#### **RESEARCH PAPER**

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## Rational design of biodegradable sulphonamide candidates treating septicaemia by synergistic dual inhibition of COX-2/PGE2 axis and DHPS enzyme

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#### ABSTRACT

A new series of co-drugs was designed based on hybridising the dihydropteroate synthase (DHPS) inhibitor sulphonamide scaffold with the COX-2 inhibitor salicylamide pharmacophore through biodegradable linkage to achieve compounds with synergistic dual inhibition of COX-2/PGE2 axis and DHPS enzyme to enhance antibacterial activity for treatment of septicaemia. Compounds **5 b**, **5j**, **5n** and **50** demonstrated potent *in vitro* COX-2 inhibitory activity comparable to celecoxib. **5j** and **50** exhibited ED<sub>50</sub> lower than celecoxib in carrageenan-induced paw edoema test with % PGE2 inhibition higher than celecoxib. Furthermore, **5 b**, **5j** and **5n** showed gastric safety profile like celecoxib. Moreover, *in vivo* antibacterial screening revealed that, **5j** showed activity against *S.aureus and E.coli* higher than sulfasalazine. While, **50** revealed activity against *E.coli* higher than sulfasalazine and against *S.aureus* comparable to sulfasalazine. Compound **5j** achieved the target goal as potent inhibitor of COX-2/PGE2 axis and *in vivo* broad-spectrum antibacterial activity against induced septicaemia in mice.



**Abbreviations:** COX-2: cyclooxygenase-2 isozyme; PGE2: prostaglandin E2; DHPS: dihydropteroate synthase enzyme; SLS: *streptococcal cytolysin S*; SLO: *streptococcal cytolysin O*; NSAIDs: non-steroidal antiinflammatory drugs

#### 1. Introduction

Prostaglandin E2 (PGE2) is an oxygenated arachidonic acid metabolite produced mainly by the action of Cyclooxygenase enzymes (COX)<sup>1</sup>. Being a pro-inflammatory mediator, PGE2 activates macrophages, neutrophils, and mast cells at early stages of inflammation<sup>2,3</sup>. Additionally, it had been demonstrated as a powerful immunosuppressant inhibiting pathogen-killing *via* alveolar macrophages and phagocytosis. As a result, it is considered a potent risk factor during inflammation<sup>4,5</sup>. Moreover, COX-2 isozyme, a key enzyme in the process of PGE2 biosynthesis, had been reported to be induced by infections of the pathogenic *Streptococcus pyogenes*. In addition, several bacterial toxins including *streptococcal cytolysin S* (SLS) and *streptococcal cytolysin O* (SLO) produced by *S. pyogenes* together with *pneumolysin* and *Clostridium difficile toxin A* have been reported as potent inducers of COX-2 expression<sup>6</sup>. besides, it had been confirmed that COX-2/PGE2 production were up-regulated and promoted biofilm formation by *Staphylococcus aureus* thereby enhances its adherence to the human fibronectin and finally motivates its growth<sup>7</sup>. Hence, blocking COX-2/PGE2 pathway introduces a new, an effective and an unexpected

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manner to overcome bacterial infections. Accordingly, celecoxib, the selective COX-2 inhibitor, had been reported to increase sensitivity of Staphylococcus aureus to antibiotics<sup>8</sup>. Interestingly, celecoxib increased the utilisation of ampicillin by bacteria by inhibiting antibiotic resistance genes as well as increasing membrane permeability<sup>9,10</sup>. Furthermore, it had been reported that the commonly available antibiotics chloramphenicol, cefuroxime and Oxytetracycline exerted a synergistic effect with celecoxib, against MRSA strains with a 4-fold reduction in the cefuroxime and chloramphenicol MICs and 2-fold decrease in the Oxytetracycline MIC<sup>11,12,13</sup>. On the other hand, sulphonamides were the first antibacterial agents to be discovered by Gerhard Domagk, since then they have been applied for different clinical indications<sup>14</sup>. Moreover, they exert their action by competitive inhibition of dihydropteroate synthase enzyme (DHPS), a significant anti-folate target<sup>15</sup>. Additionally, the sulphonamide moiety constitutes the prime pharmacophore of most selective COX-2 inhibitors as celecoxib and its derivatives<sup>16</sup>. In fact, sulphonamides comprise an interesting class of drugs with a wide scope of pharmacological activities such as antibacterial<sup>17,18</sup>, hypoglycemic<sup>19</sup>, diuretic<sup>20</sup>, carbonic anhydrase enzyme inhibitory properties<sup>21-24</sup>, antithyroid<sup>25</sup> and anti-inflammatory agents<sup>26</sup>. This may be attributed to sulphonamides' phenylamino and sulphonyl amino groups essential for various biological activities as well as metal coordination<sup>27</sup>. Furthermore, Sulfasalazine (I), Figure 1 is a sulphonamide prodrug degraded by colonic bacteria via azo reductase enzyme to sulfapyridine, an antibiotic, and 5-aminosalicylic acid, an anti-inflammatory agent, compounds with different modes of action<sup>28,29</sup>. It has been demonstrated that, sulfasalazine exerted its anti-inflammatory action via inhibiting a number of immunological processes, including lymphocyte proliferation, interleukin-2 synthesis in addition to interleukin-1 production by monocytes, but was not classified as an NSAID<sup>30</sup>. On the other hand, salicylamides had been previously reported as inhibitors of the two-component regulatory system (TCS) in bacteria<sup>31,32</sup>. Besides, "5-substituted salicylamides" mainly designed to act as topical antibiotics, were serendipitously discovered to have innate anti-inflammatory activity. Therefore, those 5-substituted salicylamides as were referred to as lipophilic, antibacterial, anti-inflammatory drugs (LAADs). Salifluor (II), Figure 1, the first-generation lead drug candidate of LAADs, had

an excellent efficacy against a wide variety of Gram-positive and Gram-negative oral organisms. Trifluorosal (III), Figure 1, a secondgeneration LAAD-type salicylamide, underwent early-stage pharmaceutical development for gingivities treatment. Among the third generation, naphthafluor (IV), Figure 1, is currently in development as a treatment for acne as well as gingivitis<sup>33</sup>. Besides, salicylamide derivatives V, Figure 1 exhibited significant COX-2 inhibition with significant COX-2 selectivity comparable to celecoxib<sup>34</sup>. As well, salicylamide derivatives have been regarded as one of the promising candidates owing to their interesting biological activities, including antimycobacterial<sup>35,36</sup>, antifungal<sup>37</sup>, antibacterial<sup>31,38</sup>, antineoplastic<sup>39,40</sup>, antianthelminthic<sup>41</sup>, antiparasitic<sup>42</sup> as well as anti-inflammatory activities<sup>43,44</sup>. In view of the aforesaid facts, designing new candidates targeting COX-2/DHPS enzymes together with PGE2 production would be more effective in combating inflammatory bacterial infections. Thus, in the current study, we utilised the DHPS inhibiting properties of sulphonamides and the COX-2 inhibiting properties of salicylamides as well as their beneficial antibacterial effects to provide a monotherapy approach targeting COX-2 and DHPS enzymes being effective against bacterial infections together with their sequential inflammatory actions with lower evidence of resistance. Accordingly, herein we designed some new biodegradable molecules combining sulphonamide moiety linked to N-Substituted-2-hydroxybenzamides through azo linkage. The newly synthesised compounds were evaluated in vitro for their COX-1/COX-2 inhibitory activities and their antibacterial activities against human pathogenic bacteria including a number of Gram-positive and Gram-negative bacteria and the most active compounds were further evaluated in vivo for their anti-inflammatory and antibacterial efficacies. In addition, their effect on PGE2 production and ulcerogenic effect were also evaluated. Moreover, molecular docking studies were performed to profile the binding pattern of the potential dual COX-2/DHPS inhibitors with the active site of the targeted COX-2 and dihydropteroate synthase (DHPS) enzymes. Furthermore, in silico predictions of physicochemical parameters, drug likeness score and acquiescence to the Lipinski's rule of five (RO5) were carriedout to the biologically active compounds to estimate their suitability to act as possible orally active dug candidates.



#### 2. Experimental

#### 2.1. Chemistry

All chemicals were bought from commercial sources and used directly without further purification. Open-glass capillaries were used in measuring melting points on Stuart Scientific melting point apparatus (SMP10) and are uncorrected. The reactions' rates were followed up by thin layer chromatography (TLC) on silica gel pre-coated Merck aluminium GF254 plates, and the spots were visualised at  $\lambda$  254 nm using UV-lamp for few seconds' exposure to iodine vapours. Thermo Scientific Nicolet iS20 Fourier Transform Infra-red Spectrometer was used for recording Infra-red spectra (IR), using KBr discs, Faculty of Pharmacy, Mansoura University. Proton nuclear magnetic resonance spectra (<sup>1</sup>H-NMR) were scanned on JNM-ECA II 500 MHz JEOL Spectrometer at the NMR unit, Faculty of Science, Mansoura University and on Bruker Avance III 400 MHz Spectrometer at Centre for Drug Discovery Research and Development-Faculty of Pharmacy, Ein Shams University using deuterated dimethylsulphoxide (DMSO-d<sub>6</sub>) as a solvent. Data were interpreted as chemical shifts expressed in  $\delta$ values (ppm) relative to tetramethylsilane (TMS) as internal standard. Signal splitting type was indicated by one of the following letters: s = singlet, d = doublet, t = triplet, q = quartette, dd = doubletof doublet and m = multiplet. <sup>13</sup>C-NMR spectra were scanned on JNM-ECA II 125 MHz JEOL Spectrometer at the NMR unit, Faculty of Science, Mansoura University. Electron impact mass spectra (EI-MS) were scanned on Direct Inlet part mass analyser in Thermo Scientific GCMS model ISQ at the Regional Centre for Mycology and Biotechnology, Al-Azhar University, Cairo. Elemental analyses (C, H, N and S) was the tool used for checking purity of the new compounds using FLASH 2000 CHNS/O analyser, Thermo Scientific at the Regional Centre for Mycology and Biotechnology (RCMB), Al-Azhar University. The results were within ±0.4% of the calculated values for the proposed formulae. ChemDraw Professional 16.0.1.4. was used for naming the new compounds according to the naming algorithm developed by CambridgeSoft Corporation compounds 2a<sup>45</sup> and 2 b-2d<sup>46,47,48</sup> were prepared as previously reported.

