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Synthesis, biological evaluation and *in silico* studies of novel N-(4-(2-(2-cyano-3-(substituted phenyl)acryloyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl)propanamides

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In the present study novel N-(4-(2-(2-cyano-3-(substituted phenyl)acryloyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl)propanamides have been synthesized and characterized by IR, ^{1}H and ^{13}C NMR and Mass data. *In vitro* antioxidant and antiinflammatory activities have been evaluated for the synthesized compounds **5, 6a-i**. The molecular properties, antiinflammatory potential and toxicity profile for the synthesized compounds have been predicted by *in silico* tools such as Molinspiration, PASS analysis and Osiris property explorer respectively. Compounds **6b** (4-OH), **6d** (4-OCH_3), and **6e** (3,4-di-OCH_3) have emerged as the most promising candidates for further development based on *in vitro* evaluation.

Keywords: Inflammation, Ibuprofen, NSAIDs, amides, *in silico* studies

Inflammation is a physiological response characterized by the accumulation of body fluids at the affected site. It functions as the body's first line of defense, indicating the presence of injury, infection, or any physiological disturbance. However, if inflammation persists for a prolonged period, it can contribute to the development of various diseases[1]. Inflammation is often accompanied by swelling, redness and pain. Antiinflammatory drugs are given to control inflammatory responses. NSAIDs are most predominantly used to treat inflammation[2]. Ibuprofen is commonly prescribed drug used to treat inflammation[3]. Ibuprofen is non-selective COX inhibitor which also inhibits cytoprotective COX-I enzyme[4]. Prolonged use of Ibuprofen results in adverse effects like gastric ulcers, hepatotoxicity and renal toxicity[5]. To overcome these adverse effects and improve selectivity, structural modifications are introduced at the carboxyl group. Such modifications have yielded a variety of derivatives exhibiting diverse biological activities, including antimicrobial[6], antifungal[7], anticancer[8–10], antitubercular[11], analgesic and antiinflammatory properties[12–17].

In the present study Ibuprofen was conjugated with *para*-aminobenzoic acid (PABA), an antitubercular agent. Subsequently, cyanoacetylation was carried out, followed by a Knoevenagel condensation at the active methylene group, yielding compounds containing tyrphostin moieties, known tyrosine kinase inhibitors that play a crucial role in anticancer activity[18,19]. Moreover, obtained amide functionality containing Ibuprofen derivatives have many pharmacological activities such as anthelmintic, antibacterial[20], antifungal, antiulcer, anti-HIV, antihypertensive, and neuroleptic properties[21]. The designed molecules were synthesized and characterized by IR, ^{1}H, ^{13}C and Mass data. *In vitro* antioxidant properties were estimated by 1,1-diphenyl-2-picrylhyrdrazyl (DPPH) scavenging and nitric oxide (NO) free radical scavenging assays. *In vitro* antiinflammatory activity was evaluated by erythrocyte membrane stabilization and protein denaturation methods. Further, *in silico* analysis was performed using online computational tools, such as Molinspiration, PASS analysis, and Osiris Property Explorer to assess drug-likeness, antiinflammatory potential, and the toxicity parameters.

Materials and Methods

Ibuprofen, *para*-aminobenzoic acid and aldehydes, were procured from Avra synthesis private limited, Himedia labs and SR CHEM and all other chemicals

were of AR grade. Melting points were examined in capillary tubes using melting point apparatus (Bio-Technics, India) and were reported uncorrected. Precoated silica gel plates (60 F_{254} plates, Merck Germany) were used to perform TLC using benzene: ethylacetate (1:1) ratio as the solvent system and spots were detected in Ultraviolet (UV) chamber (Bio-Technics, India-R/340/OB). Infrared (IR) spectra were documented using Fourier Transform InfraRed (FTIR) spectrophotometer Schimadzu, Japan, Bruker OPUS_7.5.18. and the absorption bands were expressed in cm^{-1} using potassium bromide (KBr) pellet method. ^1H and ^{13}C NMR spectra were recorded on SA Varian, SA Algilent spectrometer operating at 400 MHz for proton and 100 MHz for carbon measurements. Tetramethylsilane (TMS) was used internal standard. Chemical shifts were documented as parts per millions (δ ppm) and dimethylsulfoxide (DMSO-d_6) as a solvent. Agilent with ESI was used to record mass spectra.

Experimental Section

Synthesis of N-(4-(2-(2-cyanoacetyl)hydrazine-1-carbonyl) phenyl)-2-(4-isobutylphenyl)propanamide (5)

Ibuprofen (**1**) (2.06 gm, 0.01mole) was added to dry toluene (25 mL) and thionyl chloride (2 mL) was added slowly with constant shaking. At 80°C the reaction mixture was maintained on a water bath for 2 hrs and residual thionyl chloride was evaporated under reduced pressure [22]. Slight yellow colored acid chloride (**2**) obtained was used immediately in the next step. Compound (**2**) (2.24 gm, 0.01 mole) was added to 10 mL tetrahydrofuran (THF) and subsequently added to the beaker containing PABA (1.37 gm, 0.01mole), 20 mL THF, few drops of triethylamine and continued stirring for 3hrs at RT. The solvent was removed under vacuum to obtain 4-(2-(4-Isobutylphenyl) propanamido) benzoic acid (**3**)[21]. To Compound (**3**) (3.25 gm, 0.01 mole) in dioxan (20 mL), thionyl chloride 3 mL was added, refluxed for 3hrs to obtain 4-(2-(4-Isobutylphenyl) propanamido)benzoyl chloride (**4**) an orange-colored liquid on removing excess of dioxan on rotary evaporator. Meanwhile 2-cyanoacetic acid hydrazide was prepared as described by Bondock *et al*.[23] 2-Cyanoacetic acid hydrazide (0.99 gm, 0.01mol), sodium bicarbonate ($NaHCO_3$) (0.84 gm, 0.01 mol), (THF) (8 mL) and distilled water (H_2O) (4 mL) were mixed in a beaker. Compound (**4**) (3.43 gm, 0.01 mol) in THF (6 mL) was added slowly over a period of 30 min with continuous stirring to the above mixture. The reaction mixture at RT (RT) was stirred for 3 hrs. The solvent was evaporated under reduced pressure and the obtained solid was rinsed with water [24,25] and subsequently dried. The product obtained *N*-(4-(2-(2-cyanoacetyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl)propanamide (**5**) was recrystallized with absolute ethanol and used in the next step.

