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ORIGINAL ARTICLE

Synthesis of *tert*-butyl (substituted benzamido)phenylcarbamate derivatives: anti-inflammatory activity and docking studies

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Abstract A series of new *tert*-butyl 2-(substituted benzamido) phenylcarbamate **(4a–4j)** were synthesized by the condensation of *tert*-butyl 2-amino phenylcarbamate (**3**) with various substituted carboxylic acid in the presence of EDCI and HOBt as coupling reagent, obtain in excellent yields. The structures of all newly synthesized compounds were characterized spectroscopically and evaluated for in vivo anti-inflammatory activity compared to the standard drug, indomethacin, by using the carrageenan-induced rat paw edema protocol. Most of the compounds exhibited a promising anti-inflammatory activity within 9 to 12 h, the percentage of inhibition values ranging from 54.239 to 39.021%. The results revealed that the compounds **4i** and **4a** exhibited better or equivalent anti-inflammatory activity with the percentage of inhibition of 54.239 and 54.130%, respectively, which was comparable to standard drug. In addition to experimental results, in silico docking studies was used as a tool to verify and expand the experimental outcomes.

Keywords Amide derivatives · Anti-inflammatory activity · Paw edema · Docking studies

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Introduction

Inflammation is a key area of research for various pharmaceutical companies. Arthritis, asthma, allergy, multiple sclerosis, and additional diseases which cause inflammation and pain are widely prevalent throughout the world. For the treatment of pain and inflammation, various drugs such as indomethacin, ibuprofen, aspirin, nimisulide, celecoxib, rofecoxib, and dichlofenac are available in the market [1, 2]. But long-term use of these drugs causes various side effects such as ulceration, renal failure, gastrointestinal bleeding, and heart stroke [3–5]. All these indicate that there is a need for safer anti-inflammatory drugs. These serious side effects are limiting the use of non steroidal anti-inflammatory drugs (NSAIDs) in common inflammation cases. Interestingly, the replacement of the carboxylic groups by amide groups in NSAID drugs such as indomethacin, meclofenamic acid, and ketoprofen conferred the compound's greater selectivity for cyclooxygenase-2 over the cyclooxygenase-1 enzyme [6, 7]. This enzyme exists in the form of COX-1, which plays a crucial cytoprotective role in the gastrointestinal tract, whereas the second form COX-2 is responsible for the production of PGs during inflammation [8]. The inhibition of prostanoids was produced by COX-2 which might be decided to the anti-inflammatory, analgesic, and antipyretic affects of NSAIDs [9]. Therefore, development of novel compounds with anti inflammatory agents like an alternative to NSAIDs and improved safety profile is of believable importance.

Amide formation from carboxylic acids and amines is a fundamental reaction in organic, biological, medicinal, polymer, and material chemistry for which a great amount of research is still pursued [10]. The amide derivatives were associated with a broad spectrum of biological activities including anti-inflammatory [11], antidegenerative [12], antiplatelet [13], anticancer [14, 15], antimicrobial [16–18], urokinase

inhibitor [19], antituberculosis [20, 21], anticonvulsant [22], insecticide [23], and antitumor [24]. And amide derivatives possessing anti-inflammatory activities have been reported in the literature [25–27]. Encouraged by these results, it was attempted in the present study to synthesize a novel series of *tert*-butyl 2-(substituted benzamido) phenylcarbamate analogues (**4a**–**4j**) and screening for their anti-inflammatory activity. The results revealed that several derivatives showed promising activities. The in silico docking study of all the compounds was also performed to provide the binding modes of COX-2 enzyme.

Materials and methods

Chemistry

All starting materials, reagents, and solvents were commercially available and used after purification. All the melting points are uncorrected and were determined in open capillary tubes using sulfuric acid bath. IR spectra were recorded on Perkin-Elmer 1000 instrument using KBr pellet. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ calibrated solvents on a VARIAN spectrometer at 500 and 125 MHz, respectively. Chemical shift signals are given in δ (parts per million) relative to TMS, and coupling constants *(J)* are expressed in Hertz (Hz). Combinations of the following abbreviations are used to describe NMR spectra: s (singlet), d (doublet), t (triplet) q (quartet), and m (multiplet). Flash column chromatography was performed using silica gel (Merck, 60–120 Mesh). Commercially available reagents were used as supplied and some of them were distilled before use. All reactions were performed in oven-dried glassware. Electron Spray Ionization (ESI) and high-resolution mass spectra were recorded on a QSTARXL hybrid MS/MS system (Applied Bio systems, USA) under electrospray ionization.

Preparation of tert-butyl 2-amino phenyl carbamate (3)

A mixture of 0.800-g compound **2** (3.361 mmol, 1.0 eq) and 0.779 g $FeCl_3$ (3.361 mmol, 1.0 eq) was dissolved in methanol (10 cm^3), and $N_2H_4{\cdot}H_2O$ (5 cm^3) were added at room temperature. The reaction mixture was heated under reflux for 3 h. The completion of the reaction was monitored by TLC. The contents were cooled to room temperature, concentrated under reduced pressure to remove the solvent. Then, it was basified with saturated $NaHCO_3$ solution and extracted with DCM; it was washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated to obtained crude solid which was purified by column chromatography eluting with 20% ethyl acetate in hexane to produce compound **3** as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.25 (s, 1H), 8.00 (d, 1H, J = 1.06 Hz); 7.40 (dd, 1H, J = 6.80, 2.14 Hz); 6.79 (dd, 1H, J = 6.76, 2.14 Hz), 6.30 (s, 2H, NH_2), 1.38 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 152.5, 141.5, 125.5, 125.1, 122.8, 118.9, 114.5, 79.5, 28.4 ppm; IR (KBr): $\bar{\nu}$ = 3355.7, 3100.4, 1685.3, 1642.2 cm^{-1}; MS (ESI+): m/z = 209.15 ($[M + H]^+$).

