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Synthesis, Characterization and Anti-inflammatory Activity of 2-(4-chlorobenzyl)-5-(di(5-substituted-1*H*-indol-3-yl)methyl)-6-(4-substituted phenyl)imidazo[2,1-*b*][1,3,4]thiadiazoles

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In present study, the anti-inflammatory potential of a series of 2-(4-chlorobenzyl)-5-(di(5-substituted-1*H*-indol-3-yl)methyl)-6-(4-substituted phenyl)imidazo[2,1-*b*][1,3,4]thiadiazoles (**5a-e**) were evuluated after characterizing through ^{1}H NMR, ^{13}C NMR spectral studies. Initial toxicity and anti-inflammatory efficacy of synthesized compounds is evaluated against RAW264.7 macrophages cell line to derive at their IC_{50} values. Compounds **5a-e** are further tested for their anti-inflammatory activity in Swiss albino rats using the 1% carrageenan-induced paw edema model. Compound **5a** is taken as the lead compound and is further considered for behavioural evaluations such as stair climbing and motility exercises. Paw edema is analyzed at time intervals of 0, 1, 2, 3, 4 and 24 h and stair climbing and motility tests are surveyed after 24 h of exposure to the test compound. The results of present study show that the compound **5a** significantly reduces the paw thickness by 68% at $p < 0.001$ and increased the stair climbing (1.5 folds) and motility (1.6 folds) when compared to the 1% carrageenan rat group. Conclusively the results of present study show that compound **5a** exhibits strong anti-inflammatory potential that can be used as a preclinical template for further investigations as an alternative therapy to the current NSAIDs.

Keywords: Imidazo[2,l-*b*][1,3,4]thiadiazole derivative, Anti-inflammatory activity, Paw edema, Stair climbing.

INTRODUCTION

Inflammation is a complex defensive mechanism of the body tissue that cover features of redness, pain and swelling [1]. Although non-steroidal anti-inflammatory drugs (NSAIDs) are the most favoured line of treatment they can lead to gastro-intestinal complications during long term use leading to suppression of physiological prostaglandin production in these tissues [2,3]. Since NSAIDs cause immune suppression after long use research in this area, should address alternatives that are safer and come with minimal side effects [4-7].

Imidazo[2,1-*b*][1,3,4]thiadiazole derivatives exhibit wide range of biological activities [8]. The imidazo[2,1-*b*][1,3,4]-thiadiazole scaffold makes up the core structure of pharmacologically active molecules possessing antimicrobial, anticonvulsant, diuretic, anticancer, antitubercular, analgesic and anti-inflammatory properties [9-11]. The imidazo[2,1-*b*][1,3,4]thiadiazole construct are widely explored as a reliable source for discovering novel biologically active molecules since the 1,3,4-thiadiazole moiety is pharmaceutically suitable as a "hydrogen binding domain" while the imidazole nucleus is responsible for the biological activities of compounds containing it [12-15].

Thus taking leads from the above broad-spectrum benefits of imidazo[2,1-*b*][1,3,4]thiadiazole scaffold, we synthesized imidazo[2,l-*b*][1,3,4]thiadiazole derivatives (**5a-e**) for their proposed anti-inflammatory potential after having characterizing them through LCMS and NMR. Among the synthesized compounds, **5a** displayed a good correlation in the analytical and spectral data to the proposed structures. Compounds of the **5a-e** series were further studied for their cytotoxic potential in mouse macrophage cell line RAW 264. Compound **5a** was

selected as the lead compound for anti-inflammatory investigations based upon its IC_{50} value 35 µM. **5a** was orally gavaged in Swiss albino rats using 1% carrageenan-induced paw edema. The edema was compared to standard anti-inflammatory drug indomethacin. Paw thickness was assessed at various time points as 1, 2, 3, 4 and 24 h. Further stair climbing and motility tests were performed after 24 h of exposure to 1% carrageenan. The results of current study show that compound **5a** significantly reduces the paw thickness and increases the climbing and motility in the animal after injection of compound **5a**. Conclusively the results of present study show that compound **5a** exhibits strong anti-inflammatory potential that can be used as a preclinical template for further investigation as an alternative line of treatment to NSAID therapy.