General procedure for synthesis of *N*-(4-(2-(2-cyano-3-(substituted phenyl)acryloyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl)propane mide), 6a-i

N-(4-(2-(2-cyanoacetyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl) propanamide (**5**) (0.01mol, 4.06 g), substituted benzaldehydes **(a-i)** (0.01mol) were added to 50 mL of toluene and were refluxed for 5-6 hrs in presence of 0.35 mL piperidine and 1.3 mL of acetic acid[26]. The reaction progress was monitored by TLC. After completion, the mixture was cooled to RT, and the resulting solid was collected by filtration. The crude product was then recrystallized using rectified spirit.

N-(4-(2-(2-Cyanoacetyl)hydrazine-1-carbonyl) phenyl)-2-(4-isobutylphenyl) propanamide, 5: White solid. m.p.208-210°C. Yield 87%. IR (KBr): 3449 (NH str, amide), 2956 (CH str, alkyl), 2222 (CN str, nitrile), 1665 (-CO str, amide-I), 1521 cm^{-1} (NH, bending of amide-II); ^1H NMR (400 MHz, DMSO-d_6): δ 0.83-0.85 (d, 6H, $(CH_3)_2$), 1.40-1.41 (d, 3H, CH_3), 1.78-1.81 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 3.74 (s, 2H, CH_2), 3.80-3.83 (q, 1H, CH), 7.10-7.12 (d, 2H, Ar), 7.28-7.30 (d, 2H, Ar), 7.68-7.71 (d, 2H, Ar) 7.80-7.82 (d, 2H, Ar), 10.30 (s, 1H, NH), 10.38 (bs, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6): 18.61, 22.16 (2C) 23.80, 29.57, 44.20, 45.67, 115.63, 118.38 (2C), 126.33, 126.97 (2C), 128.96 (2C), 128.96 (2C), 138.83, 139.58, 142.49, 161.78, 164.76, 172.84; MS: *m/z* 407 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-phenylacryloyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl)propana mide, 6a**: White solid. m.p.182-184°C. Yield 60%. IR (KBr): 3349 (NH str, amide), 2942 (CH str, alkyl), 2221 (CN str, nitrile), 1655 (-CO str, amide-I), 1531 cm^{-1} (NH, bending of amide-II); ^1H NMR (400 MHz, DMSO-d_6): δ 0.82-0.84 (d, 6H, $(CH_3)_2$), 1.43-1.44 (d, 3H, CH_3), 1.77-1.86 (m, 1H, CH), 2.39-2.44 (d, 2H, CH_2), 3.82-3.89 (q, 1H, CH), 7.05-7.08 (d, 2H, Ar), 7.10-7.12 (d, 2H, Ar), 7.28-7.30 (m, 3H, Ar) 7.50-