Spectroscopic data of compounds 4a–4j

General procedure for the synthesis of compounds **4a**–**4j** to a stirred solution of 0.150 g benzoic acid (1.07 mmol, 1 eq), in a 5 cm^3 *N*,*N*-dimethylformamide at cooling at 0 °C then it was added 0.153 cm^3 of DIPEA (*N*,*N*-diisopropylethylamine) (1.605 mmol, 1.5 eq), 0.244 g of compound **3** (1.177 mmol, 1.1 eq) and 0.306 g EDCI (1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide.HCl) (1.605 mmol, 1.5 eq) and 0.216 g of HOBt (hydroxybenzotriazole) (1.605 mmol, 1.5 eq) successively. The reaction mixture was stirred for 30 min and it was kept at room temperature for 3 h to complete the reaction. The completion of the reaction was monitored by TLC. The crude product was diluted with water, extracted with diethyl ether, and separated the organic layer, washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated, to obtain crude solid which was purified by column chromatography eluting with 20–40% ethyl acetate in hexane, to obtain corresponding desired compounds **4a**–**4j** as solids.

Preparation of tert-butyl 2-(4-fluorobenzamido) phenylcarbamate (4a)

From the 0.150 g 4-florobenzoic acid (1.07 mmol, 1 eq), 0.153 cm^3 DIPEA (1.605 mmol, 1.5 eq), 0.244 g compound **3** (1.177 mmol, 1.1 eq), 0.306 g EDCI (1.605 mmol, 1.5 eq), and 0.216 g HOBt (1.605 mmol, 1.5 eq), the compound **4a** was obtained as white-colored solid (0.275 g, 74%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, *v*/v). m.p. 250–252 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.48 (s, 1H, NH), 8.65 (s, 1H, NH), 7.94 (d, 2H, J = 8.25 Hz, Ar-H), 7.75 (d, 2H, J = 7.98 Hz, Ar-H), 7.36–7.26 (m, 4H, Ar-H), 1.48 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 165.92, 164.66, 163.6, 154.65, 130.50, 130.02, 129.70 (2C), 125.93(2C), 124.38 (2C), 115.5 (2C), 81.20, 28.19 (3C) ppm; IR (KBr): $\bar{\nu}$ = 3266.7, 3064.2, 1693.5, 1650.4, 752.3 cm^{-1}; MS (ESI+): m/z = 331.05 ($[M + H]^+$), 348.07, 353.02; anal calcd for: $C_{23}H_{38}N_2O_3$: C, 70.73; H, 9.81; N, 7.17; O, 12.29; found: C, 70.78; H, 9.83; N, 7.20; O, 12.33.

tert-butyl 2-(4-methylbenzamido)phenylcarbamate (4b)

From the 0.150 g 4-methyle benzoic acid (1.1 mmol, 1 eq), 0.157 cm^3 DIPEA (1.65 mmol, 1.5 eq), 0.251 g compound **3** (1.21 mmol, 1.1 eq), 0.315 g EDCI (1.65 mmol, 1.5 eq) and

0.222 g HOBt (1.65 mmol, 1.5 eq) the compound **4b** was obtained as off white colored solid (0.210 g, 55.5%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, *v*/v). m.p. 210–212 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.80 (s, 1H, NH), 9.01 (s, 1H, NH), 7.87 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.35 (s, 2H, *J* = 7.93 Hz, Ar-H), 7.28 (d, 2H, *J* = 8.05 Hz, Ar-H), 7.21 (d, 2H, *J* = 7.55 Hz, Ar-H), 2.51 (s, 3H, CH_3), 1.45 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 165.73, 154.55, 152.23, 142.33, 131.24, 130.05, 129.16, 127.37, 125.80(2C), 124.46, 81.06, 28.22(3C), 21.45 ppm; IR (KBr): $\bar{\nu}$ = 3332.4, 3249.1, 3069.9, 2908.8, 1799.6, 1690.8 cm^{-1}; MS (ESI+): *m/z* = 327.08 ([M + H]$^+$), 349.04; anal calcd for: $C_{19}H_{22}N_2O_3$: C, 69.92, H, 6.79, N, 8.58, O, 14.71; found: C, 69.42, H, 6.38, N, 8.52, O, 14.75.

tert-butyl 2-(4-tert-butylbenzamido)phenylcarbamate (4c)

From the 0.150 g 4-*tert*-butylbenzoic acid (0.84 mmol, 1 eq), 0.120 cm^3 DIPEA (1.26 mmol, 1.5 eq), 0.192 g compound **3** (0.924 mmol, 1.1 eq), 0.240 g EDCI (1.26 mmol, 1.5 eq) and 0.170 g HOBt (1.26 mmol, 1.5 eq) the compound **4c** was obtained as off white colored solid (0.285 g, 83.3%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (70:30, *v*/v). m.p. 215–217 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.38 (s,1H, NH), 8.64 (s, 1H, NH), 7.99 (d, 2H, *J* = 7.85 Hz, Ar-H), 7.30 (d, 2H, *J* = 8.01 Hz, Ar-H), 7.21 (m, 2H, Ar-H), 7.10 (d, 2H, *J* = 7.01 Hz, Ar-H), 1.55 (s, 9H, $3CH_3$), 1.28 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 165.54, 157.26, 155.13, 149.70, 130.14, 128.54, 127.68(2C), 126.55(2C), 124.70(2C), 124.23(2C), 80.05, 34.03, 30.19(3C). 27.42(3C) ppm; IR (KBr): $\bar{\nu}$ = 3266.9, 3064.2, 1693.5, 1650.5, 1442.8 cm^{-1}; MS (ESI+): *m/z* = 369.12 ([M + H]$^+$), 391.16; anal calcd for: $C_{22}H_{28}N_2O_3$: C, 71.71, H, 7.66, N, 7.60, O, 13.03; found: C, 71.74; H, 7.68; N, 7.60; O, 13.05.

tert-butyl 2-(2-iodobenzamido)phenylcarbamate (4d)

