figure 4 illustrates the hydrophobic interactions formed between amino acids in 1CX2 protein and compound 2c. In Figure 5, diclofenac was shown as a control. A hydrogen bond is formed between compound 2c and receptor 2OYE. The residue His207 was involved in hydrogen bonding (2.16 bond

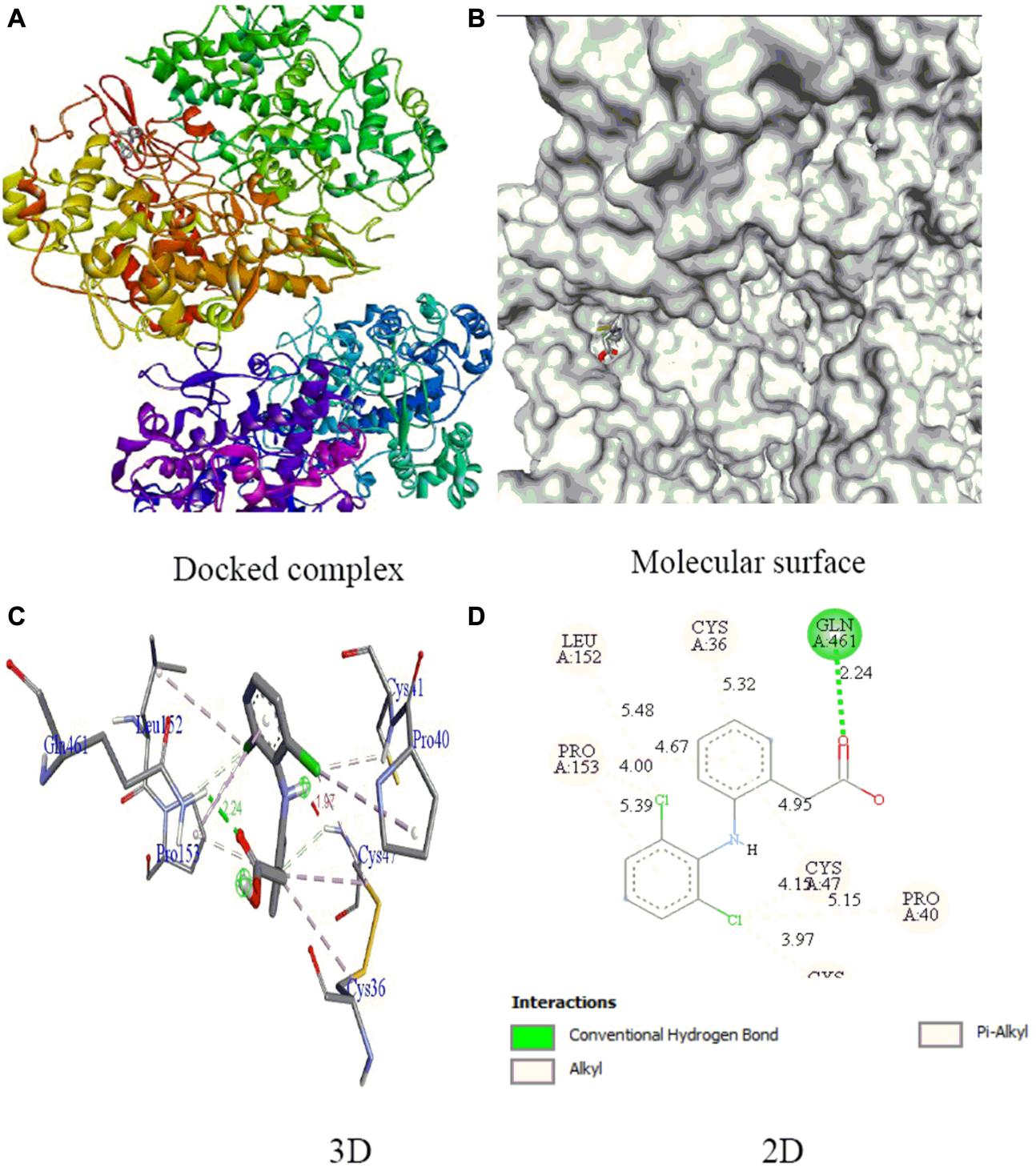


Figure 5 The binding of diclofenac with 1CX2 protein complex: (**A**) Docking complex; (**B**) Molecular surface; (**C**) 3D interaction; (**D**) 2D interaction.

length) and in hydrophobic interactions, residues His386, Val447, and Glu454 took part. The Glu454 is involved in electrostatic interactions and hydrogen bonds were formed only once between diclofenac and receptor 2OYE in the case of the control diclofenac. The hydrogen bonding interaction with residue Val349 (bond length: 2.79) was complex in nature. As a result, Tyr348, Leu352, Tyr385, Phe518, Ile523, Ala527 and Leu531 formed complex hydrophobic interactions and amino acid residues in 2OYE form hydrogen bonds and hydrophobic interactions. Figure 6 shows