7.52 (d, 2H, Ar), 7.70-7.72 (2d, 4H, Ar), 8.11 (s, 1H, -CH=), 10.32 (bs, 3H, 3NH); ^{13}C NMR (100 MHz, DMSO-d_6): 18.63, 22.17 (2C), 29.59, 44.21, 45.67, 105.96, 116.06, 121.70 (2C), 127.62, 127.91, 128.60 (2C), 128.71 (2C), 129.01 (2C), 129.60 (2C), 131.10 (2C), 132.43, 133.45, 138.85, 139.60, 151.48, 161.83, 164.94, 172.84; MS: *m/z* 495 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-(4-hydroxyphenyl)acryloyl) hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl) propanamide, 6b**: Yellow solid. m.p.176-178°C. Yield 76%. IR (KBr): 3300 (NH str, amide), 2956 (CH str, alkyl), 2224 (CN str, nitrile), 1672 (CO str, amide-I), 1514 cm^{-1} (NH bending, amide-II); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83-0.85 (d, 6H, $(CH_3)_2$), 1.40-1.42 (d, 3H, CH_3), 1.76-1.87 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 3.80-3.86 (q, 1H, CH), 6.87-6.90 (d, 2H, Ar), 7.10-7.12 (d, 2H, Ar), 7.29-7.31 (d, 2H, Ar) 7.70-7.72 (d, 2H, Ar), 7.81-7.89 (2d, 4H, Ar), 8.05 (s, 1H, -CH=), 10.33 (bs, 3H, 3NH); ^{13}C NMR (100 MHz, DMSO-d_6): 18.64, 22.27 (2C), 29.49, 42.27, 45.77, 105.56, 115.06, 115.92 (2C), 121.42 (2C), 124.23, 127.52, 128.71 (2C), 129.60 (2C), 129.71 (2C), 133.10, 140.32, 141.91, 143.21 (2C), 151.49, 157.23, 161.83, 164.94, 172.84; MS: *m/z* 511 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-(4-hydroxy-3-methoxyphenyl) acryloyl)hydrazine-1-carbonyl) phenyl)-2-(4-isobutylphenyl)propanamide, 6c**: Yellow solid. m.p.184-186°C. Yield 74%. IR (KBr): 3452 (NH str, amide), 2958 (CH str, alkyl), 2213 (CN str, nitrile), 1645 (CO str, amide-I), 1514 (NH bending, amide-II), 1258 (asym. C-O-C, str), 1026 cm^{-1} (sym. C-O-C, str); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84-0.85 (d, 6H, $(CH_3)_2$), 1.40-1.42 (d, 3H, CH_3), 1.76-1.83 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 3.82 (s, 4H, OCH_3, CH), 6.93-6.95 (d, 1H, Ar), 7.10-7.12 (d, 2H, Ar), 7.28-7.30 (d, 2H, Ar) 7.50-7.52 (d, 1H, Ar), 7.70-7.72 (d, 3H, Ar), 7.83-7.85 (d, 2H, Ar), 8.11 (s, 1H, -CH=), 10.32 (bs, 3H, 3NH); ^{13}C NMR (100 MHz, DMSO-d_6): 18.63, 22.17 (2C), 29.59, 44.21, 45.67, 55.53, 98.96, 113.29, 116.06, 116.92, 118.42 (2C), 122.86, 126.46, 126.65, 126.99 (2C), 128.39 (2C), 128.97 (2C), 138.85, 139.60, 142.45, 147.86, 151.48, 152.30, 161.83, 164.94, 172.84; MS: *m/z* 541 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-(4-methoxyphenyl)acrylo yl)hydrazine-1-carbonyl)phenyl)-2-(4-isobuty lphenyl)propanamide, 6d**: Yellow solid. m.p.190-192°C; Yield 82%. IR (KBr): 3449 (NH str, amide), 2957 (CH str, alkyl), 2215 (CN str, nitrile), 1657 (CO str, amide-I), 1518 (NH bending, amide-II), 1264 (asym. C-O-C, str), 1025 cm^{-1} (sym. C-O-C, str); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84-0.85 (d, 6H, $(CH_3)_2$), 1.40-1.42 (d, 3H, CH_3), 1.76-1.83 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 3.80-3.82 (q, 1H, CH), 7.10-7.17 (2d, 4H, Ar), 7.29-7.31 (d, 2H, Ar), 7.71-7.73 (d, 2H, Ar) 7.83-7.86 (d, 2H, Ar), 8.00-8.03 (d, 2H, Ar), 8.18 (s, 1H, -CH=), 10.33 (s, 3H, 3NH); ^{13}C NMR (100 MHz, DMSO-d_6): 18.64, 22.19 (2C), 29.62, 44.22, 45.69, 55.69, 100.77, 114.95 (2C), 116.43, 118.44 (2C), 124.24, 126.57, 127.01 (2C), 128.44 (2C), 129.00 (2C), 132.78 (2C), 138.87, 139.62, 142.51, 151.06, 161.61, 162.93, 165.04, 172.87; MS: *m/z* 525 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-(3,4-dimethoxyphenyl)acryloy l)hydrazine-1-carbonyl)phenyl) -2-(4-isobutylphe nyl)propanamide, 6e**: Yellow solid. m.p.125-127°C. Yield 63%. IR (KBr): 3449 (NH str, amide), 2957 (CH str, alkyl), 2212 (CN str, nitrile), 1614 (CO str, amide-I), 1515 (NH bending, amide-II), 1264 (asym. C-O-C, str), 1026 cm^{-1} (sym. C-O-C, str); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83-0.85 (d, 6H, $(CH_3)_2$), 1.40-1.42 (d, 3H, CH_3), 1.76-1.90 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 3.80-3.91 (m, 7H, 2-OCH_3, CH), 6.63-6.78 (2d, 4H, Ar), 7.10-7.12 (d, 2H, Ar), 7.70-7.72 (d, 2H, Ar) 7.80-7.88 (d, 2H, Ar), 8.14-8.16 (d, 1H, Ar), 8.69 (s, 1H, -CH=), 10.31 (s, 1H, NH), 10.33-10.38 (bs, 2H, 2NH) ; ^{13}C NMR (100 MHz, DMSO-d_6): 18.63, 22.19 (2C), 29.62, 44.22, 45.68, 56.04, 56.21, 98.42, 100.20, 106.87, 113.16, 116.68, 118.43 (2C), 126.61, 127.00 (2C), 128.40 (2C), 128.99 (2C), 129.79, 131.68, 138.86, 139.61, 142.47, 144.20, 160.71, 161.81, 165.01, 172.86; MS: *m/z* 555 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-(4-isopropylphenyl) acryloyl) hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl) propanamide, 6f**: White solid. m.p.183-185°C. Yield 86%. IR (KBr): 3449 (NH str, amide), 2957 (CH str, alkyl), 2212 (CN str, nitrile), 1614 (CO str, amide-I), 1515 cm^{-1} (NH bending, amide-II); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83-0.85 (d, 6H, $(CH_3)_2$), 1.21-1.22 (d, 6H, $(CH_3)_2$), 1.40-1.42 (d, 3H, CH_3), 1.76-1.81 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 2.90-2.94 (q, 1H, CH), 3.80-3.84 (q, 1H, CH), 7.10-7.12 (d, 2H, Ar), 7.29-7.34 (2d, 4H, Ar), 7.63-7.65 (d, 2H, Ar), 7.71-7.74 (d, 2H, Ar), 7.86-7.88 (d, 2H, Ar), 8.14 (s, 1H, -CH=), 10.20 (bs, 2H, NH), 10.31 (s, 1H,