From the 0.150 g 2-iodobutylbenzoic acid (0.600 mmol, 1 eq), 0.085 cm^3 DIPEA (0.900 mmol, 1.5 eq), 0.137 g compound **3** (0.66 mmol, 1.1 eq), 0.171 g EDCI (0.9 mmol, 1.5 eq) and 0.121 g HOBt (0.90 mmol, 1.5 eq) the compound **4d** was obtained as yellow colored solid (0.195 g, 67.9%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, *v*/v). m.p. 225–227 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.16 (s, 1H, NH), 8.26 (s, 1H, NH), 7.91 (d, 1H, *J* = 7.93 Hz, Ar-H), 7.64 (d, 1H, *J* = 7.17 Hz, Ar-H), 7.53–7.38 (m, 3H, Ar-H), 7.24–7.12 (m, 3H, Ar-H), 1.47(s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.10, 153.96, 152.12, 141.63, 139.92, 131.34, 131.26, 128.93, 128.08, 126.70(2C), 125.25(2C), 92.55, 80.88, 28.25 ppm; IR (KBr): $\bar{\nu}$ = 3341.2, 3246.4, 1723.8, 1648.5, 748.7 cm^{-1}; MS (ESI+): *m/z* = 438.95.([M + H]$^+$), 455.97; anal calcd for: $C_{18}H_{19}IN_2O_3$: C, 49.33; H, 4.37; I, 28.96; N, 6.39; O, 10.95; found: C, 49.30; H, 4.38; I, 28.94; N, 6.36; O, 10.94.

tert-butyl 2-(3,5-dinitrobenzamido)phenylcarbamate (4e)

From the 0.100 g 3,5-dinitrobenzoic acid (0.47 mmol, 1 eq), 0.089 cm^3 DIPEA (0.705 mmol, 1.5 eq), 0.107 g compound **3** (0.517 mmol, 1.1 eq), 0.134 g EDCI (0.705 mmol, 1.5 eq) and 0.095 g HOBt (0.705 mmol, 1.5 eq) the compound **4e** was obtained as brick red-colored solid (0.140 g, 67.6%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, *v*/v). m.p. 248–250 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.02 (s, 1H, NH), 9.42 (s, 1H, Ar-H), 8.19 (s, 1H, NH), 8.98 (d, 2H, *J* = 7.96 Hz, Ar-H), 7.79 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.40 (d, 2H, *J* = 8.5 Hz, Ar-H), 1.50 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 160.93, 153.58, 147.98, 137.33, 131.55(2C), 127.71(2C), 126.28, 127.38(2C), 123.89, 123.44, 120.53, 80.25, 27.8(3C) ppm; IR (KBr): $\bar{\nu}$ = 3222.5, 2826.1, 1772.6, 1630.5 cm^{-1}; MS (ESI+): *m/z* = 403.00 ([M + H]$^+$), 425.00; anal calcd for: $C_{18}H_{18}N_4O_7$: C, 53.43; H, 4.51; N, 13.92; O, 27.83; found: C, 53.73; H, 4.52; N, 13.90; O, 27.84.

tert-butyl 2-dodecanamidophenylcarbamate (4f)

From the 0.150 g dodecanoic acid (0.75 mmol, 1 eq), 0.107 cm^3 DIPEA (1.125 mmol, 1.5 eq), 0.175 g compound **3** (0.825 mmol, 1.1 eq), 0.214 g EDCI (1.125 mmol, 1.5 eq) and 0.151 g HOBt (1.125 mmol, 1.5 eq) the compound **4f** was obtained as white-colored solid (0.240 g, 73.8%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, *v*/v). m.p.: 260–262 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.20(s, 1H, NH), 8.04(s, 1H, NH), 7.39 (d, 2H, *J* = 7.38 Hz, Ar-H), 7.14 (m, 2H, Ar-H), 2.36 (t, 2H, CH_2), 1.67 (t, 2H, CH_2), 1.52 (s, 9H, $3CH_3$), 1.28 (s, 16H, CH_2), 0.88 (t, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 178.82, 172.65, 154.27, 130.68, 129.91, 125.24(2C), 124.44(2C), 80.80, 37.21, 31.83, 29.52(3c), 29.26, 29.02, 28.23(3C), 25.72, 24.68, 14.03 ppm; IR (KBr): $\bar{\nu}$ = 3420.3, 3380.6, 1680.6, 1648.2 cm^{-1}; MS (ESI+): *m/z* = 391.05 ([M + H]$^+$), 413.2; anal. calcd for: $C_{23}H_{38}N_2O_3$: C, 70.73, H, 9.81, N, 7.17; O, 12.29. Found: C, 70.72, H, 9.84, N, 7.18, O, 12.29.

tert-butyl 2-(4-chloro-2,5-difluorobenzamido) phenylcarbamate(4 g)

From the 0.100 g 4-chloro-2,5-difluorobenzoic acid (0.520 mmol, 1 eq), 0.074 cm^3 DIPEA (0.78 mmol, 1.5 eq), 0.118 g compound **3** (1.572 mmol, 1.1 eq), 0.148 g EDCI (0.78 mmol, 1.5 eq) and 0.105 g HOBt (0.78 mmol, 1.5 eq) the compound **4 g** was obtained as white colored solid

(0.190 g, 95.0%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (70:30, *v*/v). m.p.: 238–240 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.59 (s, 1H, NH), 8.98 (s, 1H, NH), 8.0(t, 1H, Ar-H), 7.71 (d, 1H, *J* = 8.24 Hz, Ar-H), 7.39–7.20 (m, 3H, Ar-H), 1.51 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.10, 158.55, 153.96, 150.96, 141.63, 139.92, 128.93, 128.08, 126.79, 125.27(2C), 119.24, 115.10, 80.88, 28.25(3C) ppm; IR (KBr): $\bar{\nu}$ = 34O2.4, 3305.0, 1713.3, 1665.0 cm^{-1}; MS (ESI+): *m/z* = 383.01 ([M + H]$^+$), 400.03; anal. calcd for: $C_{18}H_{17}ClF_2N_2O_3$: C, 56.48; H, 4.48; Cl, 9.26; F, 9.93; N, 7.32; O, 12.54; found: C, 56.45; H, 4.49; Cl, 9.22; F, 9.90; N, 7.25; O, 12.42.

tert-butyl 2-(2,4,5-trimethoxybenzamido)phenylcarbamate (4 h)