#### 2.1.1. General procedure for the synthesis of compounds 5a-o

The appropriate sulphonamide derivative (2 mmol) was suspended in hydrochloric acid (17%, 2 ml) then ethanol was added until a clear solution was obtained the solution was cooled from 0 °C to -5 °C and a solution of NaNO2 (2 mmol, 0.138 gm) in water (1 ml) was added dropwise. After stirring at 0 °C for 15–30 min, a solution of the appropriate N-substituted-2-hydroxybenzamide (2 mmol) in NaOH (10%, 2 ml) was added to the diazonium salt dropwise and the reaction mixture was stirred overnight at room temperature. The formed precipitate was filtered off, washed with water and crystallised from ethanol.

**2.1.1.1**. *N*-Butyl-2-hydroxy-5-((4-sulfamoylphenyl) diazenyl) benzamide (5a). Pale brown crystals; yield 95%, m.p. 227–229°C. IR (KBr, cm<sup>-1</sup>): 3529 (OH), 3377, 3269 (NH<sub>2</sub>, NH), 1646 (C=O), 1496 (N=N), 1305, 1149 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSO-d6) δ 0.92 (t, J=7.4 Hz, 3H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.31–1.42 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.52–1.61 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 3.36–3.39 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 7.08 (d, J=8.6 Hz, 1H, salicyl-C<sub>3</sub>-H),7.51 (s, 2H, sulfamoylphenyl NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 7.78–7.82 (m, 4H, 1-sulfamoylphenyl-C<sub>2,6,3,5</sub>-H), 7.97 (dd, J=8.6, 2.4 Hz, 1H, salicyl-C<sub>4</sub>-H), 8.57 (d, J=2.4 Hz, 1H, salicyl-C<sub>6</sub>-H), 9.14 (t, J=5.7 Hz, 1H, CH<sub>2</sub>-NH, D<sub>2</sub>O exchangeable), 13.48 (s, 1H, OH, D<sub>2</sub>O exchangeable) 0.13C-NMR (125 MHz, DMSO-D<sub>6</sub>) δ 13.72 (NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>- CH<sub>3</sub>), 19.67 (NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 30.82 (NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 46.47 (NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 115.31(salicyl-C<sub>3</sub>), 118.68 (salicyl-C<sub>1</sub>), 121.79 (sulfamoylphenyl-C<sub>2,6</sub>), 125.44 (salicyl-C<sub>4</sub>), 125.83(salicyl-C<sub>6</sub>), 126.77 (sulfamoylphenyl-C<sub>3,5</sub>), 144.49 (sulfamoylphenyl-C<sub>4</sub>), 150.34 (salicyl-C<sub>5</sub>), 151.68 (sulfamoylphenyl-C<sub>1</sub>), 163.42 (salicyl-C<sub>2</sub>), 168.58 (C = O). Anal. Calcd.for C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O<sub>4</sub>S (376.43): C, 54.24; H, 5.36; N, 14.88; S, 8.52. Found: C, 54.37; H, 5.48; N, 14.66; S, 8.63.

**2.1.1.2.** *N*-Butyl-2-hydroxy-5-((4-(*N*-(thiazol-2-yl) sulfamoyl) phenyl) diazenyl) benzamide (5 b). Shiny orang crystals; yield 97%, m.p. 239–241 °C. IR (KBr, cm<sup>-1</sup>): 3528 (OH), 3395, 3373 (NH), 1637 (C = O), 1491 (N = N), 1298, 1143 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSO-d6)  $\delta$  0.91 (t, J = 7.5 Hz, 3H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.30–1.40 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, 3H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, 1.30–1.40 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, 3H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, 3H, 3.41–3.47 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>, 6.88 (d, J = 4.7 Hz, 1H, thiazolyl-C<sub>5</sub>-H), 7.10 (d, J = 8.9 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.29 (d, J = 4.7 Hz, 1H, thiazolyl-C<sub>4</sub>-H), 7.94 – 8.01 (m, 5H, 1-sulfamoyl phenyl-C<sub>2,3,5,6</sub>-H and salicyl-C<sub>4</sub>-H), 8.59 (s, 1H, salicyl-C<sub>6</sub>-H), 9.12 (t, J = 5.7 Hz, 1H, CH<sub>2</sub>-NH, D<sub>2</sub>O exchangeable), 12.84 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 13.52 (s, 1H, OH, D<sub>2</sub>O exchangeable). Anal. Calcd.for C<sub>20</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub>S<sub>2</sub> (459.54): C, 52.27; H, 4.61; N, 15.24; S, 13.95. Found: C, 52.39; H, 4.72; N, 15.13; S, 14.07.

2.1.1.3. N-Butyl-2-hydroxy-5-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) diazenyl) benzamide (5c). Orang crystals; yield 95%, m.p. 228-230 °C. IR (KBr, cm<sup>-1</sup>): 3653 (OH), 3465, 3425 (NH), 1646 (C=O), 1494 (N=N), 1344,1146 (SO<sub>2</sub>).<sup>1</sup>H-NMR (400 MHz, DMSOd6)  $\delta$  0.91 (t, J=7.5 Hz, 3H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.31-1.40 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.52 – 1.60 (*m*, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH CH<sub>3</sub>), 3.41–3.48 (*m*, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 7.06 (*t*, J=4.6 Hz, 1H, pyrimidinyl-C<sub>5</sub>-H), 7.11 (d, J = 8.8 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.96 – 8.00 (m, 3H, 1-sulfamoyl phenyl- $C_{2.6}$ -H and salicyl- $C_4$ -H), 8.17 (d, J = 8.6 Hz, 2H, sulfamoyl phenyl-C<sub>3.5</sub>-H), 8.52 (d, J = 4.6 Hz, 2H, pyrimidinyl-C<sub>4,6</sub>-H), 8.59 (d, J = 2.4 Hz, 1H, salicyl-C<sub>6</sub>-H), 9.12 (t, J = 5.7 Hz, 1H, CH<sub>2</sub>-NH, D<sub>2</sub>O exchangeable), 12.04 (s, 1H, 1-sulfamoyl NH, D<sub>2</sub>O exchangeable), 13.52 (s, 1H, OH, D<sub>2</sub>O exchangeable). <sup>13</sup>C-NMR (125 MHz, DMSO-D<sub>6</sub>)  $\delta$  13.73 (NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-C CH<sub>3</sub>), 19.69 (NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 30.85 (NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 46.51 (NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 115.70 (pyrimidinyl-C<sub>5</sub>), 118.83 (salicyl-C<sub>3</sub>), 122.47 (salicyl-C<sub>1</sub>), 124.07 (sulfamoylphenyl-C<sub>2.6</sub>), 126.15  $(salicyl-C_4),$ 129.11  $(salicyl-C_6),$ 137.11(sulfamoylphenyl-C<sub>3.5</sub>) 144.53(sulfamoylphenyl-C<sub>4</sub>), 152.10 (salicyl-C<sub>5</sub>), 154.10 (sulphamoylphenyl-C<sub>1</sub>), 156.79 (pyrimidinyl-C<sub>4,6</sub>), 158.70 (pyrimidinyl-C<sub>2</sub>), 163.97 (salicyl-C<sub>2</sub>), 168.27 (C = O). Anal. Calcd.for C<sub>21</sub>H<sub>22</sub>N<sub>6</sub>O<sub>4</sub>S (454.51): C, 55.50; H, 4.88; N, 18.49; S, 7.05. Found: C, 55.62; H, 4.98; N, 18.37; S, 7.13. EIMS m/z (% relative abundance): 457.96 (2.96) (M<sup>+•</sup>+3), 454.49 (10.42) (M<sup>+•</sup>), 432.78 (18.08), 396.36 (43.52), 327.07 (28.63), 115.05 (38.74), 57.04 (48.32), 44.92 (82.56) 43.03 (100) (base peak).