EXPERIMENTAL

Chlorophenyl acetic acid, thiosemicarbazide, phenacyl bromide, ethanol, aqueous sodium carbonate and all the other chemicals involved in the chemical synthesis were procured from (Sigma-Aldrich, USA).Carrageenan was acquired from (Hi-Media, India). Standard anti-inflammatory drug indomethacin was acquired from (Recon, Bangalore, India). RAW 264.7 mouse macrophage cell line was obtained from ATCC (American type culture collection); Manassas, USA and cultured with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 µg/mL streptomycin and 100 µg/mL penicillin (Gibco BRL), maintained at 37 ºC in a 5% CO_2 incubator.

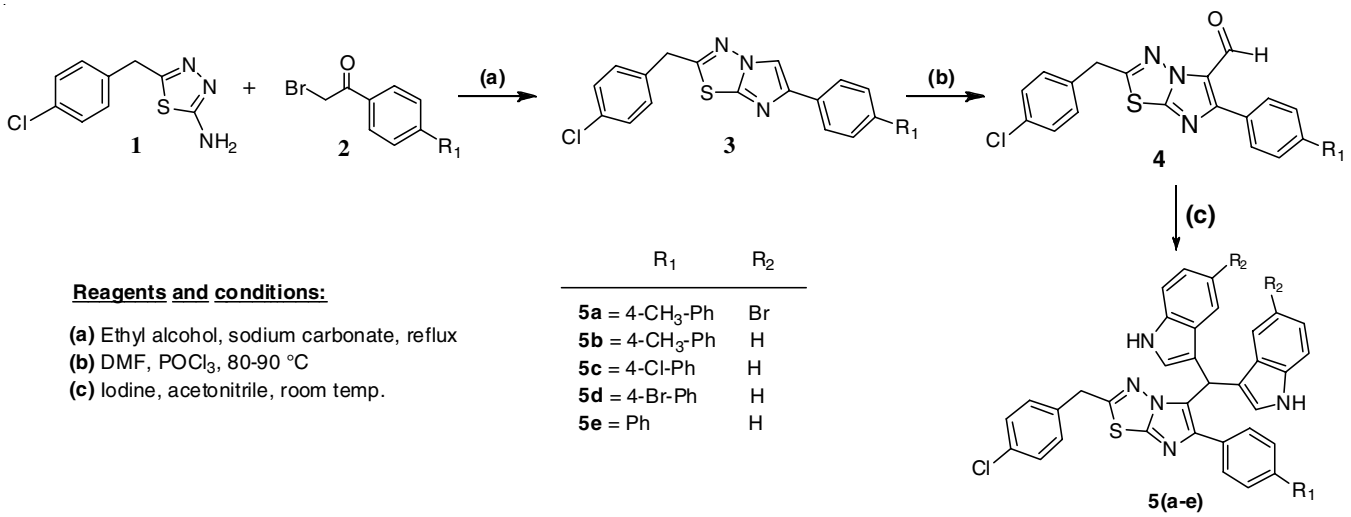
Cell cytotoxicity: All the compounds **5a-e** were screened for *in vitro* cytotoxic potential using RAW 264.7 cell line. Cells were seeded at a density of 1×10^6 cells/well in a 96-well plate and grown at standard conditions (37 °C with 5% CO_2) for 24 h and further exposed to increasing concentrations of synthesized compounds **5a-e** from a concentration range of 6.25 µM to 200 µM. Compounds that were synthesized were dissolved in DMSO as treatment group, while vehicle group were given DMSO alone. After 24 h of exposure, cells were treated with MTT (5 mg/mL) for 4 h. Insoluble formazan crystals are formed in the process that was treated with 50 µL of DMSO and the absorbance was read at a wavelength 560 nm. The results are expressed as % inhibition *vs*. concentration

Animals and care: Male Swiss albino female rats weighing around 120-150 g were used. They received standard maintenance conditions with (12:12 h light/dark cycles, 50% ± 5% humidity and temperature of 25 ± 2 °C) and were housed in polypropylene cages. Pellet diet was *ad libitum* with free access to water. All the animal investigations were permitted by the Institutional Animal Ethics Committee (IAEC), (Approval No: BCP/IAEC/EXTP/04/2018) Bharathi College of Pharmacy, Bharathi Nagara, Mandya District, India. The experiments were carried in agreement with the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines for laboratory animal facility.