NH) ; ^{13}C NMR (100 MHz, DMSO-d_6): 18.63, 22.16 (2C), 23.64 (2C), 29.58, 33.35, 44.21, 45.68, 100.77, 118.41, 122.86 (2C), 126.99 (2C), 127.11, 127.62, 128.50, 128.97 (2C), 129.00 (2C), 129.32 (2C), 132.09, 138.86, 139.59, 142.31, 147.39, 150.57, 162.34, 165.04, 172.83; MS: *m/z* 537 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-(4-(dimethylamino)phenyl) acryloyl)hydrazine-1-carbonyl) phenyl)-2-(4-iso butylphenyl)propanamide, 6g**: Orange solid. m.p.206-208°C. Yield 64%. IR (KBr): 3429 (NH str, amide), 2955 (CH str, alkyl), 2211 (CN str, nitrile), 1612 (CO str, amide-I), 1519 (NH bending, amide-II), 1372 cm^{-1} (CN str, dimethylamino); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84-0.85 (d, 6H, $(CH_3)_2$), 1.40-1.42 (d, 3H, CH_3), 1.75-1.85 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 6.40 (s, 6H, N-$(CH_3)_2$), 3.80-3.85 (q, 1H, CH), 6.83-6.85 (d, 2H, Ar), 7.10-7.12 (d, 2H, Ar), 7.29-7.31 (d, 2H, Ar) 7.70-7.72 (d, 2H, Ar), 7.83-7.91 (2d, 4H, Ar), 8.03 (s, 1H, -CH=), 10.20 (bs, 2H, 2NH), 10.31 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 18.63, 22.18 (2C), 29.60, 40.12 (2C), 44.22, 45.70, 94.91, 111.70 (2C), 117.67, 118.44 (2C), 118.59 , 126.77, 127.00 (2C), 128.39 (2C), 128.99 (2C), 132.97 (2C), 138.87, 139.61, 142.42, 151.03, 153.19, 162.50, 165.05, 172.87; MS: *m/z* 538 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-(4-fluorophenyl) acryloyl)hydr azine-1-carbonyl)phenyl)-2-(4-isobutylphenyl) propanamide, 6h**: White solid. m.p.189-191°C. Yield 65%. IR (KBr): 3287 (NH str, amide), 2955 (CH str, alkyl), 2224 (CN str, nitrile), 1653 (CO str, amide-I), 1598 (NH bending, amide-II), 1242 cm^{-1} (C-F str); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84-0.85 (d, 6H, $(CH_3)_2$), 1.39-1.42 (d, 3H, CH_3), 1.76-1.83 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 3.80-3.85 (q, 1H, CH), 7.10-7.12 (d, 2H, Ar), 7.28-7.30 (m, 3H, Ar) 7.44-7.48 (t, 1H, Ar), 7.71-7.73 (m, 3H, Ar), 7.77-7.79 (d, 1H, Ar), 7.84-7.95 (d, 1H, Ar), 8.06-8.08 (d, 1H, Ar), 8.44 (s, 1H, -CH=), 10.33 (s, 3H, 3NH); ^{13}C NMR (100 MHz, DMSO-d_6): 18.63, 22.18 (2C), 29.60, 44.22, 45.70, 106.32, 113.70, 115.67 (2C), 121.7 (2C), 126.27, 126.92, 128.30 (2C), 129.39 (2C), 129.99 (2C), 131.27 (2C), 133.12, 140.43, 142.42, 150.03, 162.50, 165.04, 165.65, 172.87; MS: *m/z* 513 $(M+1)^+$.

***N*-(4-(2-(2-Cyano-3-(4-(trifluoromethyl)phenyl) acryloyl)hydrazine-1-carbonyl)phenyl)-2-(4-iso butylphenyl)propanamide, 6i**: White solid. m.p.192-194°C. IR (KBr): 3304 (NH str, amide), 2951 (CH str, alkyl), 2205 (CN str, nitrile), 1645 (CO str, amide-I), 1596 (NH bending, amide-II), 1249 cm^{-1} (C-F str); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84-0.85 (d, 6H, $(CH_3)_2$), 1.41-1.42 (d, 3H, CH_3), 1.76-1.83 (m, 1H, CH), 2.39-2.41 (d, 2H, CH_2), 3.80-3.86 (q, 1H, CH), 7.10-7.13 (d, 2H, Ar), 7.29-7.31 (d, 2H, Ar) 7.73-7.75 (d, 2H, Ar), 7.80-7.82 (d, 2H, Ar), 7.83-7.94 (2d, 4H, Ar), 8.51 (s, 1H, -CH=), 10.32 (bs, 3H, 3NH); ^{13}C NMR (100 MHz, DMSO-d_6): 18.63, 22.18 (2C), 29.60, 44.22, 45.70, 106.91, 113.70, 121.71 (2C), 124.10 , 125.21 (2C), 127.60, 128.70 (2C), 128.90 (2C), 129.09 (2C), 129.60 (2C), 130.20, 133.10, 135.40, 140.30, 141.90, 150.90, 164.80, 165.90, 172.70; MS: *m/z* 563 $(M+1)^+$.