From the 0.100 g 4-chloro-2,5-difluorobenzoic acid (0.47 mmol, 1 eq), 0.090 cm^3 DIPEA (0.705 mmol, 1.5 eq), 0.107 g compound **3** (0.512 mmol, 1.1 eq), 0.134 g EDCI (0.705 mmol, 1.5 eq) and 0.095 g HOBt (0.705 mmol, 1.5 eq) the compound **4 h** was obtained as white colored solid (0.150 g, 72.4%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, *v*/v). m.p.: 188–190 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.02 (s, 1H, NH), 8.40 (s, 1H, NH), 7.9 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.3 (s, 1H, Ar-H), 7.18 (d, 2H, *J* = 8.6 Hz), 6.92 (s, 1H, Ar-H), 3.68 (s, 9H, 3O–CH_3), 1.51 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.59, 153.77, 152.88, 152.62, 143.3, 128.85, 129.80, 125.08, 124.78(2C), 123.86(2C), 114.08, 112.49, 96.24, 80.38, 56.59, 56.31, 56.06, 28.25(3C) ppm; IR (KBr): $\bar{\nu}$ = 3045.2, 2980.5, 1760.5, 16.95.5, 1150.9 cm^{-1}; MS (ESI+): *m/z* = 403.1 ([M + H]$^+$), 425.1; anal calcd for: $C_{21}H_{26}N_2O_6$: C, 62.67; H, 6.51; N, 6.96; O, 23.85; found: C, 62.65; H, 6.50; N, 6.98; O, 23.89.

tert-butyl 2-(4-(1H-indol-2-yl)butanamido)phenylcarbamate (4i)

From the 0.150 g 4-(1*H*-indol-2-yl)butanoic acid (0.74 mmol, 1 eq), 0.140 cm^3 DIPEA (1.11 mmol, 1.5 eq), 0.169 g compound **3** (0.812 mmol, 1.1 eq), 0.212 g EDCI (1.11 mmol, 1.5 eq) and 0.149 g HOBt (1.11 mmol, 1.5 eq) the compound **4i** was obtained as white colored solid (0.250 g, 75.7%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (70:30, *v*/v). m.p.: 262–264 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.35 (s, 1H, NH), 9.42(s, 1H, NH), 8.67 (s, 1H, NH), 7.98 (m, 4H, Ar-H), 7.63–7.42 (m, 2H, Ar-H), 7.19 (d, 2H, *J* = 8.85 Hz, Ar-H), 6.72 (s, 1H, indole-H), 2.62–2.41 (m, 4H, 2-CH_2-), 1.68 (q, 2H, −CH_2-), 1.45 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.44, 154.18, 136.30, 130.82, 129.88, 127.33(2C), 126.19, 125.29(2C), 124.56, 121.74, 119.05, 118.71, 115.03, 111.15, 80.85, 36.38, 28.21(3C), 25.83, 24.24 ppm; IR (KBr): $\bar{\nu}$ = 3074.2, 2926.9, 2855.1, 1743.39, 1665.0 cm^{-1}; MS (ESI+): *m/z* = 394.22 ([M + H]$^+$), 416.26; anal calcd for: $C_{23}H_{27}N_3O_3$: C, 70.21; H, 6.92; N, 10.68; O, 12.20; found: C, 70.22; H, 6.94; N, 10.70; O, 12.20.

tert-butyl 2-(2-bromoacetamido)phenylcarbamate (4j)

From the 0.100 g 2-bromoacetic acid (0.72 mmol, 1 eq), 0.140 cm^3 DIPEA (1.08 mmol, 1.5 eq), 0.164 g compound **3** (0.79 mmol, 1.1 eq), 0.206 g EDCI (1.08 mmol, 1.5 eq) and 0.145 g HOBt (1.08 mmol, 1.5 eq) the compound **4j** was obtained as brick red-colored solid (0.250 g, 75.7%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, *v*/v). m.p.: 180–182 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3402, 3305, 2972.6, 1717.8, 1658.2, 768.5.^{1}H–NMR- (500 MHz) (CDCl3)-d_6: δ 9.64 (s, 1H, NH), 8.99 (s, 1H, NH), 8.89 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.20–7.1.52 (m, 2H, Ar-H), 3.62 (s, 2H, −CH_2-), 1.45 (s, 9H, $3CH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.45, 154.11, 130.76, 129.77, 126.20(2C), 125.29 (2C), 80.05, 28.24(3C), 24.08 ppm; IR (KBr): $\bar{\nu}$ = 3074.2, 2926.9, 2855.1, 1743.39, 1665.0 cm^{-1}; MS (ESI+): *m/z* = 643.22 ([M + H]$^+$); anal calcd for: $C_{25}H_{32}Br_2O_6$: C, 47.43; H, 5.21; Br, 24.27; N, 8.51; O, 14.58; found: C, 47.61; H, 5.25, Br, 24.32; N, 8.47; O, 14.65.

Carrageenan-induced hind paw edema test

In this study, the in vivo anti-inflammatory activity was evaluated for all the newly synthesized compounds **4a**–**4j** using the carrageenan-induced rat paw edema method. For the determination of anti-inflammatory effect, the carrageenan-induced paw edema model was employed. Each rat was injected with a freshly prepared 0.1 cm^3 of 1% carrageen a suspension in normal saline (0.9% NaCl) into subplantar tissue of the right hind paw. The intraperitoneal administration of control, test samples (synthesized compounds) and reference drug, for the control, 10 mg/kg saline solution was administered. Paw edema was measured every 60 min for 3 h after induction of inflammation. The anti-inflammatory activity of the tested compounds and reference drug (ibuprofen 10 mg/kg) were determined as the increase in paw edema volume (control) and the results are summarized in Table 1 and as percentage inhibition (% inhibition) and summarized in Table 1. Results were expressed as the mean ± SE difference between disease control (control) ten compounds and one standard drug-treated animal using one-way analysis of variance ANOVA, followed by Dennett's test for multiple comparisons.