Synthesis of 2-(4-chlorobenzyl)-5-(di(5-substituted-1*H*-indol-3-yl)methyl)-6-(4-substituted phenyl)imidazo[2,1-*b*]-[1,3,4]thiadiazole (5a-e): 5-(4-Chlorobenzyl)-1,3,4-thiadiazol-2-amine (**1**) was prepared from 4-chlorophenyl-acetic acid and thiosemicarbazide using H_2SO_4 as solvent at 75 °C for 6 h. 2-(4-Chlorobenzyl)-6-(4-substituted phenyl)imidazo[2,1-*b*]-[1,3,4]thiadiazole derivatives (**3a-d**) were synthesized by refluxing 5-(4-chlorobenzyl)-1,3,4-thiadiazol-2-amine (**1**) with the appropriate phenacyl bromide (**2**) in ethanol for 8 h and quenching with aqueous sodium carbonate. The various phenacyl bromides (**2a-d**) were purchased from Sigma-Aldrich. 2-(4-Chlorobenzyl)-6-(4-substituted phenyl)imidazo-[2,1-*b*][1,3,4]thiadiazole-5-carbaldehydes (**4a-d**) were synthesized by using Vilsmeier-Haack condition ($POCl_3$ and DMF) on the corresponding 2-(4-chlorobenzyl)-6-(4-substituted phenyl)imidazo[2,1-*b*]-[1,3,4]thiadiazole. 2-(4-Chlorobenzyl)-5-(di(5-substituted-1*H*-indol-3-yl)methyl)-6-(4-substituted phenyl)imidazo[2,1-*b*]-[1,3,4]thiadiazoles (**5a-e**) were obtained by treating corresponding aldehydes (**4a-d**) and two equivalence of 5-substituted indoles in presence of iodine in acetonitrile at 60 ºC for 6 h [16,17] (**Scheme-I**).

Spectral data

5-(4-Chlorobenzyl)-1,3,4-thadiazol-2-amine (1): Yield 65%; m.p.: 181-183 °C; brown solid; ^{1}H NMR (400 Hz, DMSO-



Scheme-I: Synthesis of imidazo[2,1-*b*][1,3,4]thiadiazole derivative **5(a-e)**

d_6): δ ppm: 4.123 (s, 2H, -CH_2-), 7.022 (s, 2H, -NH_2), 7.260-7.80 (d, 2H, ar, *J* = 8 Hz), 7.348-7.367 (d, 2H, ar, *J* = 7.6 Hz); ^{13}C NMR (400 Hz, DMSO-d_6) δ ppm: 35.123, 129.002, 130.949, 131.998, 137.420, 157.520, 169.382; HRMS-(ESI): *m/z* calculated for [$C_9H_8ClN_3S$+H^+] = 227.0127: found = 227.0129.

6-(4-Bromophenyl)-2-(4-chlorobenzyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3a): Yield 58%; m.p.: 165-167 °C; pale yellow solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 4.430 (s, 2H, -CH_2-), 7.412 (s, 4H, arom.), 7.547-7.569 (d, 2H, arom., *J* = 8.8 Hz), 7.756-7.777 (d, 2H, arom., *J* = 8.4 Hz), 8.672 (s, 1H, -CH=); ^{13}C NMR (400 Hz, DMSO-d_6) δ ppm: 36.533, 111.205, 120.558, 127.037, 129.257, 131.495, 132.017, 132.652, 133.601, 135.413, 144.197, 145.544, 164.715; HRMS-(ESI): *m/z* calculated for [$C_{17}H_{11}BrClN_3S$ + H^+] = 405.7113: found = 405.7115.

2-(4-Chlorobenyl)-6-(4-chlorobenzyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3b): Yield 63%; m.p.: 167-170 °C; pale yellow solid; ^{1}H NMR (400MHz-DMSO-d_6): δ ppm : 4.428 (s, 2H, -CH_2-), 7.409-7.434 (d, 6H, ar, *J* = 6 Hz), 7.814-7.835 (d, 2H, ar, *J* = 8.4 Hz), 8.662 (s, 1H, -CH=); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 36.537, 111.149, 126.716, 129.090, 129.236, 131.473, 132.038, 132.660, 133.234, 135.384, 144.180, 145.522, 164.640; HRMS-(ESI): *m/z* calculated for [$C_{17}H_{11}C_{l2}N_3S$+ H^+] = 361.2603: found = 361.2605.