Biological Activity

In vitro antioxidant activity

Evaluation of *in vitro* antioxidant properties carried out by using DPPH radical and nitric oxide (NO) radical scavenging assays

DPPH radical scavenging assay

Test solutions of 100 µM concentration were prepared by using absolute ethanol and were added to 100 µM DPPH in absolute alcohol. All the test tubes were kept at RT for about 20 min in a dark place and the absorbance of the samples were determined using UV visible absorbance spectrophotometer at 517 nm [27,28]. Control experiment was performed without a test compound. Ascorbic acid and Ibuprofen were used as a standard reference.

$$\% \text{ Reduction of DPPH} = \frac{A_{control} - A_{test}}{A_{control}} \times 100 \quad \ldots (1)$$

Nitric oxide free radical scavenging assay

To phosphate buffer saline *p*H 7.4 was added sodium nitroprusside (5 mM) and was incubated for 150 min at 25°C with test compounds of 100 µM concentration prepared in absolute ethanol. From the incubated solution, 2 mL was withdrawn and mixed with 2 mL of Griess reagent, then allowed to stand for 30 min[29,30]. The control experiment was performed without a test compound. Ascorbic acid and Ibuprofen were used as standard reference.

$$\% \text{ Nitric oxide free radical scavenging activity} = \frac{A_{control} - A_{test}}{A_{control}} \times 100 \quad \ldots (2)$$

In vitro antiinflammatory activity

Erythrocyte membrane stabilization and protein denaturation methods were followed to assess *in vitro* antiinflammatory activity.

Erythrocyte membrane stabilization method

The erythrocyte suspension was prepared as described by Anosike *et al*.[31] Test sample was prepared by taking 0.5 mL (100 µM) test solution, 1 mL phosphate buffer (*p*H 7.4, 0.15 M) and 2mL of hyposaline (0.36%) and 0.5 mL of erythrocyte suspension (10% v/v). Control was prepared by omitting the test solution. Ibuprofen was used as standard reference. At 37 ºC all the assay mixtures were incubated for 30 min and at 3000 rpm they were centrifuged. For hemoglobin content supernatant solution was estimated using UV spectrophotometric method at 560 nm.

$$\% \text{ Protection} = 100 - \frac{Optical\ density_{test}}{Opical\ density_{control}} \times 100 \quad \ldots (3)$$

Protein denaturation method

Egg albumin solution (5% w/v) 0.2 mL, phosphate buffer saline (*p*H 6.4) 2.8 mL and test solution (100 µM) 2 mL were taken. Control experiment was carried out with distilled water in place of test compound. Ibuprofen was taken as reference standard. Test samples, control and standard were left aside for 15 min. Heat the samples to 70 ºC for 5 min and after cooling under tap water the absorbance was measured at 660 nm.[32]

$$\% \text{ Inhibition of protein denaturation} = \frac{A_{control-A_{test}}}{A_{control}} \times 100 \quad \ldots (4)$$

In silico ADMET prediction

Molinspiration

By using molinspiration the drug-likeness of the synthesized compounds (**5, 6a-i**) was assessed based on Lipinski's Rule of Five. The chemical structures of compounds (**5, 6a-i**) were generated, and molecular properties such as log P, topological polar surface area (TPSA), molecular weight, number of hydrogen bond donors and acceptors, rotatable bonds, and molecular volume were calculated. Percentage absorption was calculated using the formula 109-(0.345*TPSA). These parameters were used to predict the compound's absorption, distribution, metabolism, and elimination (ADME) characteristics.[33]

PASS analysis

PASS is a software tool designed for evaluating the biological potential of the drug-like molecule. Based the chemical structure PASS predicts the probable biological activities, the drug-like molecule possess before the chemical synthesis.[34,35]