2.1.1.4. N-Butyl-2-hydroxy-5-((4-(N-(4-methylpyrimidin-2yl) sulfamoyl) phenyl) diazenyl) benzamide (5d). Deep orang crystals; yield 97%, m.p. 235–238 °C. IR (KBr, cm<sup>-1</sup>): 3676 (OH), 3525, 3394 (NH), 1643 (C=O), 1495 (N=N), 1347, 1149 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSO-d6)  $\delta$  0.91 (t, J=7.3 Hz, 3H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.30 – 1.39 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.52 – 1.60 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 2.32 (s, 3H,pyrimidinyl-4-CH<sub>3</sub>), 3.30–3.36 (m, 2H, NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 6.91 (d, J=5.2 Hz, 1H, pyrimidinyl-C<sub>5</sub>-H), 7.11 (d, J=8.9 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.95–7.99 (m, 3H, 1-sulfamoyl phenyl-C<sub>2,6</sub>-H, salicyl-C<sub>4</sub>-H), 8.17 (d, J=8.6 Hz, 2H, 1-sulfamoyl phenyl-C<sub>3,5</sub>-H), 8.32 (d, J=5.2 Hz, 1H, pyrimidinyl-C<sub>6</sub>-H), 8.60 (d, J=2.4 Hz, 1H, salicyl-C<sub>6</sub>-H), 9.13 (t, J=5.6 Hz, 1H, CH<sub>2</sub>-NH, D<sub>2</sub>O

exchangeable), 11.96 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 13.53 (s, 1H, OH, D<sub>2</sub>O exchangeable). Anal. Calcd.for  $C_{22}H_{24}N_6O_4S$  (468.53): C, 56.40; H, 5.16; N, 17.94; S, 6.84. Found: C, 56.52; H, 5.17; N, 17.83; S, 6.94.

2.1.1.5. N-Benzyl-2-hydroxy-5-((4-sulfamoylphenyl) diazenyl) benzamide (5e). Orang crystals; yield 95%, m.p. 250-252°C. IR (KBr, cm<sup>-1</sup>): 3452 (OH), 3400, 3361 (NH<sub>2</sub>), 3268 (NH), 1640 (C = O), 1494 (N = N), 1337, 1150 (SO<sub>2</sub>). <sup>1</sup>H- NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  4.56 (s, 2H, -benzyl-CH<sub>2</sub>), 7.12 (d, J = 9.0 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.28 (s, 2H, benzyl-C<sub>2,6</sub>-H), 7.33–7.37 (m, 3H, benzyl-C<sub>3,4,5</sub>-H),7.53 (s, 2H, sulfamoylphenylNH<sub>2</sub>, D<sub>2</sub>O exchangeable), 7.97–8.03 (m, 5H, sulfamoylphenyl-C<sub>2,3,5,6</sub>-H and salicyl-C<sub>4</sub>-H), 8.64 (s, 1H, salicyl-C<sub>6</sub>-H), 9.71 (s, 1H, CH<sub>2</sub>-NH, D<sub>2</sub>O exchangeable), 13.30 (br.s, 1H, OH, D<sub>2</sub>O exchangeable) 0.13 C-NMR (125 MHz, DMSO-d<sub>6</sub>)  $\delta$  42.65 (benzyl-CH<sub>2</sub>), 116.13 (salicyl-C<sub>3</sub>), 118.95 (salicyl-C<sub>1</sub>), 122.66 (sulfamoylphenyl-C<sub>2,6</sub>), 126.06 (salicyl-C<sub>4</sub>), 126.60 (salicyl-C<sub>6</sub>), 127.09 (benzyl-C<sub>2,6</sub>), 127.49 (benzyl-C<sub>4</sub>), 128.49 (benzyl-C<sub>3,5</sub>), 138.82 (sulfamoylphenyl- $C_{3,5}$ ), 144.46 (benzyl- $C_1$ ), 145.40 (sulfamoylphenyl- $C_4$ ), 152.00 (salicyl-C<sub>5</sub>), 153.68 (sulfamoylphenyl-C<sub>1</sub>), 163.91(salicyl-C<sub>2</sub>), 168.13 (C = O). Anal. Calcd.for  $C_{20}H_{18}N_4O_4S$  (410.45): C, 58.53; H, 4.42; N, 13.65; S, 7.81. Found: C, 58.79; H, 4.55; N, 13.42; S, 7.89. EIMS m/z (% relative abundance): 410.42 (4.8) (M<sup>+•</sup>), 393.47 (10.02), 314.40 (13.69), 167.46 (27.82), 96.14 (32.86), 69.04 (100) (base peak), 53.96 (37.56), 43.98 (43.98).

**2.1.1.6.** *N*-Benzyl-2-hydroxy-5-((4-(*N*-(*thiazol-2-yl*) sulfamoyl) phenyl) diazenyl) benzamide (5f). Deep brown crystals; yield 90%, m.p. 281–283 °C. IR (KBr, cm<sup>-1</sup>): 3566 (OH), 3450, 3371 (NH), 1642 (C = O), 1493 (N = N), 1297, 1139 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSO-d6)  $\delta$  4.55 (d, J = 5.4 Hz, 2H, benzyl-CH<sub>2</sub>), 6.86 (d, J = 4.5 Hz, 1H, thiazolyl-C<sub>5</sub>-H), 7.08 (d, J = 9.2 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.27 (d, J = 4.5 Hz, 2H, benzyl-C<sub>2,6</sub>-H), 7.32 – 7.38 (*m*, 4H, benzyl-C<sub>3,4,5</sub>-H and thiazolyl-C<sub>4</sub>-H), 8.61 (d, J = 2.5 Hz, 1H, salicyl-C<sub>6</sub>-H), 9.86 (*t*, J = 5.4 Hz, 1H, CH<sub>2</sub>-NH, D<sub>2</sub>O exchangeable), 12.06 (*s*, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 13.33 (*s*, 1H, OH, D<sub>2</sub>O exchangeable). Anal. Calcd.for C<sub>23</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub>S<sub>2</sub> (493.56): C, 55.97; H, 3.88; N, 14.19; S, 12.99. Found: C, 56.09; H, 3.99; N, 14.08; S, 13.11.

2.1.1.7. N-Benzyl-2-hydroxy-5-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) diazenyl) benzamide (5 g). Deep yellow crystals; yield 93%, m.p. 275–277 °C. IR (KBr, cm<sup>-1</sup>): 3568 (OH), 3393, 3356 (NH), 1648 (C=O), 1493 (N=N), 1337,1145 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSOd6)  $\delta$  4.55 (d, J=5.4 Hz, 2H, benzyl-CH<sub>2</sub>), 7.06 (t, J=4.8 Hz, 1H, pyrimidinyl-C<sub>5</sub>-H), 7.13 (d, J = 8.9 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.24–7.29 (m, 2H, benzyl-C<sub>2,6</sub>-H), 7.33 – 7.39 (m, 3H, benzyl-C<sub>3,4,5</sub>-H), 7.98 (d, J=7.8 Hz, 2H, sulfamoyl phenyl-C<sub>2.6</sub>-H), 8.02 (dd, J=8.9, 2.6, 1H, salicyl-C<sub>4</sub>-H), 8.17 (d, J = 7.8 Hz, 2H, sulfamoyl phenyl-C<sub>3,5</sub>-H), 8.52 (d, J = 4.8 Hz, 2H, pyrimidinyl-C<sub>4,6</sub>-H), 8.63 (d, J = 2.6 Hz, 1H, salicyl-C<sub>6</sub>-H), 9.65 (t, J = 5.4 Hz, 1H, CH<sub>2</sub>-NH, D<sub>2</sub>O exchangeable) 12.06 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 13.30 (s, 1H, OH, D<sub>2</sub>O exchangeable). <sup>13</sup>C-NMR (126 MHz, DMSO-D6)  $\delta$  42.62 (benzyl-CH<sub>2</sub>), 115.94 (pyrimidinyl-C<sub>5</sub>), 118.77(salicyl-C<sub>3</sub>), 122.45(salicyl-C<sub>1</sub>), 126.04 (sulfamoylphenyl-C<sub>2,6</sub>), 126.57 (salicyl-C<sub>4</sub>), 127.05 (salicyl-C<sub>6</sub>), 127.44 (benzyl-C<sub>2,6</sub>), 128.44 (benzyl-C<sub>4</sub>), 129.06 (benzyl-C<sub>3,5</sub>), 132.94 (sulfamoylphenyl-C<sub>3,5</sub>), 138.74 (sulfamoylphenyl-C<sub>4</sub>), 141.74 (benzyl-C<sub>1</sub>), 144.59 (salicyl-C<sub>5</sub>), 154.05 (sulfamoylphenyl-C<sub>1</sub>), 156.76 (pyrimidinyl-C<sub>4.6</sub>), 158.50 (pyrimidinyl-C<sub>2</sub>), 163.62 (salicyl-C<sub>2</sub>), 168.09 (C = O). Anal. Calcd. for  $C_{24}H_{20}N_6O_4S$  (488.52): C, 59.01; H, 4.13; N, 17.20; S, 6.56. Found: C, 59.13; H, 4.24; N, 17.09; S, 6.64.