Acute toxicity All animals used in the inflammatory experiments were observed for 24 h and mortality of animals recorded where present for each group at the end of observation

Table 1 The anti-inflammatory activity of the *tert*-butyl 2-(substituted benzamido) phenyl carbamate (**4a–4j)** derivatives and reference drug (ibuprofen) in carrageenan-induced rat paw edema assay, values are expressed as mean ± SEM (mm) and percentage inhibition of inflammation

Compound codes	Mean paw volume ± SEM and (% of inhibition)				
	1 h	3 h	6 h	9 h	12 h
4a	0.823 ± 0.000 (26.714)	0.752 ± 0.000 (41.433)	0.524 ± 0.017 (54.553)	0.626 ± 0.010 (40.550)	0.422 ± 0.014 (54.130)***
4b	0.783 ± 0.015 (30.276)	0.651 ± 0.010 (49.299)	0.606 ± 0.010 (47.441)	0.652 ± 0.011 (38.081)	0.551 ± 0.012 (40.108)
4c	0.631 ± 0.012 (43.811)	0.593 ± 0.017 (53.816)	0.513 ± 0.008 (55.507)	0.417 ± 0.011 (60.398)	0.492 ± 0.025 (46.521)
4d	0.733 ± 0.015 (34.728)	0.510 ± 0.015 (60.280)	0.632 ± 0.015 (45.186)	0.582 ± 0.014 (44.729)	0.427 ± 0.008 (53.586)**
4e	0.782 ± 0.017 (30.365)	0.403 ± 0.012 (48.364)	0.625 ± 0.011 (45.793)	0.752 ± 0.011 (28.584)	0.561 ± 0.013 (39.021)
4f	0.676 ± 0.008 (39.804)	0.681 ± 0.015 (39.018)	0.651 ± 0.006 (43.538)	0.553 ± 0.020 (47.483)	0.425 ± 0.006 (53.804)**
4g	0.634 ± 0.012 (43.544)	0.653 ± 0.000 (49.143)	0.654 ± 0.020 (43.278)	0.602 ± 0.010 (42.830)	0.492 ± 0.014 (46.521)
4h	0.702 ± 0.008 (37.488)	0.652 ± 0.005 (49.221)	0.513 ± 0.011 (55.507)	0.535 ± 0.012 (49.192)	0.429 ± 0.012 (53.369)*
4i	0.822 ± 0.010 (26.803)	0.725 ± 0.006 (43.535)	0.682 ± 0.014 (40.849)	0.675 ± 0.014 (35.897)	0.421 ± 0.020 (54.239)***
4j	0.893 ± 0.008 (20.480)	0.642 ± 0.073 (50.000)	0.625 ± 0.010 (45.793)	0.622 ± 0.017 (40.930)	0.427 ± 0.011 (53.586)**
Indomethacin	0.851 ± 0.006 (24.220)	0.752 ± 0.011 (41.433)	0.681 ± 0.008 (40.936)	0.527 ± 0.011 (49.952)	0.421 ± 0.012 (54.239)***
Disease control	1.123 ± 0.008	1.284 ± 0.006	1.153 ± 0.017	1.053 ± 0.088	0.920 ± 0.011
Normal control					

**The mean difference is significant at the $P < 0.05$ level; ***the mean difference is significant at the $P < 0.01$ level; Dunnetts-tests treat one group as a control, and compare all other groups against it

period. Thus, edema volume in control (*V*c) and in groups treated with test compounds (*V*t) was calculated. The percentage of inhibition was calculated using the formula

$$\text{Percentage of inhibition} = \frac{V\text{c} - V\text{t}}{V\text{c}} \times 100$$

where *V*c is mean paw inflammation of control animal and *V*t mean paw inflammation of test in drug-administered animal.

Statistical analysis The anti-inflammatory activity was determined as increase in the paw edema volume percentage in the treated animals (Table 1). Results were expressed as the mean ± SE, and different groups were compared using one-way analysis of variance (ANOVA) followed by Dennett's test for multiple comparisons. Test where $p < 0.05$ was accepted to be a significant difference.

Preparation of test samples for bioassay All test samples (50 mg/kg) were suspended in a mixture of distilled water and 0.5% carboxyl methylcellulose (CMC) and were given intraperitoneally (i.p.) to the test animals. The animals of the control group received the same experimental handling except that the test drug treatment was replaced with appropriate volumes of the vehicle. Indomethacin (10 mg/kg) for anti-inflammatory was used as a reference drug.

Experimental animals Male Swiss albino rats (180–200 g) were used for anti-inflammatory activity. All of the animals were left for 2 days in the laboratory for acclimatization before the day of experiment. A minimum of 6 animals were used in each group. All pharmacological activities were carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) norms (Registered No:1757/PO/RcBiBt/S/14 CPCSEA), after obtaining the approval from the Animal Ethics Committee from Jeeva life science, Uppal Industrial area, Hyderabad, Telangana state, India.

Molecular docking

Crystal structure of cyclooxygenase-2 with PDB ID: 4COX [9] was retrieved from the protein data bank. GLIDE 5.6 [28] was used for molecular docking. Protein was prepared using protein preparation wizard in Maestro 9.0 applying default

parameters. A grid was generated around the active site by selecting the co-crystallized ligand. Receptor van der Waals scaling for nonpolar atoms was kept at 0.9 [29] Molecules were built using Maestro build panel and prepared by LigPrep 2.0 application. Low-energy conformation of the ligands were selected and docked into the grid generated for the protein using extra precision (XP) docking mode [30–34].