OSIRIS property explorer

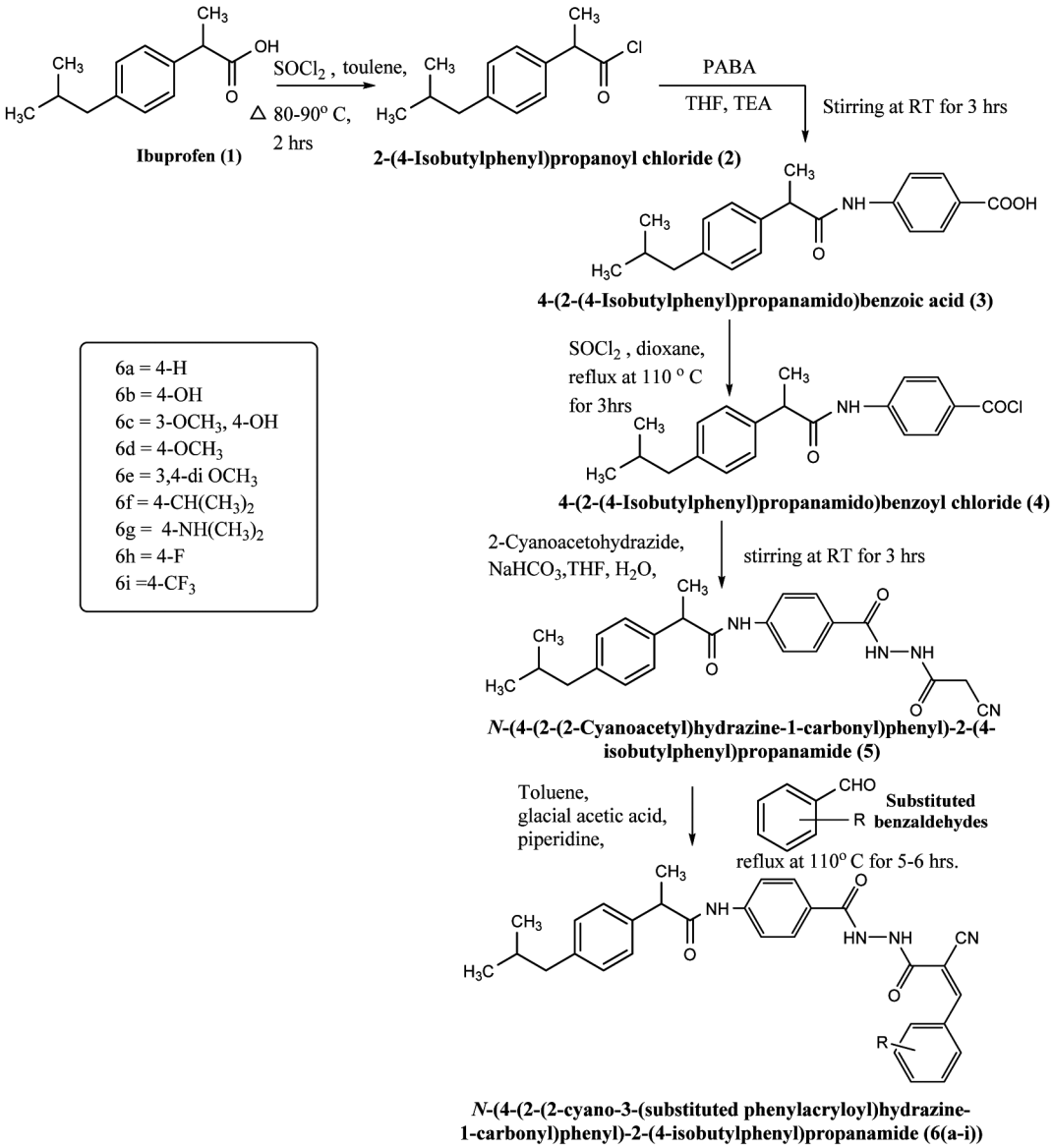
OSIRIS is a freely available online tool used for the prediction of pharmacokinetic and toxicity-related parameters of drug-like compounds. It provides toxicity indicators including mutagenicity, tumorigenicity, irritancy, and reproductive effects. The predicted results are visually interpreted through color coding: green signifies favorable, drug-like properties, while red highlights potential risks or toxic effects like mutagenicity.[36,37]

Results and Discussion

Chemistry

The main aim to synthesize novel Ibuprofen derivatives is to decrease the side effects associated with carboxylic acid group of Ibuprofen. On conjugating Ibuprofen with PABA it resulted information of amide bond between Ibuprofen and PABA where, amide derivatives have diversified pharmacological activities. Moreover, the designed molecules have structural similarity to tyrphostins that are potential anticancer agents. In step 1 thionyl chloride was added to Ibuprofen to form Ibuprofen acid chloride **(2)** and was immediately treated with PABA to obtain 4-(2-(4-Isobutylphenyl) propanamido)benzoic acid (**3**) which on further treating with thionyl chloride resulted information of 4-(2-(4-Isobutylphenyl) propanamido)benzoyl chloride (**4**). Cyanoacetylation of compound (**4**) with 2-cyano acetohydrazide resulted in formation of *N*-(4-(2-(2-Cyanoacetyl)hydrazine-1-carbonyl)phenyl)-2-(4-iso butylphenyl)propanamide (**5**) as intermediate. Various substituted benzaldehydes **(a-i)** were treated with compound **(5)** to obtain the proposed novel *N*-(4-(2-(2-cyano-3-(4-substituted phenyl)acryloyl)hydrazine-1-carbonyl) phenyl)-2-(4-isobutylphenyl) propanamide (**6a-i**) as illustrated in Scheme 1.

Compounds purity was assessed by performing thin layer chromatography (TLC) and spots were detected in UV chamber. The yields obtained were in the range of 60 to 87% for all the synthesized compounds. The intermediate **(5)** obtained was confirmed by peaks at 3449 cm^{-1} for -NH str of amides and -CN peak at 2221 cm^{-1} in IR spectra. ^{1}H NMR of the compound **(5)** showed single and broad singlet at δ 10.30 and 10.38 indicating the formation of -CO-NH- and –CO-NH-NH-CO- linkage**.** A signal for CH_2 *i.e*. active methylene group was observed at δ 3.74 for two protons. Further, the intermediate **(5)** was confirmed by appearance of signal at δ 23.80 for CH_2 (active methylene) carbon and a signal at δ 115.63 for -CN



Scheme 1 — Synthesis of novel N-(4-(2-(2-cyano-3-(substituted phenyl)acryloyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl) propanamides (**6a-i**)

carbon as observed in ^{13}C NMR. Appearance of three signals at δ 161.78, 164.76 and 172.84 confirms the formation of -CO-NH- and –CO-NH-NH-CO- linkage in the intermediate (**5**). In mass spectrum M+Na peak was obtained at 407 *m/z* for the intermediate **(5)**.

In IR spectra compounds **(6a-i)** were confirmed by the presence of -CN peak in the range of 2205-2224 cm^{-1}.