**2.1.1.8. 4-**((**4**-Hydroxy-**3-**(*piperidine-1-carbonyl*) *phenyl*) *diazenyl*) *benzenesulfonamide* (**5** *h*). Yellow crystals; yield 95%, m.p. 220–222 °C. IR (KBr, cm<sup>-1</sup>): 3568 (OH), 3384, 3346 (NH<sub>2</sub>), 1681 (C = O), 1482 (N = N), 1349, 1148 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSO-d6)  $\delta$  1.41–1.61 (*m*, 6H, piperidinyl-C<sub>3,4,5</sub>-H), 3.14–3.25, 3.51–3.65 (2m, 4H, piperidinyl-C<sub>2,6</sub>-H), 7.08 (d, *J* = 8.7 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.52 (*s*, 2H, sulfamoyl NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 7.71 (d, *J* = 2.5 Hz, 1H, salicyl-C<sub>6</sub>-H), 7.89 (dd, *J* = 8.7, 2.5 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.95 – 8.01 (*m*, 4H, sulfamoyl phenyl-C<sub>2,3,5,6</sub>-H), 10.87 (*s*, 1H, OH, D<sub>2</sub>O exchangeable). Anal. Calcd. for C<sub>18</sub>H<sub>20</sub>N<sub>4</sub>O<sub>4</sub>S (388.44): C, 55.66; H, 5.19; N, 14.42; S, 8.25. Found: C, 55.78; H, 5.29; N, 14.30; S, 8.36.

2.1.1.9. 4-((4-Hydroxy-3-(piperidine-1-carbonyl) phenyl) diazenyl)-N-(thiazol-2-yl) benzenesulfonamide (5i). Orang crystals; yield 90%, m.p. 164–166 °C. IR (KBr, cm<sup>-1</sup>): 3651 (OH), 3451 (NH), 1574 (C = O), 1481 (N = N), 1328, 1138  $(SO_2)$ . <sup>1</sup>H-NMR (400 MHz, DMSOd6)  $\delta$  1.42–1.61 (*m*, 6H, piperidinyl-C<sub>3,4,5</sub>-H), 3.14–3.25, 3.51–3.65  $(2 \text{ m}, 4\text{H}, \text{piperidinyl-C}_{2.6}\text{-H}), 6.87 \text{ (d}, J = 4.5 \text{ Hz}, 1\text{H}, \text{thiazolyl-C}_{5}\text{-H}),$ 7.07 (d, J = 8.7 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.28 (d, J = 4.5 Hz, 1H, thiazolyl-C<sub>4</sub>-H), 7.69 (d, J = 2.4 Hz, 1H, salicyl-C<sub>6</sub>-H), 7.87 (dd, J = 8.7, 2.4 Hz, 1H, salicyl-C<sub>4</sub>-H), 7.91-7.99 (m, 4H, sulfamoyl phenyl-C<sub>2.3,5,6</sub>-H), 10.87 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 12.86 (s, 1H, OH, D<sub>2</sub>O exchangeable) 0.13 C-NMR(126 MHz, DMSO-D6)  $\delta$  24.05 (piperidinyl-C<sub>4</sub>), 25.63 (piperidinyl-C<sub>3.5</sub>), 47.34 (piperidinyl-C<sub>2.6</sub>), 108.60 (thiazolyl-C<sub>5</sub>), 116.41(salicyl-C<sub>3</sub>), 119.31(salicyl-C<sub>1</sub>), 122.53(sulfamoylphenyl-C<sub>2,6</sub>), 124.63(salicyl-C<sub>4</sub>), 125.66(salicyl-C<sub>6</sub>), 126.17 (thiazolyl-C<sub>4</sub>), 127.19 (sulfamoylphenyl-C<sub>3,5</sub>), 143.51(sulfamoylphenyl-C<sub>4</sub>), 144.93 (salicyl-C<sub>5</sub>), 153.69 (sulfamoylphenyl-C<sub>1</sub>), 157.39 (salicyl- $C_2$ ),165.61(C = O),169.07(thiazolyl- $C_2$ ). Anal. Calcd.for  $C_{21}H_{21}N_5O_4S_2$  (471.55): C, 53.49; H, 4.49; N, 14.85; S, 13.60. Found: C, 53.61; H, 4.59; N, 14.73; S, 13.68.

2.1.1.10. 4-((4-Hydroxy-3-(piperidine-1-carbonyl) phenyl) diazenyl)-N-(pyrimidin-2-yl) benzenesulfonamide (5j). Orang crystals; yield 93%, m.p. 267–269 °C. IR (KBr, cm<sup>-1</sup>): 3651 (OH), 3448 (NH), 1581 (C = O), 1498 (N = N), 1349, 1166 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSOd6)  $\delta$  1.41–1.60 (*m*, 6H, piperidinyl-C<sub>3,4,5</sub>-H), 3.14–3.24, 3.52–3.66 (2 m, 4H, piperidinyl-C<sub>2,6</sub>-H), 7.04–7.08 (*m*, 2H, pyrimidinyl-C<sub>5</sub>-H, salicyl-C<sub>3</sub>-H), 7.70 (d, *J* = 2.2 Hz, 1H, salicyl-C<sub>6</sub>-H), 7.87 (dd, *J* = 8.5, 2.2 Hz, 1H, salicyl-C<sub>4</sub>-H), 7.95 (d, *J* = 8.5 Hz, 2H, sulfamoyl phenyl-C<sub>2,6</sub>-H), 8.14 (d, *J* = 8.5 Hz, 2H, sulfamoyl phenyl-C<sub>3,5</sub>-H), 8.51 (d, *J* = 4.8 Hz, 2H, pyrimidinyl-C<sub>4,6</sub>-H), 10.90 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 12.02 (br.s, 1H, OH, D<sub>2</sub>O exchangeable). Anal. Calcd.for C<sub>22</sub>H<sub>22</sub>N<sub>6</sub>O<sub>4</sub>S (466.52): C, 56.64; H, 4.75; N, 18.01; S, 6.87. Found: C, 56.68; H, 4.87; N, 17.89; S, 6.88.

2.1.1.11. 4-((4-Hydroxy-3-(piperidine-1-carbonyl) phenyl) diazenyl)-N-(4-methylpyrimidin-2-yl) benzenesulfonamide (5k). Orang crystals; yield 97%, m.p. 208–210 °C. IR (KBr, cm<sup>-1</sup>): 3652 (OH), 3567, 3448 (NH), 1612 (C = O), 1472 (N = N), 1337, 1165 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSO-d6)  $\delta$  1.41–1.62 (*m*, 6H, piperidinyl-C<sub>3.4.5</sub>-H), 2.32 (s, 3H, pyrimidinyl-4-CH<sub>3</sub>), 3.15-3.25, 3.52-3.66 (2m, 4H, piperidinyl-C<sub>2.6</sub>-H), 6.90 (d, J = 5.3 Hz, 1H, pyrimidinyl-C<sub>5</sub>-H), 7.19 (d, J = 8.8 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.74 (d, J = 2.4 Hz, 1H, salicyl-C<sub>6</sub>-H), 7.88 (dd, J = 8.8, 2.4 Hz, 1H, salicyl-C<sub>4</sub>-H), 7.94 (d, J = 8.6 Hz, 2H, sulfamoyl phenyl- $C_{2,6}$ -H), 8.14 (d, J = 8.6 Hz, 2H, sulfamoyl phenyl-C<sub>3.5</sub>-H), 8.31 (d, J = 5.3 Hz, 1H, pyrimidinyl-C<sub>6</sub>-H), 10.89 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 11.99 (br.s, 1H, OH, D<sub>2</sub>O exchangeable) 0.13 C-NMR (125 MHz, DMSO-D<sub>6</sub>)  $\delta$  23.22 (pyrimidinyl-4-CH<sub>3</sub>-C), 24.07 (piperidinyl-C<sub>4</sub>), 25.69 (piperidinyl-C<sub>3,5</sub>), 47.25 (piperidinyl-C<sub>2,6</sub>) 112.45 (pyrimidinyl-C<sub>5</sub>), 116.44 (salicyl-C<sub>3</sub>), 119.36 (salicyl-C<sub>1</sub>), 122.31(sulfamoylphenyl-C<sub>2,6</sub>), 125.62 (salicyl-C<sub>4</sub>), 126.24 (salicyl-C<sub>6</sub>),

129.25 (sulfamoylphenyl-C<sub>3,5</sub>), 137.59 (sulfamoylphenyl-C<sub>4</sub>), 145.00 (salicyl-C<sub>5</sub>), 153.93(sulfamoylphenyl-C<sub>1</sub>), 156.42 (pyrimidinyl-C<sub>6</sub>), 157.51(pyrimidinyl-C<sub>2</sub>), 159.49 (salicyl-C<sub>2</sub>), 165.62 (pyrimidinyl-C<sub>4</sub>), 168.54 (C = O). Anal. Calcd.for  $C_{22}H_{22}N_6O_5S$  (482.52): C, 54.76; H, 4.60; N, 17.42; S, 6.64. Found: C, 54.84; H, 4.64; N, 17.34; S, 6.72. EIMS m/z (% relative abundance): 482.49 (11.78) (M<sup>+</sup>•), 458.70 (48.60), 395.30 (34.33), 360.25 (41.93), 349.02 (50.06), 308.18 (51.43), 282.03 (100) (base peak), 193.02 (35.65).