2-(4-Chlorobenyl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (3c): Yield 55%; m.p.: 155-157 °C; pale yellow solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm : 4.421 (s, 2H, -CH_2-), 7.210-7.247 (t, 1H, ar, *J* = 7.6 Hz), 7.339-7.377 (t, 2H, ar, *J* = 8 Hz), 7.406 (s, 4H, ar), 7.803-7.822 (d, 2H, ar, *J* = 7.6 Hz), 8.612 (s, 1H, -CH=); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 36.508, 110.741, 125.042, 127.689, 129.080, 129.245, 131.493, 132.631, 134.305, 135.462, 145.337, 164.406; HRMS-(ESI): *m/z* calculated for [$C_{17}H_{12}ClN_3S$ + H^+] = 326.8153: found = 326.8150.

2-(4-Chlorobenyl)-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3d): Yield 68%; m.p.: 165-167 °C; pale yellow solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm : 2.273 (s, 3H, -CH_3), 4.416 (s, 2H, -CH_2-), 7.156-7.176 (d, 2H, arom., *J* = 8 Hz), 7.405 (s, 4H, arom.), 7.689-7.709 (d, 2H, arom., *J* = 8 Hz), 8.537 (s, 1H, -CH=); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 21.232, 36.523, 110.218, 125.026, 129.238, 129.627, 131.467, 131.590, 132.634, 135.479, 136.912, 145.525, 164.117; HRMS-(ESI): *m/z* calculated for [$C_{18}H_{14}ClN_3S$+ H^+] = 340.8419: found = 340.8422.

6-(4-Bromophenyl)-2-(4-chlorobenzyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4a): Yield 62%; m.p.: 138-140 °C; pale brown solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 4.532 (s, 2H, -CH_2-), 7.424 (s, 4H, arom.), 7.657-7.679 (d, 2H, arom., *J* = 8.8 Hz), 7.866-7.887 (d, 2H, arom., *J* = 8.4 Hz), 9.946 (s, 1H, CHO); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 36.447, 123.594, 123.897, 129.313, 131.116, 131.486, 131.865, 132.102, 132.804, 135.309, 151.017, 152.601, 167.390, 177.577; HRMS-(ESI): *m/z* calculated for[$C_{18}H_{11}BrClN_3OS$ + H^+] = 433.7214: found = 433.7215.

2-(4-Chlorobenzyl)-6-(4-chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4b): Yield 55%; m.p.: 157-160 °C; pale yellow solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 4.523 (s,2H, -CH_2-), 7.392-7.437 (t, 4H, arom., *J* = 8.8 Hz), 7.506-7.528 (d, 2H, arom., *J* = 8.8 Hz), 7.926-7.947 (d, 2H, arom., *J* = 8.4 Hz), 9.935 (s, 1H, -CHO); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 36.411, 123.865, 129.168, 129.304, 130.860, 131.473, 132.787, 134.810, 135.307, 150.990, 152.527, 167.393, 177.560; HRMS-(ESI): *m/z* calculated for [$C_{18}H_{11}Cl_2N_3OS$+ H^+] = 389.2704: found = 389.2701.

2-(4-Chlorobenzyl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4c): Yield 60%; m.p.: 148-150 °C; pale yellow solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 4.532 (s, 2H, -CH_2-), 7.425-7.484 (t, 7H, arom., *J* = 6.4 Hz), 7.878-7.898 (t, 2H, arom., *J* = 6.0 Hz), 9.926 (s, 1H,-CHO); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 36.411, 123.856, 129.177, 129.323, 130.014, 131.493, 132.631, 132.758, 135.394, 151.204, 154.541, 167.150, 177.587; HRMS-(ESI): *m/z* calculated for [$C_{18}H_{12}ClN_3OS$ + H^+] = 354.8254: found = 354.8259.

2-(4-Chlorobenzyl)-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]-thiadiazole-5-carbaldehyde (4d): Yield 66%; m.p.: 158-160 ºC; pale yellow solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 2.317 (s, 3H, -CH_3), 4.503 (s, 2H, -CH_2-), 7.246-7.265 (d, 2H, arom., *J* = 7.6 Hz), 7.403 (s, 4H, arom.), 7.754-7.774 (d, 2H, arom., *J* = 8 Hz), 9.897 (s, 1H, -CHO); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 21.336, 123.660, 129.190, 129.285, 129.702, 129.835, 131.438, 132.785, 135.327, 139.719, 151.121, 154.698, 166.858, 177.407; HRMS-(ESI): *m/z* calculated for [$C_{19}H_{14}ClN_3OS$ + H^+] = 368.8520: found = 368.8524.