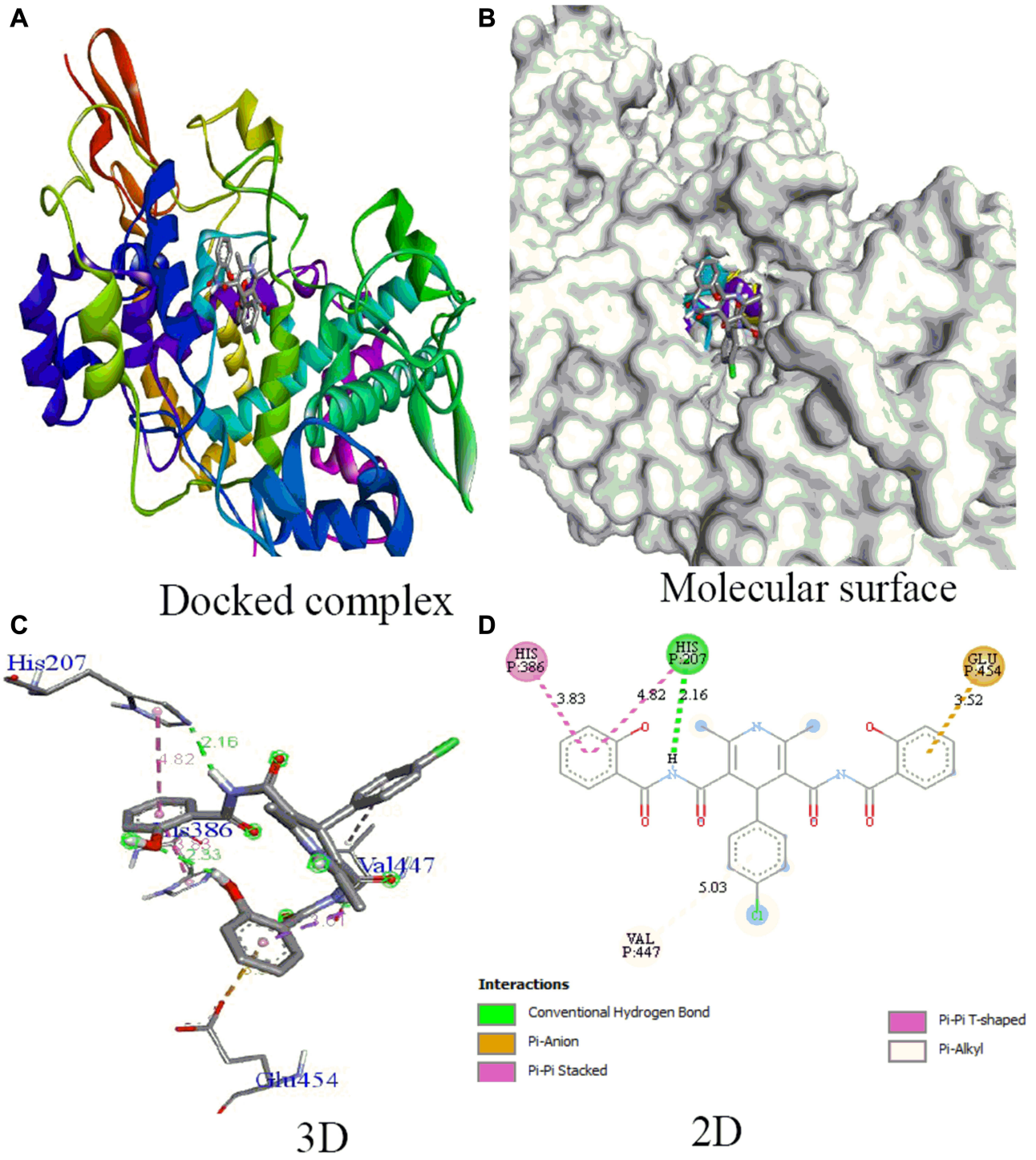


Figure 6 The binding of 2c with2OYE protein complex: (**A**) Docking complex; (**B**) Molecular surface; (**C**) 3D interaction; (**D**) 2D interaction.

a protein containing compound 2c, and Figure 7 shows diclofenac as a control. The results display that compound 2c has a remarkable inhibition capability than the control, diclofenac and other compounds in both COX proteins, these results are shown in Table 2.