**2.1.1.12. 4**-((**4**-Hydroxy-3-(morpholine-4-carbonyl) phenyl) diazenyl) benzenesulfonamide (5 l). Pale brown crystals; yield 93%, m.p. 272–274 °C. IR (KBr, cm<sup>-1</sup>): 3450 (OH), 3340 (NH<sub>2</sub>), 1610 (C = O), 1489 (N = N), 1345, 1154 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSOd6)  $\delta$  3.53–3.69 (m, 8H, morpholinyl-H), 7.11 (d, *J* = 8.8 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.50 (*s*, 2H, sulfamoyl NH<sub>2</sub>, D<sub>2</sub>O exchangeable), 7.77 (d, *J* = 2.4 Hz, 1H, salicyl-C<sub>6</sub>-H), 7.91 (dd, *J* = 8.8, 2.4 Hz, 1H, sali cyl-C<sub>4</sub>-H), 7.96 – 8.01 (m, 4H, sulfamoyl phenyl-C<sub>2,3,5,6</sub>-H), 11.00 (*s*, 1H, OH, D<sub>2</sub>O exchangeable). Anal. Calcd.for C<sub>17</sub>H<sub>18</sub>N<sub>4</sub>O<sub>5</sub>S (390.41): C, 52.30; H, 4.65; N, 14.35; S, 8.21. Found: C, 52.41; H, 4.77; N, 14.25; S, 8.29.

2.1.1.13. 4-((4-Hydroxy-3-(morpholine-4-carbonyl)phenyl)diazenyl)-N-(thiazol-2-yl)benzenesulfonamide (5 m). Brown crystals; yield 95%, m.p. 235–237 °C. IR (KBr, cm<sup>-1</sup>): 3653 (OH), 3454 (NH), 1603 (C = O), 1483 (N = N), 1411, 1141 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSOd6)  $\delta$  3.52–3.69 (*m*, 8H, morpholinyl-H), 6.86 (d, J = 4.6 Hz, 1H, thiazolyl-C<sub>5</sub>-H), 7.14 (d, J = 8.9 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.27 (d, J = 4.6 Hz, 1H, thiazolyl-C<sub>4</sub>-H), 7.74 (d, J = 2.5 Hz, 1H, salicyl-C<sub>6</sub>-H), 7.88 (dd, J = 8.9, 2.5 Hz, 1H, salicyl-C<sub>4</sub>-H), 7.92-7.98 (m, 4H, sulfamoyl phenyl-C<sub>2.3.5.6</sub>-H), 11.06 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 12.87 (br.s, 1H, OH, D<sub>2</sub>O exchangeable) 0.13 C-NMR (125 MHz, DMSO-D<sub>6</sub>)  $\delta$  46.62 (morpholinyl-C<sub>3.5</sub>), 66.50 (morpholinyl-C<sub>2.6</sub>), 108.62 (thiazolyl-C<sub>5</sub>), 116.52 (salicyl-C<sub>3</sub>), 119.32 (salicyl-C<sub>1</sub>), 120.87 (sulfamoylphenyl-C<sub>2,6</sub>), 124.91(salicyl-C<sub>4</sub>), 125.16 (salicyl-C<sub>6</sub>), 125.55 (thiazolyl-C<sub>4</sub>), 129.02(sulfamoylphenyl-C<sub>3.5</sub>), 137.16 (sulfamoylphenyl-C<sub>4</sub>), 145.96 (salicyl-C<sub>5</sub>), 153.08 (sulfamoylphenyl-C<sub>1</sub>), 159.79 (salicyl-C2), 168.64 (C = O), 169.21(thiazolyl-C<sub>2</sub>). Anal. Calcd.for C<sub>20</sub>H<sub>19</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub> (473.52): C, 50.73; H, 4.04; N, 14.79; S, 13.54. Found: C, 50.85; H, 4.12; N, 14.68; S, 13.58. EIMS m/z (% relative abundance): 476.32 (3.62) (M<sup>+•</sup>+3), 473.46 (13.41) (M<sup>+•</sup>), 401.27 (19.48), 393.28 (38.19), 371.15 (17.19), 157.01 (15.13), 125.34 (20.48), 84.94 (24.86), 70.99 (34.08), 44.41 (100) (base peak).

2.1.1.14. 4-((4-Hydroxy-3-(morpholine-4-carbonyl) phenyl) diazenyl)-N-(pyrimidin-2-yl) benzenesulfonamide (5n). Shiny brown crystals; yield 95%, m.p. 241–243 °C. IR (KBr, cm<sup>-1</sup>): 3651 (OH), 3450 (NH), 1583 (C=O), 1500 (N=N), 1335, 1162 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSO-d6) δ 3.51–3.67 (m, 8H, morpholinyl-H), 7.05 (t, J = 4.9 Hz, 1H, pyrimidinyl-C<sub>5</sub>-H), 7.11 (d, J = 8.8 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.75 (d, J = 2.5 Hz, 1H, salicyl-C<sub>6</sub>-H), 7.89 (dd, J = 8.8, 2.5 Hz, 1H, salicyl-C<sub>4</sub>-H), 7.95 (d, J = 8.6 Hz, 2H, sulfamoyl phenyl-C<sub>2.6</sub>-H), 8.15 (d, J = 8.6 Hz, 2H, sulfamoyl phenyl-C<sub>3,5</sub>-H), 8.51 (d, J = 4.9 Hz, 2H, pyrimidinyl-C<sub>4.6</sub>-H), 11.03 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 11.97 (s, 1H, OH,  $D_2O$  exchangeable). <sup>13</sup>C-NMR (125 MHz, DMSO-D6)  $\delta$  46.91(morpholinyl-C<sub>3.5</sub>), 66.35 (morpholinyl-C<sub>2.6</sub>), 116.57(pyrimidinyl-C<sub>5</sub>), 119.22(salicyl-C<sub>3</sub>), 122.49(salicyl-C<sub>1</sub>), 123.19(sulfamoylphenyl- $C_{26}$ ), 124.78(salicyl-C<sub>4</sub>), 126.50(salicyl- $C_6$ ), 129.05 (sulfamoylphenyl-C<sub>3.5</sub>), 132.15(sulfamoylphenyl-C<sub>4</sub>), 145.02(salicyl-C<sub>5</sub>), 154.13(sulfamoylphenyl-C<sub>1</sub>), 156.80(pyrimidinyl-C<sub>4,6</sub>), 157.54 (pyrimidinyl-C<sub>2</sub>), 158.39 (salicyl-C<sub>2</sub>), 165.98 (C = O). Anal. Calcd.for C<sub>21</sub>H<sub>20</sub>N<sub>6</sub>O<sub>5</sub>S (468.49): C, 53.84; H, 4.30; N, 17.94; S, 6.84. Found: C, 53.92; H, 4.34; N, 17.83; S, 6.92.

2.1.1.15. 4-((4-Hydroxy-3-(morpholine-4-carbonyl) phenyl) diazenyl)-N-(4-methylpyrimidin-2-yl) benzenesulfonamide (5o). Shiny brown crystals; yield 95%, m.p. 205–207 °C. IR (KBr, cm<sup>-1</sup>): 3652 (OH), 3450 (NH), 1612 (C = O), 1472 (N = N), 1337, 1165 (SO<sub>2</sub>). <sup>1</sup>H-NMR (400 MHz, DMSO-d6)  $\delta$  2.32 (s, 3H, pyrimidinyl-4-CH<sub>3</sub>), 3.51–3.66 (m, 8H, morpholinyl-H), 6.90 (d, J = 5.3 Hz, 1H, pyrimidinyl-C<sub>5</sub>-H), 7.19 (d, J = 8.8 Hz, 1H, salicyl-C<sub>3</sub>-H), 7.74 (d, J = 2.4 Hz, 1H, salicyl-C<sub>6</sub>-H), 7.88 (dd, J = 8.8, 2.4 Hz, 1H, salicyl-C<sub>4</sub>-H), 7.94 (d, J = 8.6 Hz, 2H, sulfamoyl phenyl-C<sub>2,6</sub>-H), 8.14 (d, J = 8.6 Hz, 2H, sulfamoyl phenyl-C<sub>3,5</sub>-H), 8.31 (d, J = 5.3 Hz, 1H, pyrimidinyl-C<sub>6</sub>-H), 11.21 (s, 1H, sulfamoyl NH, D<sub>2</sub>O exchangeable), 11.85 (s, 1H, OH, D<sub>2</sub>O exchangeable). Anal. Calcd.for C<sub>22</sub>H<sub>22</sub>N<sub>6</sub>O<sub>5</sub>S (482.52): C, 54.76; H, 4.60; N, 17.42; S, 6.64. Found: C, 54.84; H, 4.71; N, 17.32; S, 6.68.

#### 2.2. Biological evaluation

#### 2.2.1. In vitro COX-1 and COX-2 enzymatic assay

All the synthesised target compounds were screened for their COX-1 and COX-2 inhibitory activities according to the previously reported methods<sup>49,50</sup> (page S34, supplementary file).

#### 2.2.2. Carrageenan-induced paw edoema in mice

According to the reported procedures for carrageenan-induced paw edoema test in mice<sup>51</sup>, this test was carried out for compounds **5 b**, **5j**, **5n** and **5o**. (Approved by HU-IACUC)<sup>52</sup> (Pages S34 and S35, supplementary file).

#### 2.2.3. Determination of ED<sub>50</sub>

 $ED_{50}$  of compounds **5 b**, **5j**, **5n** and **5o** was calculated as reported<sup>53</sup>. (Page S35, supplementary file).

#### 2.2.4. Estimation of rat plasma PGE2

Plasma PGE2 concentration of compounds **5 b**, **5j**, **5n** and **5o** was also measured as previously reported<sup>54</sup>. (Approved by HU-IACUC)<sup>52</sup> (Page S35, supplementary file).

#### 2.2.5. Gastric ulcerogenic activity

The acute gastric ulcerogenic effect of compounds **5 b**, **5j**, **5n** and **50** in adult male Wistar rats was evaluated as previously reported<sup>55</sup>. (Approved by HU-IACUC)<sup>52</sup> (Pages S35 and S36, supplementary file).