The ^{1}H NMR of all the molecules exhibited a specific singlet signal for -CH=C (benzylidene) at δ 8.05-8.69 indicating the formation of link between substituted benzaldehydes and N-(4-(2-(2-Cyanoacetyl)hydrazine-1-carbonyl)phenyl)-2-(4-iso butylphenyl) propanamide **(5)** by Knoevenagel condensation reaction.

In ^{13}C of all the synthesized molecules showed a signal at δ 145.12-161.20 for benzylidene carbon. Disappearance of CH_2 signal (active methylene group) at δ 23.80 further confirms the condensation of the intermediate with the substituted benzaldehydes and formation of the derivatives (**6a-i**) in ^{13}C NMR.

The MASS spectra of all synthesized compounds showed specific molecular ion $(M+1)^+$ peaks

Table 1 — *In vitro* antioxidant and *in vitro* antiinflammatory activities of *N*-(4-(2-(2-cyano-3-(substituted phenyl)acryloyl) hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl)propanamide

S. No.	Compd	Substituent	*In vitro* antioxidant activity		*In vitro* antiinflammatory activity	
			% Inhibition at 100 μM by DPPH method	% Inhibition at 100 μM by nitric oxide radical scavenging method	% Protection at 100 μM by Erythrocyte membrane stabilization method	% Inhibition at 100 μM by Protein denaturation method
1	**5**	–	69.83	71.32	65.46	70.27
2	**6a**	4-H	75.70	73.28	67.08	71.35
3	**6b**	4-OH	76.95	78.70	64.62	74.85
4	**6c**	3-OCH_3, 4-OH	75.05	70.58	71.31	72.35
5	**6d**	4-OCH_3	74.35	74.68	73.31	74.27
6	**6e**	3,4-di OCH_3	80.44	78.49	75.54	73.85
7	**6f**	4-$CH(CH_3)_2$	76.43	69.88	72.31	70.82
8	**6g**	4-$N(CH_3)_2$	71.65	73.31	71.31	72.48
9	**6h**	4-F	73.42	68.57	67.00	69.82
10	**6i**	4-CF3	72.17	69.97	66.85	68.42
11	Ibuprofen	–	74.24	73.10	70.00	73.10
12	Ascorbic acid	–	83.52	78.34	–	–

*Average of triplicate measurements

corresponding to their respective molecular weights.

In vitro antioxidant activity

The *in vitro* antioxidant activity of the compounds **5** and **6a–6i** revealed a clear influence of aromatic substituents on biological activity. In the DPPH radical scavenging assay, most derivatives showed improved activity compared to the intermediate **5** (69.83%) and Ibuprofen (74.24%) the reference standard. Among all, the 3,4-dimethoxy derivative (**6e)** exhibited the highest antioxidant potential (80.44%), approaching the standard ascorbic acid (83.52%). Compounds with electron-donating groups such as 4-OH (**6b**) (76.95%), 3-OCH_3,-4-OH (**6c**) (75.05%), and 4-OCH_3 (**6d**) (74.35%) also demonstrated strong activity, indicating that electron-rich substituents favor radical scavenging. In contrast, the fluoro (**6h**) and trifluoromethyl (**6i**) derivatives showed relatively lower inhibition, suggesting reduced antioxidant capability for electron-withdrawing groups.

A similar pattern was observed in the nitric oxide radical scavenging assay, where compound **6b** (4-OH) (78.70%) and **6e** (3,4 dimethoxy) (78.49%) displayed the highest activity, both comparable to the standard ascorbic acid (78.34%). The intermediate (**5**) (71.32%) and Ibuprofen (73.10%) showed moderate inhibition. The slight reduction in activity for **6h** (4-F) (68.57%) and **6i** (69.97%) (4-CF_3) further supports the negative impact of strong electron-withdrawing substituents on antioxidant performance.

In vitro antiinflammatory activity

The antiinflammatory activity as assessed by erythrocyte membrane stabilization and protein denaturation assays, showed a consistent improvement for methoxy-substituted derivatives. The 3,4-dimethoxy compound **6e** again demonstrated the highest protection in the membrane stabilization assay (75.54%), surpassing both compound **5** (65.46%) and Ibuprofen (70.00%). Compounds **6c** (3-OCH_3, 4-OH), **6d** (4-OCH_3), and **6f** (4-$CH(CH_3)$) also exhibited strong membrane-protective effects. In the protein denaturation assay, several derivatives including **6b** (4-OH), **6d** (4-OCH_3), **6e** (3,4-dimethoxy), and **6g** (4-N-(CH_3)) showed inhibition above 72%, and were comparable or superior to Ibuprofen (73.10%). Meanwhile, the fluoro (**6h**) and CF_3 (**6i**) derivatives showed comparatively lower protection, consistent with their antioxidant results. The *in vitro* antioxidant and antiinflammatory activities results were represented in the Table 1.