5-(*Bis*(5-bromo-1*H*-indol-3-yl)methyl)-2-(4-chlorobenzyl)-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazole (5a): Yield 55%; m.p.: 228-230 ºC; white solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 2.313 (s, 3H, -CH_3-), 4.263 (s, 2H, -CH_2-), 6.251 (s, 1H, -CH-), 7.095-7.141 (t, 8H, arom., *J* = 13.6), 7.233-7.252 (d, 2H, arom., *J* = 7.6 Hz), 7.302-7.348 (t, 4H, arom., *J* = 18.4 Hz), 7.460-7.480 (d, 2H, arom., *J* = 8 Hz), 11.177 (s, 2H, -NH-); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 26.031, 35.466, 41.024, 116.317, 118.798, 118.891, 125.710, 128.653, 129.730, 131.084, 132.755, 133.196, 133.781, 134.437, 135.729, 136.898, 137.134, 140.221, 141.872, 146.251, 148.876, 167.202; HRMS-(ESI): *m/z* calculated for [$C_{35}H_{24}Br_2ClN_5S$ + H^+] = 742.9246: found = 742.9248.

2-(4-Chlorobenzyl)-5-(di(1*H*-indol-3-yl)methyl)-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]Thiadiazole (5b): Yield 62%; m.p.: 202-204 °C; white solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 2.306 (s, 3H, -CH_3-), 4.279 (s, 2H, -CH_2-), 6.350 (s, 1H, -CH-), 6.786-6.824 (t, 2H, arom., *J* = 15.2 Hz), 6.985-7.045 (m, 6H, arom.), 7.108-7.129 (d, 2H, arom., *J* = 7.6 Hz), 7.208-7.227 (d, 2H, arom., *J* = 7.6 Hz), 7.311-7.365 (m, 4H, arom.), 7.498-7.518 (d, 2H, arom., *J* = 8 Hz), 10.915 (s, 2H, -NH-); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 21.243, 30.770, 36.338, 112.050, 114.747, 118.378, 118.942, 121.362, 124.644, 125.823, 126.736, 127.915, 129.033, 129.586, 131.032, 132.365, 135.493, 136.714, 136.888, 141.164, 143.790, 162.166; HRMS-(ESI): *m/z* calculated for [$C_{35}H_{26}ClN_5S$ + H^+] = 585.1324: found = 585.1328.

2-(4-Chlorobenzyl)-6-(4-chlorophenyl)-5-(di(1*H*-indol-3-yl)methyl)imidazo[2,1-*b*][1,3,4]Thiadiazole (5c): Yield 54%; m.p.: 182-184 °C; white solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 4.293 (s, 2H, -CH_2-), 6.371 (s, 1H, -CH-), 6.794-

6.831 (t, 2H, arom., *J* = 14.8 Hz), 6.988-7.037 (m, 6H, arom.), 7.118-7.139 (d, 2H, arom., *J* = 8.4 Hz), 7.315-7.367 (m, 4H, arom.), 7.437-7.458 (d, 2H, arom., 8.4 Hz), 7.607-7.628 (d, 2H, arom., *J* = 8.4 Hz), 10.938 (s, 2H, -NH-); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 30.944, 36.338, 112.071, 114.307, 118.439. 119.003, 121.413, 124.756, 126.387, 126.695, 128.992, 129.053, 129.535, 131.063, 132.222, 132.407, 133.965, 135.421, 136.755, 139.913, 144.148, 162.802; HRMS-(ESI): *m/z* calculated for [$C_{34}H_{23}Cl_2N_5S$ + H^+] = 605.5509: found = 605.5516.

6-(4-Bromophenyl)-2-(4-chlorobenzyl)-5-(di(1*H*-indol-3-yl)methyl)imidazo[2,1-*b*][1,3,4]Thiadiazole (5d): Yield 57%; m.p.: 178-180 °C; white solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 4.291 (s, 2H, -CH_2-), 6.373 (s, 1H, -CH-), 6.794-6.832 (t, 2H, arom., *J* = 15.2 Hz), 6.990-7.038 (m, 6H, arom.), 7.116-7.138 (d, 2H, arom., *J* = 8.8 Hz), 7.315-7.367 (m, 4H, arom.), 7.544-7.596 (m, 4H, arom.), 10.938 (s, 1H, -NH-), 10.942 (s, 1H, -NH-); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 30.953, 36.333, 112.083, 114.292, 118.446, 119.001, 120.820, 121.414, 124.760, 126.414, 126.696, 129.061, 129.849, 131.065, 131.901, 132.407, 134.324, 135.423, 136.756, 139.938, 144.180, 162.820; HRMS-(ESI): *m/z* calculated for [$C_{34}H_{23}BrClN_5S$ + H^+] = 650.0019: found = 650.0023.