#### 2.2.6. Antibacterial screening

**2.2.6.1.** *Inhibition-zone measurements.* Inhibition zones of the target compounds was calculated as previously reported<sup>56</sup>. (Page S36, supplementary file).

*2.2.6.2. Minimal inhibitory concentration (MIC) measurement.* MIC of test compounds was measured according to the reported methods<sup>57</sup>. (Page S36, supplementary file).

2.2.6.3. Minimal bactericidal concentration (MBC) measurement. MBC tests were also carried out as reported<sup>58</sup>. (Page S37, supplementary file).

**2.2.7.** In vivo antibacterial screening in mice (bacteremic infection) The *in vivo* antibacterial activity of compounds **5 b**, **5j**, **5n** and **5o** against *E.coli* and *Staphylococcus aureus* using sulfasalazine as a reference drug<sup>59</sup> was performed as reported<sup>60</sup>. (Approved by HU-IACUC)<sup>52</sup> (Pages S37 and S38, supplementary file).

#### 2.3. Docking studies

Molecular Operating Environment (MOE 2018.0802) software<sup>61,62</sup> https://www.chemcomp.com/Research-Citing\_MOE.htm, Chemical Computing Group (Chemical Computing Group, Quebec, Canada, Montreal, Canada) was utilised in performing Molecular docking studies. Protein preparation was performed as previously reported<sup>63</sup>, page S38, supplementary file.

## 2.4. In silico prediction of physicochemical properties, drug likeness score, pharmacokinetics, toxicity profile and ligand efficiency metrics

In the present study, prediction of the physicochemical properties was performed using Molinspiration chemoinformatic server, pharmacokinetics by Pre-ADMET calculator, drug likeness score and toxicological effects by Osiris property explorer, page S38, supplementary file.

#### 3. Results and discussion

#### 3. 1. Chemistry

The synthetic routes for the preparation of the intermediates and target compounds are outlined in Scheme 1. Aminolysis reaction of methyl salicylate (1) with the selected primary or secondary amines was employed to obtain N-Substituted-2-hydroxybenzamides (2a-d) in good yields and with pure regioselectivity as reported previously<sup>45,64</sup>. The target azo compounds (5a-o) were attained by diazotisation of the appropriate sulphonamide with NaNO<sub>2</sub> and HCl at 0 °C followed by addition of the appropriate benzamide (2a-d). Moreover, integrity of the structures of the newly synthesised compounds was justified by microanalysis, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and MS data (see chemistry experimental section and supporting information). The IR spectra of compounds 5a-o were characterised by the absorption bands specific for OH, NH, C = O, N = N and  $SO_2$  functional groups. <sup>1</sup>H-NMR spectra of compounds **5a-d** were characterised by appearance of one D<sub>2</sub>O exchangeable triplet signal around  $\delta$  9.12 ppm stands for n-butyl NH proton in addition to two D<sub>2</sub>O exchangeable singlets stands for sulfamoyl NH proton in the range  $\delta$  11.96 – 12.84 ppm for **5 b**, 5c and 5d and sulfamoyl NH<sub>2</sub> protons at  $\delta$  7.51 ppm for 5a together with OH proton around  $\delta$  13.52 ppm. Moreover, compounds **5e-g** were distinguished by appearance of one signal around  $\delta$  4.55 ppm corresponding to benzyl CH\_2 protons, three  $D_2O$  exchangeable signals; in the range  $\delta$  9.65 – 9.9.86 ppm corresponding to n-benzyl NH proton, around  $\delta$  12.06 ppm corresponding to sulfamoyl NH proton for **5f** and **5g**, around  $\delta$  7.53 ppm corresponding to sulfamoyl NH<sub>2</sub> protons for **5e** and around  $\delta$ 13.30 ppm corresponding to OH proton. Besides, compounds **5 h-k** were differentiated by three multiplets in the ranges  $\delta$ 1.41-1.62, 3.14-3.25 and 3.51-3.66 ppm corresponding to the ten piperidinyl protons, in addition to one D<sub>2</sub>O exchangeable singlet around  $\delta$  10.90 ppm corresponding to sulfamoyl NH proton for **5***i*, **5j** and **5k** and around  $\delta$  7.52 ppm corresponding to sulfamoyl NH<sub>2</sub> protons for **5** h together with one  $D_2O$  exchangeable singlet in the range  $\delta$  10.87–12.86 ppm corresponding to OH proton.

Furthermore, compounds **5 I-o** were distinguished by appearance of multiplet signal in the range  $\delta$  3.51–3.69 ppm stands for the eight morpholinyl protons together with two D<sub>2</sub>O exchangeable singlets; around  $\delta$  7.50 ppm corresponding to sulfamoyl NH<sub>2</sub> protons for **5** I, in the range  $\delta$  11.03–11.21 ppm corresponding to sulfamoyl NH proton for **5 m**, **5n** and **5o** and in the range  $\delta$ 11.00–12.87 ppm corresponding to OH proton. <sup>13</sup>C-NMR spectra proved the appearance of C = O signal at its expected region  $(\delta 165.61 - 168.64 \text{ ppm})$ . It also showed the presence of signals assigned to n-butyl moiety around  $\delta$  13.72, 19.67, 30.82 and 46.47 ppm for compounds **5a and 5c**, signal assigned to benzyl-CH<sub>2</sub> around  $\delta$  42.65 ppm for compounds **5e** and **5g**, three signals around  $\delta$  24.05, 25.63 and 47.34 ppm corresponding to piperidinyl carbons for compounds 5i and 5k, in addition to two signal around  $\delta$  46.62 and 66.50 ppm assigned to morpholinyl carbons for compounds 5 m and 5n. Finally, MS spectra showed the molecular ion peak ( $M^{+\bullet}$ ) at m/z 454.49 for **5c**, at 410.42 for **5e**, at 482.49 for 5k and at 473.46 for 5m.

#### 3.2. Biological evaluation

#### 3.2.1. In vitro COX-1 and COX-2 enzymatic assay

Compounds 5a-o were screened for their in vitro COX-1/COX-2 inhibitory activities using an ovine COX-1/human recombinant COX-2 assay kit (Catalog no. 560131; Cayman Chemicals Inc. Ann Arbour, MI, USA). Celecoxib and Rofecoxib were used as reference selective COX-2 inhibitors and indomethacin and Diclofenac sodium were used as reference non-selective COX inhibitors. The data recorded in Table 1 revealed that all compounds were more potent COX-2 inhibitors than Diclofenac sodium. Compared to indomethacin, compounds 5 b, 5j, 5n and 5o were more potent COX-2 inhibitors while compounds 5c, 5i and 5l were equipotent. Moreover, compounds 5d, 5g, 5k and 5m were nearly comparable to indomethacin. All compounds exhibited weak inhibition of COX-1 compared to indomethacin. Considering Celecoxib, compound 5j displayed equipotent COX-2 inhibitory activity while compounds 5 b, 5c, 5i, 5 l, 5n and 5o were nearly comparable to Celecoxib. Interestingly, all compounds showed balanced weak COX-1 and more potent COX-2 inhibition with selectivity indices (SI) ranging from 58 to 239. The resulted SI values exceeded those for the non-selective COX inhibitors while being inferior to selective COX-2 inhibitors. This could also be considered advantageous by avoiding the cardiovascular side effects of the highly selective COX-2 inhibitors<sup>65</sup>. Careful inspection of the structures of the tested compounds revealed that compounds 5 b, 5j, 5n and 5o were the most potent COX-2 inhibitors in this series. The highest activity was observed with the piperidin-1-yl and sulfadiazine derivative 5j. Replacement of 4-sulfamoylphenyl moiety in compound 5a with thiazol-2-yl (5b), pyrimidin-2-yl (5c) and 4-methylpyrimidin-2-yl (5d) respectively seemed to greatly enhance COX-2 inhibitory activity with higher selectivity indices. As observed with compounds 5e-g, introduction of benzyl moiety decreased COX-2 inhibitory activity. Moreover, the presence of 4-sulfamoylphenyl moiety in compounds 5e and 5h did not enhance COX-2 inhibitory activity making them the least active compounds in this series. Concerning compounds 5i-5k, 5l, 5n and 5o, introduction of piperidin-1-yl and morpholin-1-yl moieties to methyl salicylate portion of the compound significantly enhanced COX-2 inhibitory activity comparable to that of Celecoxib.