Overall, the results indicate that electron-donating substituents, particularly methoxy groups, significantly enhance both antioxidant and antiinflammatory activities. Compounds **6b** (4-OH), **6d** (4-OCH_3), and 3,4-dimethoxy derivative **6e,**

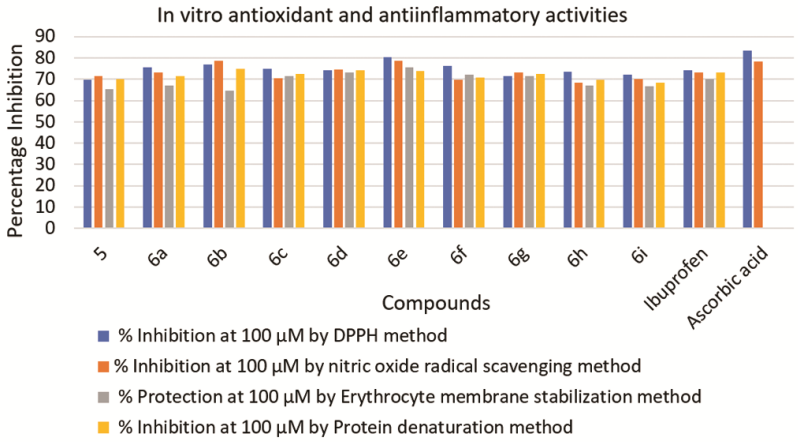


Fig. 1 — The *in vitro* antioxidant and antiinflammatory activities of the synthesised compounds (**5**, **6a-i**)

Table 2 — Molinspiration of *N*-(4-(2-(2-cyano-3-(substituted phenyl)acryloyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl)propanamides using *in silico* ADMET prediction

S. No.	Compd	mlogP	TPSA	N atom	Mol. Wt.	NoN	NOHNH	n violation	N rot	Volume	% Abs
1	5	2.75	111.09	30	406.49	7	3	0	8	383.41	70.67
2	6a	5.37	111.09	37	494.5	7	3	1	9	465.44	70.67
3	6b	4.89	131.31	38	510.5	8	4	1	9	473.45	63.70
4	6c	4.71	140.55	40	540.6	9	4	1	10	499.00	60.51
5	6d	5.42	120.32	39	524.6	8	3	2	10	490.98	67.49
6	6e	5.01	129.55	41	554.6	9	3	2	10	516.53	64.31
7	6f	6.88	111.09	40	536.6	7	3	2	10	515.38	70.67
8	6g	5.47	114.32	40	537.6	8	3	2	10	511.34	69.56
9	6h	5.53	111.09	38	512.5	7	3	2	9	470.37	70.67
10	6i	5.26	111.09	41	562.5	7	3	2	10	496.73	70.67

emerged as the most potent compound across all assays, while electron-withdrawing groups such as -F and -CF_3 tended to reduce activity. These findings highlight the importance of substituent electronics in modulating the pharmacological profile of the synthesized derivatives and serves as the lead molecules for further *in vivo* analysis. The *in vitro* antioxidant and antiinflammatory activities were represented graphically in the Fig. 1.

In silico ADMET prediction

Molinspiration

Among the synthesized compound (**5**) showed zero violations and compound **6a-c** exhibited only one violation. Whereas, compounds (**6d-i)** have more than 2 violations *i.e.* they have molecular weight and mlogP values greater than the prescribed limits of 500 Daltons and 5 respectively. All the compounds have% absorption in the range of 60.51 -70.67. Hence compounds (**5, 6a-c**) obeyed Lipinski rule of five indicating they might have good oral bioavailability. The data is represented in Table 2.

Pass Analysis

From the PASS analysis it was observed that all the compounds were predicted to have antinflammatory potential between the range of 0.238-0.375. The intermediate **5** exhibited highest pa values of 0.375 among the series indicating a comparatively higher antiinflammatory potential.

Osiris property explorer

From the osiris property explorer prediction all the synthesized compounds were predicted to be safe and do not have any mutagenicity, tumorigenicity, irritant and reproductive effects. Except compound **6b** which was predicted to possess reproductive toxicity, compound **6g** possess mutagenicity and tumorigenicity. The data is represented in the Table 3.

Table 3 — Antiinflammatory activity and toxicity risk assessment by PASS analysis and OSIRIS property explorer of *N*-(4-(2-(2-cyano-3-(substituted phenyl)acryloyl)hydrazine-1-carbonyl)phenyl)-2-(4-isobutylphenyl)propanamides

S. No.	Compd	Substituent	PASS Analysis	Toxicity Prediction			
			Pa value; antiinflammatory activity	Mutagenicity	Tumorigenic	Irritant	Reproductive Effect
1	**5**	–	0.375	G	G	G	G
2	**6a**	4-H	0.321	G	G	G	G
3	**6b**	4-OH	0.308	G	G	G	R
4	**6c**	3-OCH_3, 4-OH	0.331	G	G	G	G
5	**6d**	4-OCH_3	0.292	G	G	G	G
6	**6e**	3,4-di OCH_3	0.298	G	G	G	G
7	**6f**	4-$CH(CH_3)2$	0.275	G	G	G	G
8	**6g**	4-$N(CH_3)_2$	0.238	R	R	G	G
9	**6h**	4-F	0.303	G	G	G	G
10	**6i**	4-CF_3	0.274	G	G	G	G

Low Risk = Green (G) High Risk = Red (R)

Conclusion

In the present study a total of ten compounds were synthesized and characterized by their physical and spectral data. All the synthesized compounds were evaluated for their *in vitro* antioxidant and antiinflammatory activities. Compounds **6b** (4-OH) [31], **6d** (4-OCH_3), and **6e** (3,4-di OCH_3) consistently demonstrated superior antioxidant and antiin flammatory activities across all *in vitro* assays. The presence of electron-donating hydroxyl and methoxy substituents appears to significantly enhance radical-scavenging ability and membrane-stabilizing properties, contributing to their overall improved pharmacological profiles. Owing to their potent and well-balanced activities, these derivatives stand out as promising lead candidates and there is a need for further investigation through *in vivo* studies to validate their therapeutic potential.

Consent for publication

Not applicable.

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Supplementary Information

Supplementary information is available in the website https://nopr.niscpr.res.in/handle/123456789/5877.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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