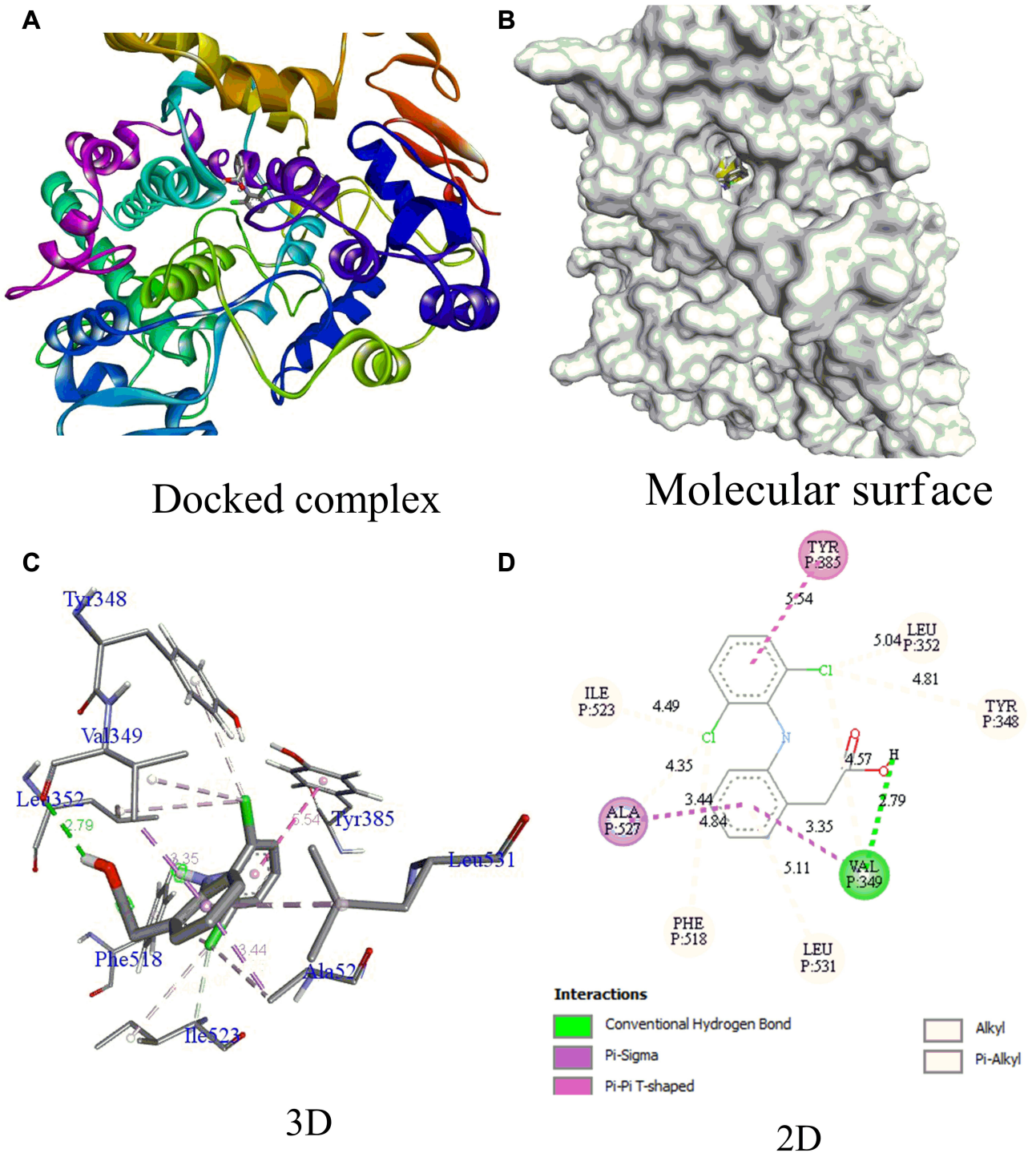


Figure 7 The binding of diclofenac with 2OYE protein: (**A**) Docking complex; (**B**) Molecular surface; (**C**) 3D interaction; (**D**) 2D interaction.

ADME and Molecular Property Prediction

Bioactive compounds are increasingly used as therapeutic agents due to their bioavailability in the case of oral doses.[60] According to the study, hydrogen binding capacity, reduction of molecular flexibility, intestinal absorption, and low polar surface area were the main predictors.[61] With one violation of Lipinski's "rule of five" for MW >500 daltons, compound 2c obeys Lipinski's "rule of five" without violation; and the control diclofenac obeys Lipinski's "rule of five" without violation (Table 3). The number of rotatable bonds and the binding of receptors or channels define the conformational

Table 2 Compound 2c and Diclofenac Interact with COX Enzyme Proteins 1CX2 and 2OYE