#### 3.2.2. Carrageenan-induced paw oedema in mice

Although most of the newly synthesised compounds exhibited a promising *in vitro* anti-inflammatory activity, it is important to



Scheme 1. Synthesis of the target co-drugs (5a-o).

assess their efficacy inside the biological system this can be attributed to their azo co-drug nature. As a result, they must be subjected to azo reductase enzyme, a metabolising enzyme in the colon, liberating two active metabolites; the sulphonamide portion and the 5-amino salicylamide portion being ready for systemic absorption. Consequently, the synthesised compounds, **5 b**, **5 j**, **5 n** and **50** showing the most potent and selective COX-2 inhibitory activity were subjected to *in vivo* carrageenan-induced paw oedema bioassay in mice using celecoxib and Diclofenac as reference drugs. The results illustrated in Table 2 and Figure 2 revealed that, after 2 h, both **5 j** and **50** exhibited similar pharmacokinetic profiles to celecoxib as revealed from their rapid onset of action. Interestingly, after 2 h, compound **5j** showed more potent inhibition of paw edoema than both celecoxib and Diclofenac. To conclude the anti-inflammatory activity of the tested compounds, the anti-inflammatory activity after 8 h was taken as a point for comparison which showed that, compound **5j** showed the best % inhibition of paw oedema (88.50%) being more than that was displayed by both celecoxib (70.87%) and Diclofenac (69.13%). Furthermore, compound **5o** displayed comparable inhibition of paw oedema (72.17%) to that of celecoxib and Diclofenac. Compounds **5b** and **5n** also showed moderate anti-inflammatory activities (56.09% and 60.87%, respectively). Furthermore, the ED<sub>50</sub> values (Table 2) showed that all compounds were nearly equipotent (10.94–18.69  $\mu mol/kg$ ). However, compound **5**j was found to be the most potent among this series with ED\_{50} = 10.94  $\mu mol/kg$  exceeding those for celecoxib (13.07  $\mu mol/kg$ ) and Diclofenac (11.46  $\mu mol/kg$ ). In addition, the ED\_{50} of compound **50** (13.27  $\mu mol/kg$ ) was the same as those for celecoxib.

#### 3.2.3. Estimation of rat plasma PGE2

Estimating serum levels of PGE2 is a critical parameter to assess the *in vivo* anti-inflammatory efficacies of COX-2 inhibitors. Consequently, compounds **5 b**, **5j**, **5n** and **5o** were subjected to estimation of PGE2 in rat serum and results were summarised in Figure 3 (Table 3, page S4 supplementary file). The results showed that, the most active compound was **5j** recording plasma PGE2% inhibition of 83.25% higher than reference celecoxib (% inhibition = 71.18) and Diclofenac (% inhibition = 79.10). Moreover, compound **5o** (72.92%) showed PGE2-diminishing

Table 1. In vitro COX-1 and COX-2 inhibitory  $IC_{50}$  values and COX SI values of the target compounds (5a-o).

	IC <sub>5</sub>	ο μΜ	SIC	
Compound ID	COX-1 <sup>a</sup>	COX-2 <sup>b</sup>	COX-1/COX-2	
Celecoxib	14.7 ± 0.06	$0.05 \pm 0.0003$	294	
Rofecoxib	$14.5 \pm 0.06$	$0.03 \pm 0.0006$	483.3	
Indomethacine	$0.1 \pm 0.003$	$0.08 \pm 0.0003$	1.25	
Diclofenac sodium	$3.8 \pm 0.03$	$0.84 \pm 0.003$	4.5	
5a	$9.6 \pm 0.06$	$0.11 \pm 0.0003$	87.3	
5b	$12.2 \pm 0.06$	$0.07 \pm 0.0005$	187.7	
5c	$11.2 \pm 0.08$	$0.08 \pm 0.0003$	145.5	
5d	$10.6 \pm 0.08$	$0.09 \pm 0.001$	116.5	
5e	$8.3 \pm 0.06$	$0.12 \pm 0.003$	69.2	
5f	$9.7 \pm 0.06$	$0.11 \pm 0.003$	88.2	
5g	$10.5 \pm 0.08$	$0.10 \pm 0.0003$	109.4	
5h	$8.2 \pm 0.12$	$0.14 \pm 0.000$	58.6	
5i	$11.2 \pm 0.11$	$0.08 \pm 0.0008$	136.6	
5j	$12.2 \pm 0.06$	$0.05 \pm 0.0005$	239.2	
5k	$10.3 \pm 0.08$	$0.09 \pm 0.0003$	111.9	
51	$10.2 \pm 0.08$	$0.08 \pm 0.001$	134.2	
5m	$9.9 \pm 0.00$	$0.10 \pm 0.0008$	102.1	
5n	$10.6 \pm 0.08$	$0.07 \pm 0.0005$	153.6	
50	$12.9\pm0.05$	$0.06\pm0.0008$	211.5	

<sup>a,b</sup>Concentration of the compound that causes 50% inhibition of enzymatic activity of cyclooxygenase 1 and 2 (COX-1 and COX-2), respectively and all values are expressed as Mean  $\pm$  SEM of triplicate determinations. <sup>c</sup>COX-2 selectivity index: (COX-1 <sub>IC50</sub>/COX-2 <sub>IC50</sub>).

activity comparable to that of celecoxib but slightly lower than that of Diclofenac. Also, **5 b** and **5 n** were acceptable PGE2-lowering agents with % PGE2-inhibition of 65.70% and 55.18%, respectively. Finally, the results showed good relevance between selective COX-2 inhibitory activity and decreasing production of plasma PGE2 as one of the prime mediators released through COX-2 enzyme pathway.

#### 3.2.4. Gastric ulcerogenic activity

Inclusive monitoring of the isolated fasted-rat stomachs revealed a normal stomach texture for compounds **5 b**, **5j** and **5n** as well as the reference celecoxib whereas compound **50** expressed signs of gastric ulcers and hyperaemia as the reference Diclofenac. Furthermore, the degree of inflammatory reactions of the tested compounds in the gastric layers was confirmed by histopathological examination (Figure 4). The results disclosed the gastro-intestinal safety profile of compounds **5 b**, **5 j** and **5 n** as well as celecoxib. Nevertheless, the tested compound **50** exhibited erosion in the gastric layers as the reference Diclofenac.

#### 3.2.5. In vitro antibacterial screening

All the newly synthesised compounds were evaluated for their in vitro antibacterial activities against the human pathogens: Staphylococcus aureus (RCMB 0100183), Staphylococcus epidermidis (RCMB 0100183), Streptococcus mutans (RCMB 0100172), and Bacillus subtilis (RCMB 0100162) as examples of Gram-positive bacteria and Pseudomonas aeruginosa (RCMB 0100243), Escherichia coli (RCMB 010052), Salmonella typhi (RCMB 0100104), Shigella dysenteriae (RCMB 0100542) and Proteus vulgaris (RCMB 010085) as examples of Gram-negative bacteria using Ampicillin and levofloxacin as standard Gram-positive and Gram-negative antibacterial agents respectively[6] (Table 5, page S5 supplementary file) see experimental section. The results showed that, most of the tested compounds did not exhibit significant in vitro antibacterial activity, whereas compounds (5 b, 5i, 5j, 5k, 5m, 5n, and 5o) displayed weak in vitro antibacterial activity this could be assigned to the fact that the investigated compounds were azo co-drugs. Hence, metabolic biotransformation by azo-reductase enzyme into their active metabolites is a requirement for expressing their activities.

Table 2. Effects of the target compounds 5 b, 5j, 5n and 5o on carrageenan-induced paw edoema in mice (mm), their percentage anti-inflammatory activity and their ED<sub>50</sub> values (µmol/kg) (95% confidence level).

		Thickness of edoema (mm) <sup>b</sup>					
Compound NO.ª	0h	2h	4h	6h	8h	$ED_{50}$ ( $\mu mol$ /kg) (95% confidence level	
Control (carrageenan)	$0.69 \pm 0.004$	$0.95 \pm 0.02$	$1.44 \pm 0.03$	$2.05 \pm 0.03$	$2.03 \pm 0.01$		
Celecoxib	$\textbf{0.62}\pm\textbf{0.01}$	$0.89 \pm 0.01^{\circ}$	$0.79 \pm 0.01^{\circ}$	$0.73 \pm 0.03^{\circ}$	$0.67 \pm 0.02^{\circ}$	13.07 (10.65 – 15.93)	
Diclofenac	$0.69\pm0.004$	$1.06 \pm 0.03^{\circ}$	(+5.1470) $0.89 \pm 0.01^{\circ}$	(04.35%) $0.80 \pm 0.01^{\circ}$	$0.71 \pm 0.01^{\circ}$	11.46 (9.98 – 13.16)	
5 b	$0.68\pm0.01$	(-11.58%) $0.95 \pm 0.04$	(38.19%) 1.19±0.01	(60.98%) 1.11 ± 0.01	(69.13%) 1.01 ± 0.02	18.69 (16.41 – 21.10)	
5j	$0.67\pm0.02$	(0%) 0.80 ± 0.01 <sup>c</sup>	(20.83%) $0.70 \pm 0.01^{\circ}$	(45.85%) $0.60 \pm 0.01^{\circ}$	(56.09%) $0.53 \pm 0.01^{\circ}$	10.94 (9.48 – 12.65)	
5n	$0.66\pm0.004$	(15.79%) 1.03 ± 0.03	(51.39%) 1.29 ± 0.01	(70.73%) $0.99 \pm 0.05$	(88.50%) 0.90 ± 0.01	14.39 (11.69 – 17.53)	
50	$0.65\pm0.004$	(-8.42%) $0.89 \pm 0.01^{\circ}$ (6.32%)	(10.42%) $0.82 \pm 0.02^{\circ}$ (43.06%)	(51.71%) $0.75 \pm 0.02^{\circ}$ (63.41%)	(60.87%) 0.64 ± 0.02 <sup>c</sup> (72.17%)	13.27 (11.81 – 14.88)	

Data were analysed by one-way ANOVA followed by Tukey's Karmer post hoc test for multiple comparisons.

<sup>a</sup>Dose level for all compounds, po: 10 µmol/kg b.wt.