2-(4-Chlorobenzyl)-5-(di(1*H*-indol-3-yl)methyl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (5e): Yield 63%; m.p.: 169-171 °C; white solid; ^{1}H NMR (400 Hz, DMSO-d_6): δ ppm: 4.283 (s, 2H, -CH_2-), 6.370 (s, 1H, -CH-), 6.782-6.818 (t, 2H, arom., *J* = 14.4 Hz), 6.972-7.033 (m, 6H, arom.), 7.108-7.128 (d, 2H, arom., *J* = 8 Hz), 7.312-7.428 (m, 7H, arom.), 7.608-7.626 (d, 2H, arom., *J* = 7.2 Hz), 10.929 (s, 2H, -NH-); ^{13}C NMR (400 Hz, DMSO-d_6): δ ppm: 30.788, 36.304, 112.064, 114.603, 118.349, 118.952, 121.365, 124.673, 126.103, 126.696, 127.669, 127.971, 129.002, 129.031, 131.045, 132.349, 135.141, 135.491, 136.688, 141.066, 143.975, 162.412; HRMS-(ESI): *m/z* calculated for [$C_{34}H_{24}ClN_5S$ + H^+] = 571.1059: found = 571.1063.

Experimental layout: 20 male Swiss albino female rats were distributed into 4 groups with 5 animals in each (n = 5):

Group A– Control (oral gavaging with saline)

Group B –1% carrageenan (100 µL)

Group C – Animals were orally gavaged with indomethacin (10 mg/kg b.wt)

Group D – Animals were orally gavaged with compound **5a** (150 mg/kgb.wt).

Induction of acute inflammation: This was conducted following protocols from the reported literature [16,17]. In short, anti-inflammatory activity was determined by the carrageenan-induced rat paw edema test. Acute inflammation (edema) was induced 1 h after the oral administration of **5a**, by intraplantar injection of 100 µL of 1% carrageenan (Hi-Media, India) saline solution into the left hind paw of the rats. The paw volume (mL) was measured using plethysmometre (UgoBasile 7140, Varese, Italy) at 1, 2, 3, 4 and 24 h after carrageenan injection.

Edema volume:

$$\text{Edema volume (mL)} = V_t - V_o$$

where V_o is the mean paw volume before carrageenan intraplantar injection, V_t is the mean paw volume at t hours.

Stair climbing activity test: Stair climbing activity test was done following a previous protocol [18]. Briefly, rats were previously acclimatized to climb a staircase that had steps at a distance of 5, 10 and 15 cm. The climbing ability of rats was evaluated based on a rewards placed on the steps at different distances with step 2 at 10 cm having water and step 3 at 15 cm having food. A score of 0 was awarded to the rat if it did not climb; 1, for climbing onto step 1; 2, for climbing onto step 2 and 3, if the rat could climb all the three steps.

Motility test: A motility test was done following a previous protocol [19,20]. Briefly, rats were observed for 5 min and scored according to the following scorecard:

0- If the rat walked with difficulty and avoided touching the toes of the inflamed paw to the floor.

1- If the rat walked with little difficulty, but with toe touching the floor.

2- If the rat walked easily.

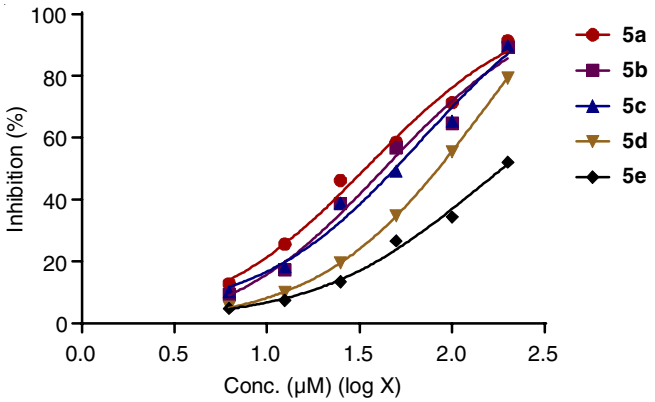
3- If the rat could climb all three steps.

Statistical analysis: Data were reported as Mean ± SEM and analyzed statistically with one and two way ANOVA followed by Turkeys multiple-comparison test with significance at $p < 0.001$.