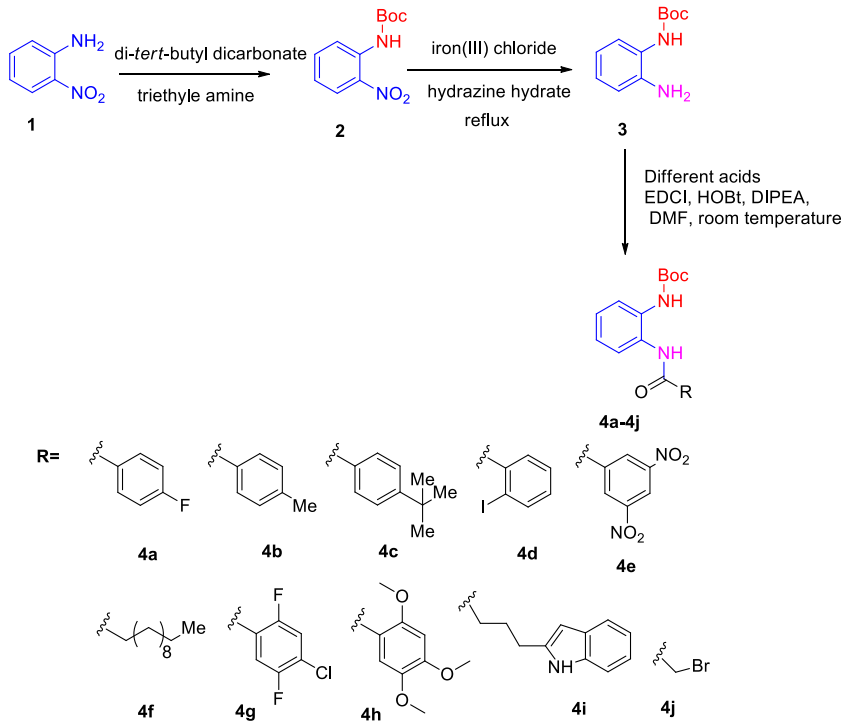
Results and discussion

Chemistry

The synthesis of the *tert*-butyl 2-(substituted benzamido) phenylcarbamate derivatives **(4a–4j)** were illustrated in Scheme 1. The compound 2-nitroaniline (**1**) was allowed to undergo amine protection via nucleophilic addition reaction by the tertiary butoxy carbonic anhydride (Boc) instantaneously in the presence of triethylamine to produced *tert*-butyl (2-nitrophenyl) carbamate (**2**) in moderate to be good yield [35]. The structure of the compound was established on the basis of its spectral properties (IR, ^{1}H NMR, MS, and elemental analysis). The IR spectrum showed the presence of absorption bands at 3360 and 1680 cm^{-1} due to NH and C = Os functional group, respectively. ^{1}H NMR spectrum showed broad singlet signals at 9.19 ppm, assigned to NH groups; the signal of up-field singlet was assigned to tertiary butoxy-proton at 1.45 ppm, in addition to the multiple signals of aromatic protons in the region of 8.53–7.78 ppm. The MS of the compound displayed an intense molecular ion peak at *m/z* 239 [M] $^+$ corresponding to molecular formula $C_{11}H_{14}N_2O_4$.

The resultant compound *tert*-butyl(2-nitrophenyl)carbamate (**2**) further undergoes reduction with hydrazine hydrate (NH_2-NH_2.H_2O) in methanol to obtained the compound *tert*-butyl(2-aminophenyl)carbamate (**3**) in excellent yield. The infrared spectrum of compound **3** showed characteristic absorptions corresponding to amine (−NH_2) is 3367 cm^{-1} and it was also confirmed by the ^{1}H NMR which showed broad singlet at 6.28 ppm corresponding to amine protons attached to benzene ring and singlet signal assigned at 9.15 ppm corresponding to NH proton of adjusted phenyl ring. All the other aromatic protons of compound **3** were observed at the expected regions. MS showed a molecular ion peak at *m/z* 209.0 [M]$^+$, corresponding to a molecular formula $C_{11}H_{16}N_2O_4$.

Finally, the compound *tert*-butyl(2-aminophenyl)carbamate (**3**) was underwent amidation coupling with different carboxylic acids, and combination of 1-(3-dimethyl amino propyl)-3-ethylcarbodiimide.HCl (EDCI) as the carboxylate activator and hydroxyl benzotriazole (HOBt) at room temperature in presence DIPEA as base to obtain the corresponding *tert*-butyl 2-(substituted benzamido)phenylcarbamate **(4a–4j)** in moderate to be good yields [36] indicated in Scheme 1. Moreover, amide synthesis using coupling reagent EDCI is low recrimination when used with HOBt reagent by product both easily soluble in water. The structures of all the newly synthesized compounds **4a–4j** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and MS and elemental analysis spectra data are included experimental section. In the IR spectra of the compound **4a** amide NH- and Boc-protected NH group absorption peak appeared at 3266 and



Scheme 1 Synthesis of *tert*-butyl (substituted benzamido)phenylcarbamate **4a–j**

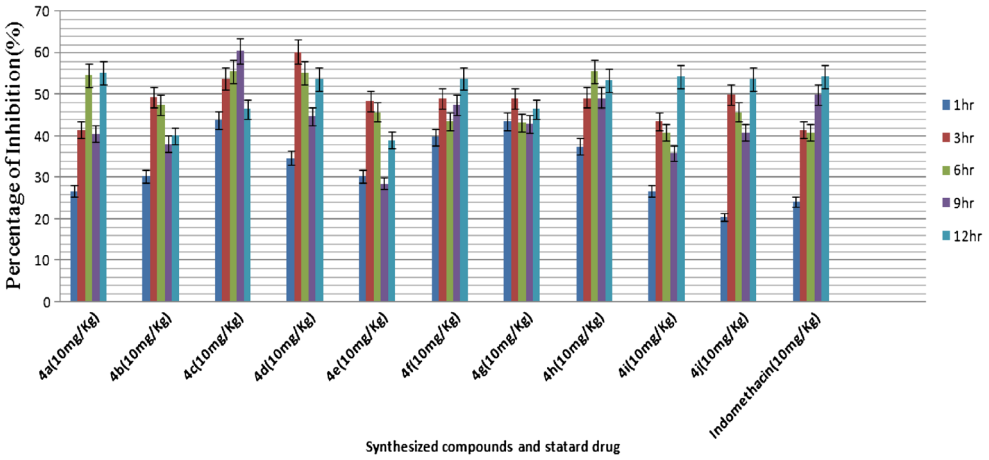


Fig. 1 Graphical representation percentage of inhibition of compounds **4a–4j** along with the standard drug

3034 cm^{-1} respectively. The absorption peak appeared at 1693 cm^{-1} was attributed to C = O group of phenylcarbamate. In addition, the absorption bands, corresponding to C = O of the amide moiety, were observed at 1650 cm^{-1}. The vibration frequency of C–H and C = C group possible to identify substitution pattern on benzene appeared at 2980 and 1693 cm^{-1}, respectively. In addition, substituted mono halo on benzene ring absorption strong peak appeared at 769 cm^{-1}. Thus, the data of IR spectroscopy supported the formation of the desired structure. The ^{1}H NMR spectrum showed signals of two singlets which were assigned to amide and phenyl carbamate at 9.48 and 9.05 ppm, respectively. A signal of Boc protons appears at 1.48 ppm in addition to the signal of downfield singlet of aromatic protons in the region of 8.00–6.80 ppm. The structure was further confirmed by ^{13}C NMR signals of carbon atoms of the two carbonyl groups appeared at 165.9 and 164.6 ppm. Boc linkage carbon exhibited resonance peaks at 81.2, 28.19 ppm. The carbon atoms of aromatic ring appeared at 154.6–115.4 ppm. MS showed a molecular ion peak at *m/z* 331.05 $[M]^{+}$, corresponding to a molecular formula $C_{23}H_{38}N_2O_3$. All the synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures as shown in Scheme 1.