Compounds	1CX2 Protein			2OYE Protein		
	Binding Affinity (kcal/mol)	No. of H- Bonds	H-Bonding Residues	Binding Affinity (kcal/mol)	No. of H- Bonds	H-Bonding Residues
2a	−7.9	1	lys546	−7.2	0	-
2b	−8.1	2	Arg44,Tyr122,	−6.5	0	-
2c	−9.7	3	Arg44,Tyr122,lys546	−8.7	1	His207
2d	−8.2	1	Tyr12	−7.6	0	-
2e	−9.1	3	Arg44,Tyr122,lys546	−7.1	0	-
2f	−8.3	1	Tyr12	−6.9	0	-
2g	−8.0	1	Tyr12	−6.2	0	-
3a	−6.2	0	-	−5.2	0	-
3b	−6.9	0	-	−5.9	0	-
3c	−7.6	1	Tyr12	−6.5	0	-
3d	−5.3	0		−6.1	0	-
3e	−5.9	0	-	−5.9	0	-
3f	−6.6	0	-	−6.3	0	-
3g	−6.8	0	-	−6.0	0	-
Diclofenac	−7.5	1	Gln461	−8.2	1	Val349

changes which can be measured by counting the number of rotatable bonds in the molecules. In both compounds, 2c and diclofenac, there are less than 10 rotatable bonds and there are no chirality centers, hence have low conformational flexibility. In addition to passive molecular transport across membranes, the topological polar surface area (tPSA) is correlated to the blood-brain barrier.[53] Compound 2c and diclofenac have tPSA value of 144.83 and 49.33 $Å^2$, respectively. In contrast, compound 2c does not meet the criteria for gastro-intestinal absorption and is to be given later via oral administration, while diclofenac does. The latter was expected to penetrate the blood–brain barrier more readily (tPSA > 90 $Å^2$) than compound 2c. The side effects of the central nervous system are compacted or abstracted in the case of compound 2c, whereas diclofenac is free of such harmful effects. The absorption percentages of compound 2c and diclofenac were 59.04% and 82.98%, respectively, which indicatethe diclofenac has high bioavailability than 2c. The oral administrative route creates an acceptable bioavailability (>50%). Compound 2c has only poor water solubility (-logS value of −6.29), whereas that of diclofenac was moderate (-logS value of −4.65). When 2c was administered, liver dysfunction was not anticipated as a side effect, because it has been projected as non-inhibitors of CYP2D6 and diclofenac has the property of inhibiting the enzyme CYP2D6 as expected. The *P*-glycoprotein (P-gp) belongs to the family ofATP-binding cassette transporters. It is involved in intestinal absorption, drug metabolism, and brain diffusion, and its inhibition may alter the bioavailability and protection.[62] As a result of the extra accumulation of phospholipids in tissues, drug-induced phospholipidosis occurs.[63] According to our study, diclofenac and compound 2c are not P-gp substrates nor they induce phospholipidosis. Compound 2c has a respectable ADME and toxicity profile, low gastro-intestinal absorption, and no blood–brain barrier penetration. As a result, compound 2c was recognized as having drug-like properties as perLipinski's "rule of five", with only one exception, ie, the violation of MW > 500; the projected parameters are acceptable within the allowed range.

Table 3 ADME Properties of Diclofenac and 2c

Comp.	tPSA[a] (Topological Polar Surface Area)	%Abs[b] (Absorption)	MW[c] (Molecular Weight)	RoB[d] (Number of Rotatable Bonds)	HBD[e] (Number of Hydrogen Bond Donors)	HBA [f] (Number of Hydrogen Bonds Acceptors)	MR [g] (Molar Refractivity)	IlogP [h] (MlogP) (Logarithm of Compound Partition Coefficient Between n-Octanol and Water)	LogS [i] (Logarithm of Water Solubility)	CYP2D6 Inhibitor
Rule	≤140 Å^2	>50	≤500	≤10	≤5	≤10	40–130	<5	>-4	-
2c	144.83	59.04	547.99	9	5	6	148.48	3.45 (2.98)	−6.29	No
Diclofenac	49.33	82.98	296.15	4	2	2	77.55	1.98 (3.84)	−4.65	Yes

Conclusions

Newly designed compounds (2a-g and 3a-g), dihyropyridines with substitution of various amide group at C-3, and C-5 positions showed higher analgesic activity. Particularly, compound 2c was the best analgesic agent compared to the standard drug, serving as a new class of active 1,4-dihydropyridne derivatives. The molecular docking study supports that 2c might be a potent compound than the control diclofenac in inhibiting COX enzyme proteins. According to ADME properties, compound 2c has no toxicity and can be accepted as a drug-like molecule. However, further studies will be done to elucidate the exact mechanism(s) of the analgesic activity of title compounds. In this study, we found that 1,4-dihydropyridine hybrid benzamides are more effective than conventional NSAIDs. A lower dose combination of 1,4-dihydropyridine hybrid benzamide is more effective for analgesic activity compared to conventional NSAIDs. According to our findings, the combination product was significantly more effective than the single drug, which may be attributed to a different mechanism of action. However, there are more chances of side effects with combination products. It is imperative to conduct detailed and in-depth studies on combinations of drugs.

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Disclosure

The authors report no conflicts of interest in this work.

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