RESULTS AND DISCUSSION

Design and chemistry: All compounds that were synthesized displayed a good correlation in the spectral and analytical data to the proposed structures.

Cell cytotoxicity: Compounds of series **5(a-e)** were screened *in vitro* for their anti-inflammatory properties against RAW 264.7 cell line. IC_{50} was prepared by nonlinear regression analysis and graph plotted by percentage inhibition against concentration of series from 6-200 µM. The results of our study showed that among all the compounds tested **5a** induced the highest anti-inflammatory potential with an IC_{50} value of 35 µM. Molecule **5b**, **5c**, **5d and 5e** displayed IC_{50} values of 43, 68, 147 and 135 µM. Therefore, **5a** was taken as lead compound (Fig. 1).



	5a	5b	5c	5d	5e
log IC_{50}	1.549	1.637	1.832	2.167	2.129
IC_{50}	35.39	43.37	68.00	146.9	134.6

Fig. 1. Effect of **(5a-e)** on the toxicity of RAW 264.7 cell line. IC_{50} was prepared by nonlinear regression analysis and graph plotted by percentage inhibition against concentration of series from 6-200 µM

Carrageenan induced paw edema: In this study, 100 µL of 1% carrageenan intraplantar injections were given to the rear paw of rats to induce inflammatory responses (Group B). Carrageenan was further superimposed with either indomethacin that served as a positive control (Group C) or with the test compound **5a** (Group D), **5b** (Group E), **5c** (Group F), **5d** (Group G), **5e** (Group H) to derive at their anti-inflammatory efficacy [21]. The results of our study show that edema was highest at 4 h with various groups showing the following mean edema scores

Group A: 0.44 ± 0.012; Group B: 1.37 ± 0.053
Group C: 0.544 ± 0.007; Group D: 0.614 ± 0.035
Group E: 1.32 ± 0.093; Group F: 1.26 ± 0.0149
Group G: 1.304 ± 0.114; Group H: 1.320 ± 0.035

However, after 24 h the edema scores decreased drastically with (Group C) showing 71% and (Group D) exhibiting a 68% decrease in inflammation rates as compared to 1% carrageenan group (Group B) that served as a positive control. Responses of (Group E, F, G and H) were comparable to 1% carrageenan group (Group B) and displayed poor anti-inflammatory responses as compared to (Group D).Group A showed increased edema with a mean value of 0.44 ± 0.009 at $p < 0.001$ (Fig. 2).

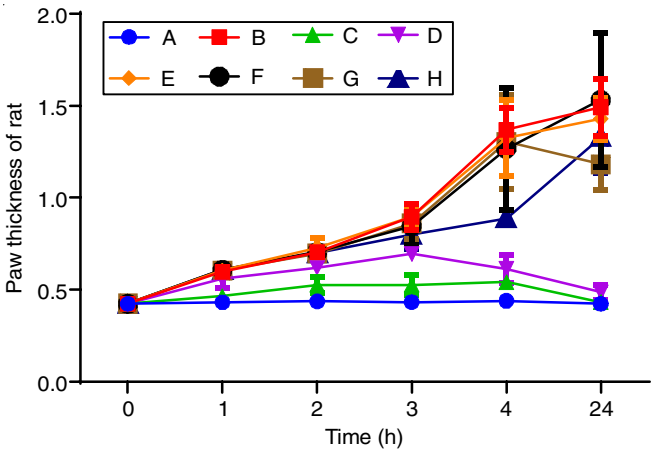


Fig. 2. Change in paw thickness at t = 0, 1, 2, 3, 4 and 24 h; n = 5 (signicant at $p < 0.001$). Edema was induced by injecting 0.1 mL of 1% solution of carrageenan into the subplantar surface of the left hind paw. Group A: normal; Group B: carrageenan control; Group C: indomethacin; Group D: **5a**; Group E: **5b**; Group F: **5c**; Group G: **5d**; Group H: **5e**

Stair climbing activity: In this study, we analyzed the anti-inflammatory potential of compound **5a** through the stair climbing capacity exercise. Rats were inflicted with 100 µL of 1% carrageenan intraplantar injections given in the rear paw of rats to induce inflammatory responses (Group B). The results of present study showed that when compared to (Group B) positive control, (Group C) exhibited 1.7 fold increase and (Group D) 1.5 fold increase in the recovery for stair climbing capacity of the rats. Therefore, compound **5a** shows comparable responses to indomethacin and also qualifies as an anti-inflammatory agent (Fig. 3).