Anti-inflammatory activity

The in vivo anti-inflammatory activity [37–39] evaluation of title compounds **(4a–4j)** were carried out using carrageenan-induced paw edema assay of Winter et al. and the result are summarized in Table 1. The Male Swiss albino rats at 10 mg/kg body weight and the standard drug, indomethacin

Table 2 Docking scores and ADME properties of synthesized compounds

Title	Dock score	M.Wt	QPlogPo/w[a]	QPlogS[b]	QPPCaco[c]	QPlogBB[d]	QPPMDCK[e]	% Human oral absorption[f]
4a	−8.49	330.36	3.479	−4.568	1470.54	−0.338	1358.19	100
4b	−5.33	326.39	3.566	−4.626	1538.17	−0.448	787.91	100
4c	−7.93	368.47	4.457	−5.293	1616.79	−0.486	831.53	100
4d	−5.98	438.26	3.786	−4.68	1709.55	−0.271	1676.76	100
4e	−5.17	402.36	2.038	−4.125	39.209	−2.087	14.92	54.44
4f	−6.60	390.56	5.636	−6.428	1552.48	−1.135	795.84	100
4g	−8.08	382.79	4.109	−5.322	1490.37	−0.163	3798.53	100
4 h	−5.62	402.45	3.673	−4.513	1971.05	−0.544	1030.11	100
4i	−9.56	393.48	4.588	−5.565	965.68	−0.891	476.38	100
4j	−6.20	329.19	2.502	−3.323	1426.09	−0.29	1414.68	100
IMC	−11.1	357.79	4.261	−5.119	190.46	−0.609	258.70	92.697

IMC indomethacin

[a] Predicted octanol/water partition coefficient log P (acceptable range—2.0–6.5)

[b] Predicted aqueous solubility in mol/L (acceptable range—6.5–0.5)

[c] Predicted caco cell permeability in nm/s (acceptable range: <25 is poor and >500 is great)

[d] Predicted blood brain barrier permeability (acceptable range—3–1.2)

[e] Predicted apparent MDCK cell permeability in nm/s (acceptable range: <25 is poor and >500 is great)

[f] Percentage of human oral absorption (acceptable range: <25 is poor and >80% is high

used for anti-inflammatory activity, all the exhibited derivatives of anti-inflammatory activities that listed for every 3 h with potency that increased with time. The results were expressed as the increase in paw volume at various time intervals in comparison to the initial values shower in Table 1. The increase of volumes in percentage was calculated by subtracting the initial paw volumes from the paw volumes obtained after the carrageenan agent was injected.

The inflammatory activity was tested for ten desired compounds. Most of the tested compounds have shown an better result in comparison with the standard drug, indomethacin, may be due to amide moiety enhancing the activity. Among them the compounds **4a**, **4d**, **4 h**, **4i**, and **4j** showed maximum activity and remaining compounds **4b, 4c,** and **3g** showed moderate to good activity after 12 h observation. In the present investigation, the highest activity of compound **4a** and **4i** than standard may be attributed to the presence of highly electronegative florosubstituted on phenyl ring and indole moiety, respectively, have been playing vital role for activity. It is found that the compounds with viz. electron