Motility: The aim of this experiment was to study the motility responses of the animal after inflicting them with 1% carrageenan (Group B) along with Group C. and Group D. The results of present study show that motility of the animal was

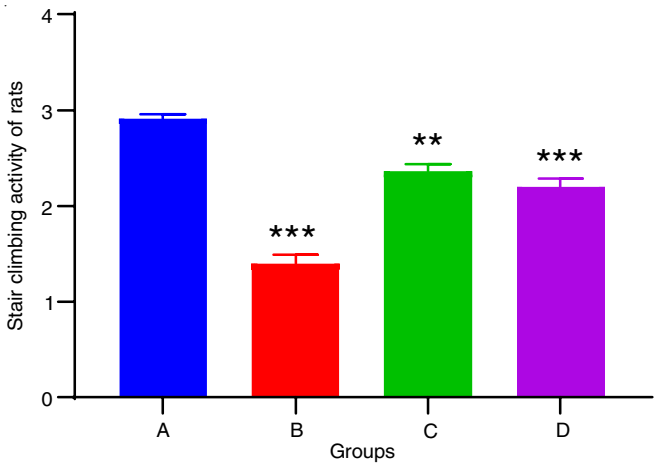


Fig. 3. Drugs were administered half-hour orally before injecting inflammation. Stair climbing activity was learnt at the time of peak inflammation (4 h for carrageenan). Group A: normal; Group B: carrageenan control; Group C: indomethacin; Group D: treated. Data are presented as mean ± SEM (**$p < 0.01$; *** $p < 0.001$)

decreased by 51% (Group B), 15% (Group C) and 20% (Group D) as compared to the control group. As compared to (Group B) indomethacin (Group C) showed a recovery by 1.7 folds and compound **5a** (Group D) showed a recovery of 1.6 folds. This is interesting as 5a shows comparable results to (Group C). Compound **5a** representing (Group D) was able to revert the motility of the animal by 31% when compared to (Group B). Compound **5a** qualifies as an anti-inflammatory chemical as it showed comparable responses to indomethacin, the standard anti-inflammatory drug (Group C) (Fig. 4).

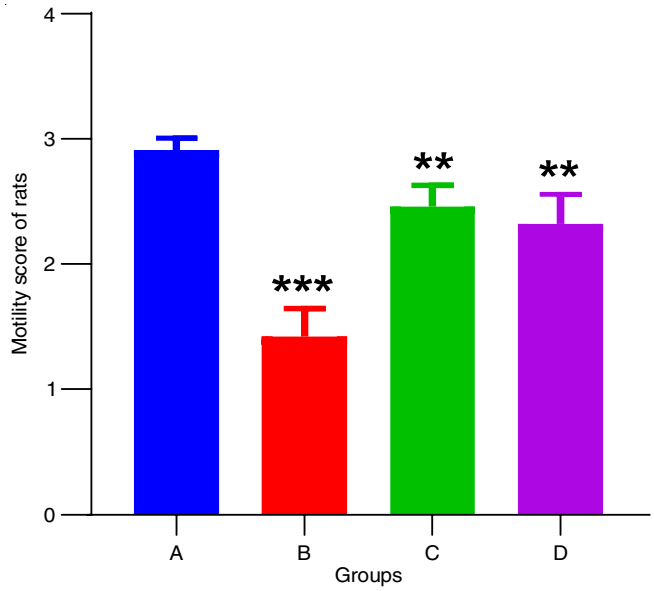


Fig. 4. Motility score was learnt at the time of peak inflammation (4 h for carrageenan); Group A: normal; Group B: carrageenan control; Group C: indomethacin; Group D: treated. Data are presented as Mean ± SEM (**$p < 0.01$; ***$p < 0.001$)

Conclusion

The present study reveals that the synthesized compound imidazo[2,1-*b*][1,3,4]thiadiazole derivatives **(5a-e)** show the

strong anti-inflammatory potential. It successfully reduced the edema scores in tested groups C and D when compared to group B (1% carrageenan) that served as a positive control. These results are further substantiated from the data received from the stair climbing and motility exercise in the rats that complement its anti-inflammatory nature. Conclusively, compound **5a** successfully qualifies as an anti-inflammatory chemical and this study is valuable as **5a** exhibits a promising approach for the treatment of inflammatory diseases and its anti-inflammatory potential can be exploited for designing a novel class of NSAID drugs shortly.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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