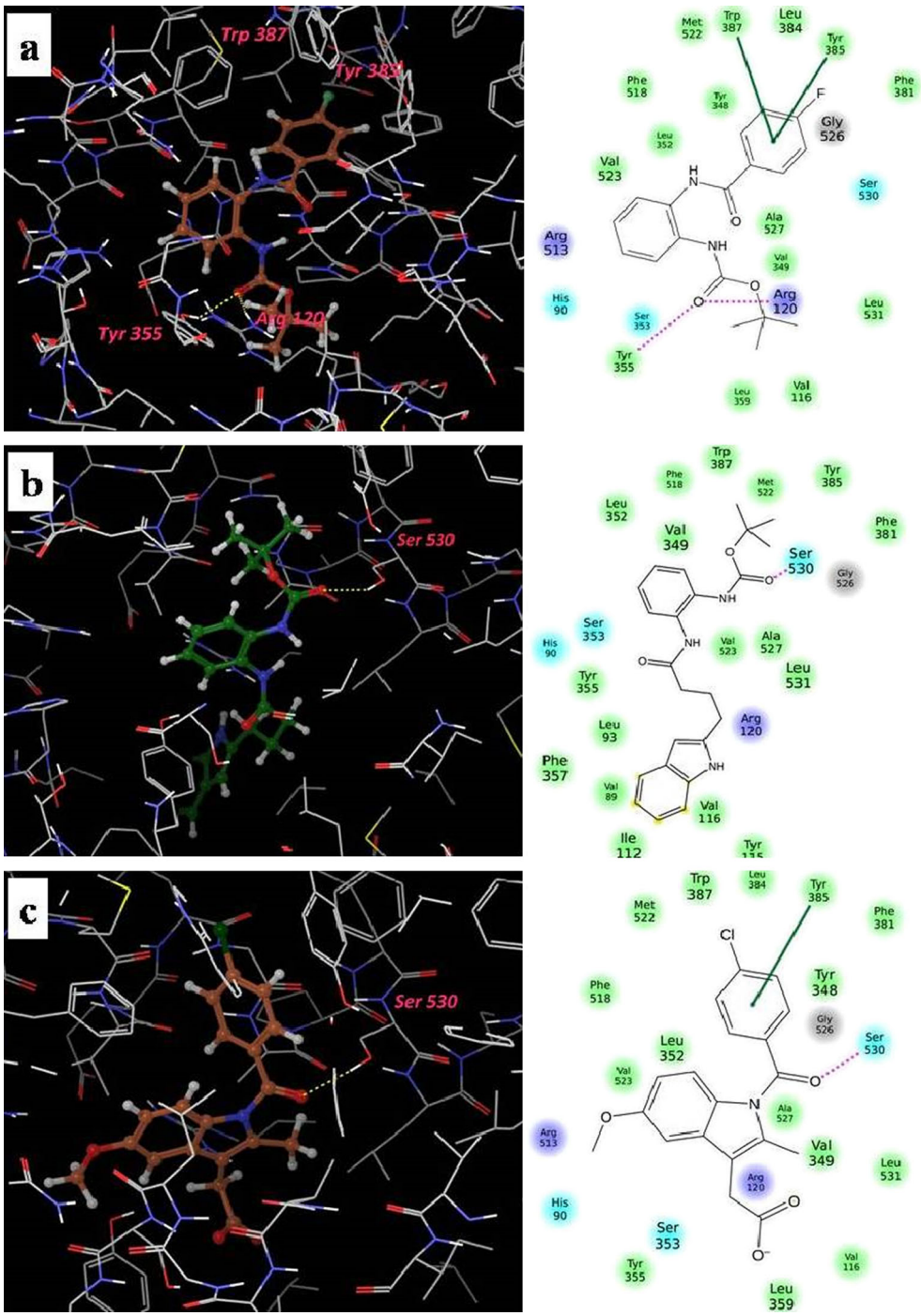


Fig. 2 Dock poses conformation of active compounds **4a** (**a**), **4i** (**b**), and standard drug indomethacin (**c**) in COX-2 protein active site

withdrawing nitro group **4e** (39.021%), showed less activity. All the synthesized compounds with their anti-inflammatory activity and percentage of inhibition are presented in Table 1. It was found that better anti-inflammatory activity for **4a** (54.130%) and **4i** (54.239%) compared to standard drug after 12-h intervals was observed in the present experiment (Fig. 1). The degree of anti-inflammatory action in ascending order in percentage of inhibition is 39.021(**4e**)<40.108(**4b**)<46.521(**4c**)-=46.521(**4g**)<53.369(**4h**)<53.586(**4j**)=53.586(**4d**)<53.804(**4f**)- < 54.130(**4a**) < 54.239(**4i**), and 54.239 standard drug, indomethacin, by the percentage of inhibition of carrageenan-induced paw edema showed in Table 1. In all the experiments after the 12-h observation, it showed a normal stage in the entire paw but it was observed (disease control) carrageenan paw with some inflammatory and also showed normal paw. The feet of rat paw edema injection of carrageen injection after 3 h, it was considered 0 h showed in Table 1.

In silico molecular docking

To gain more insight into the interactions of the high active compounds in the series, molecular docking of **4a**–**4j** was performed into the active site of COX-2. The molecules were deeply embedded into the hydrophobic pocket in the active site similar to indomethacin (standard). The compounds showed hydrogen bond interaction with Ser 530, Try 355, and Arg 120, π-π interactions with Trp 387, Tyr 385 amino acids, and the range of dock score from −9.56 to −5.17 kcal/mol. The docking score of all the compounds are depicted in Table 2. The best active compound **4a**, showed two hydrogen bond interactions with Try 355 and Arg 120 (bond length of 2.389 and 1.720 A^o) with a dock score of −8.49 kcal/mol. Additionally, it showed two π-π interactions with Trp 387 and Tyr 385 when compared to standard drug indomethacin, this can be explained in terms of hydrophobic interaction with the COX-2 protein active site (Fig. 2a). Another compound **4i** showed one hydrogen bond interaction with Ser 530, like Indomethacin, with the dock score value of −9.56 kcal/mol (bond length of 2.069 A^o) (Fig. 2b). Dock pose conformation of indomethacin showed in Fig. 2c, the dock score value of −11.14 kcal/mol(bond length of 1.989 A^o). Bonding interactions and bond lengths of active compounds with COX-2 protein was tabulated in Table 3. Finally compounds **4a** and **4i** were found to be potent and have high docking score with the COX-2 binding site.

ADME (absorption, distribution, metabolism, and excretion) properties of synthesized compounds were evaluated computationally using QikProp module of Schrodinger (QikProp3.4, Schrödinger,LLC, New York, NY, 2010) and were analyzed for drug-likeness by applying Lipinski's rule of five (Table 2) are calculated by QikProp. All these pharmacokinetic parameters were found to be good and acceptable range.

In addition a regression analysis between dock score (binding affinity) and degree of anti-inflammatory activity values of the synthesized molecules were carried out. It gave a correlation coefficient *r* of 0.828 representing significant correlation between binding affinity (dock score) and degree of anti-inflammatory activity.

Conclusions

In conclusion, a new class of *tert*-butyl 2-(substituted benzamido) phenylcarbamate **(4a–4j)** has been synthesized from *tert*-butyl 2-amino phenylcarbamate (**3**) and characterized by spectroscopic techniques. All the synthesized compounds were evaluated for their anti-inflammatory. Among the entire compound, the compounds **4a, 4d, 4f, 3i** and **3j** were showed maximum activity within 9 to 12 h. Moreover, molecular docking study was performed to provide the binding patterns of the compound **4a** and **4i** into the binding sites of COX-2 (PDB code: 4COX) enzymes. The study showed that **4a** and **4i** has favorable orientation within the COX-2 enzyme binding site and has a high docking score. In view of these studies, these compounds could be a subject of further investigations for searching potential new anti-inflammatory molecules.

Table 3 Bonding interactions and bond lengths of active compounds with COX-2 protein

Title comp.	Bonding interactions	Protein amino acids	Bond length (A^o)
4a	Two hydrogen bonding interactions	Tyr 355 and Arg 120	2.389 and 1.720
	Two hydrophobic π-π interactions	Trp 387 and Tyr 385	
4c	One hydrogen bond interaction	Arg 120	2.162
4f	One hydrogen bond interaction	Arg 120	2.083
4 g	One hydrogen bond interaction	Ser 530	1.952
4 h	One hydrogen bond interaction	Ser 530	1.974
4i	One hydrogen bond interaction	Ser 530	2.069
IMC	One hydrogen bond interaction	Ser 530	1.989
	One hydrophobic π-π interaction	Tyr 385	

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