<sup>b</sup>Values are expressed as Mean  $\pm$  SEM (number of animals n = 5 mice).

<sup>c</sup>Means are significantly different from the control group (P < 0.05).

<sup>d</sup>Values between parentheses: (percentage anti-inflammatory activity (AI%).



Figure 2. Effects of the target compounds 5 b, 5j, 5n and 5o on the thickness of carrageenan-induced paw edoema in mice along interval of 0 - 8 h after injecting carrageenan (mm) together with the reference drugs celecoxib and Diclofenac in a dose of  $10 \mu$ mol/kg.



Figure 3. % inhibition of rat serum PGE2 production after 8 h of injecting  $10 \,\mu$ mol/kg b.wt of the tested compounds as well as the reference drugs celecoxib and Diclofenac.

#### 3.2.6. In vivo antibacterial screening in mice

Compounds 5 b, 5j, 5n and 5o as well as sulfasalazine as a reference drug were evaluated for their in vivo antibacterial efficacy against experimental bacteremic infections caused by S. aureus as gram-positive and E.coli as gram-negative bacteria in mice and their ED<sub>50</sub> values were determined, summarised and listed in Table 3. Concerning infection caused by S. aureus, all tested compounds improved the survival rate of model mice compared to positive control group which died. Compound 5j displayed the most potent activity with ED<sub>50</sub> value of 302.1 µmol/kg being slightly potent S.aureus inhibitor than sulfasalazine (313.7 µmol/ kg). Moreover, compounds 5 b, 5n and 5o showed good antibacterial activity with  $ED_{50}$  values ranging from 352.7 to 413.1  $\mu$ mol/ kg. By investigating the inhibitory activity against E.coli, all the tested compounds displayed antibacterial activity higher than sulfasalazine activity (ED50 =  $272.7 - 415.7 \,\mu$ mol/kg vs 419.2  $\mu$ mol/ kg). Compound **5** was the most potent one against E.coli ( $ED_{50} =$ 272.7 µmol/kg). Furthermore, compound 5b was found to have equal inhibitory effects against both S. aureus and E. coli ( $ED_{50} =$ 413.1 and 415.7 μmol/kg, respectively).

#### 3.3. Docking studies of the potential dual COX-2/DHPS inhibitors

We docked the target azo-dye prodrugs (**5 b**), (**5n**), (**5o**), and (**5j**) into COX-2 active site (pdb entry 3LN1)<sup>66</sup> which showed potential

anti-inflammatory in both the *in vitro* (Table 1) and *in vivo* (Table 2 and Figure 2) testing. We also docked the expected metabolite of **(5j)** into DHPS active site (pdb entry 3TZF)<sup>67</sup> after exposure to the azo-reductase<sup>68</sup>into the biological system which showed the most potential anti-bacterial activity in the *in vivo* testing (Table 3).

## 3.3.1. Docking of the compounds (5 b), (5n), (5o), and (5j) as COX-2 inhibitors:

The docking solutions of the compounds (5 b), (5n), (5o), and (5j) (Figure 5) verified the potential activities against COX-2 as antiinflammatory agents because the drug-receptor interactions of the best poses are very comparable to that of the co-crystallised ligand inhibitor, celecoxib (Figure 5). All the docked compounds interacted with the Leu 338, Arq499, and Ser 339 amino acids which are redundantly reported<sup>69</sup> as a part of the polar side pocket of COX-2 active site and interaction with it is required for the selective inhibition of the enzyme. On the other side, we docked compound (5f) as one of the compounds of the least potencies against COX-2 (Table 1) to realise the reasons stand behind such low potency. Docking solution of (5f) showed failure of the compound to relax freely into the active site in a way to interact with the polar side pocket amino acids except for Ser339. And, it lacks the carboxylic acid group which might have enabled it interact with Arg120 as an alternative path to inhibit the enzyme<sup>69</sup>.

### 3.3.2. Docking of compound (5j-metabolite) as a potential DHPS inhibitor:

Docking solution of **(5j**-metabolite) revealed that the compound interacted with the receptor active site with acceptable conformity to the co-crystallised ligand inhibitor, Sulfamethoxazole (Figure 6). The metabolite interacted with Pro64, and Lys221but failed to interact with Phe28 and Ser222 amino acids. It is worthy to mention that the metabolite failed to sit into the active site aligned with the sulfamethoxazole but preferred to invert on its horizontal axis in the opposite direction to relax freely. This might be a reason for the failure of the compound to interact with both Phe28, and Ser222 amino acids as shown in (Figure 6).



Figure 4. The ulcerogenic effect of the tested compounds 5 b, 5j, 5n and 5o as well as celecoxib and Diclofenac as reference drugs on gastric layers.

Table 3. The protective effects of promising compounds and the reference drug, sulfasalazine against bacteremic infections in mice  $ED_{50}$  values in  $\mu$ mol/kg/day (95% confidence limit).

organism	Challenge dose(CFU/mouse)	drug	ED <sub>50</sub> (µmol/kg/day)	95% confidence limit
Staphylococcus aureus	9.5x10 <sup>6</sup>	Sulfasalazine	313.7	(84.62 – 523.8)
		5b	413.1	(318.5 – 511.4)
		5j	302.1	(214.7 – 373.3)
		5n	397.6	(78.40 – 583.3)
		50	352.7	(315.4 – 398.1)
Escherichia coli	7.1x10 <sup>3</sup>	sulfasalazine	419.2	(194.5 – 690.8)
		5b	415.7	(275.6 – 534.5)
		5j	272.7	(157.1 – 386.2)
		5n	367.1	(166.9 – 565.7)
		50	306.4	(160.0 – 445.5)

# 3.4. In silico prediction of the physicochemical properties, drug likeness score, pharmacokinetics, toxicity profile and ligand efficiency metrics

Early prediction of the physicochemical and pharmacokinetic properties of new drug candidates is an infrastructure for lead optimisation as well as drug development process<sup>70</sup>. Accordingly, the *in silico* physicochemical characters, drug likeness, pharmacokinetic parameters, toxicity profile and ligand efficiency metrics of the most active compounds **5 b**, **5 j**, **5 n** and **5 o** were predicted by Molinspiration<sup>71</sup>. Pre-ADMET<sup>72</sup> and Osiris property explorer<sup>73</sup> online soft wares (Table 6, pages S7 and 8 supplementary file).

The calculated values predicted that all the tested compounds comply with Lipinski's rule of 5 and satisfied Veber's criteria. Moreover, the results predicted good oral bioavailability and an acceptable molecular flexibility. Additionally, their percentages of absorption (%ABS; calculated as %ABS =  $109-0.345 \times TPSA$ )<sup>74</sup>, as well as their solubility satisfied the solubility requirement (>0.0001 mg/L)<sup>75,76</sup>. Furthermore, all the compounds expected to have low cell permeability in both Caco-2 and MDCK cell models but with low CNS penetration. Meanwhile, all of them were predicted to be excellently absorbed and strongly bound to plasma proteins (>90%)<sup>76,77</sup>. In another context, the values of LE and LLE for the most active compounds regarding their COX-2 inhibitory activity were calculated by the reported equations<sup>78</sup>, then compared to celecoxib as a reference drug. The results expected the expediency of these compounds as lead-like candidates (for more details see pages S6, 7 and 8 supplementary file).

#### 4. Conclusion

Seeking for damping bacterial resistance together with broadening spectrum of the biological activities there by dominating inflammatory bacterial infections, our study employed pharmacophoric hybridisation strategy by combining the antibacterial features of sulphonamides with the anti-inflammatory and antibacterial features of salicylamides through azo-linkage to design a novel series of biodegradable compounds that may be powerful tools to combat resistant bacteria as well as their consecutive inflammatory diseases. The newly synthesised compounds were challenged in vitro for their expected antibacterial and COX-inhibitory activities. Compounds 5b, 5j, 5n and 5o had been identified as the most potent COX-2 inhibitors among the series with IC<sub>50</sub> values ranging from 0.05  $\mu$ m to 0.07  $\mu$ m and SI values from 153.6 to 239.2. Moreover, all compounds displayed moderate in vitro antibacterial activities with MIC values ranging from  $25 - 200 \,\mu$ g/ml which may be attributed to their prodrug nature and they become active only inside the biological system after being exposed to azo reductase enzyme. Consequently, the most active COX-2 inhibitors 5b, 5i, 5n and 50 were evaluated for their in vivo anti-inflammatory and antibacterial activities. Surprisingly, compounds 5j and 5o were found to be the most potent inhibitors of carrageenan induced paw oedema in mice with % inhibition 88.50% and 72.17%, respectively and  $ED_{50}$  values of 10.94  $\mu$ mol/kg and 13.27  $\mu$ mol/ kg, respectively. In addition, investigating rat serum concentration of PGE2 revealed that all of the four compounds are

Tyr 334



5n (C)

50 (D)





5j (E)

5f (F)



Figure 5. 2 D-style of docking solutions of the potential COX-2 inhibitors, 5 b; 5n; 5o; 5j, and Celecoxib as the co-crystallised ligand inhibitor in the active site of COX-2 (PBD code 3LN1). Besides, the docking solution of compound (5f) as the least potent inhibitor to visualise its distinct binding pattern with the same active site. Amino acid residues presented by the protein sequence code of three letters and numbers; interaction forces presented by green dotted lines which are categorised according to the description scheme associated with the figure.

potent PGE2 inhibitors with % inhibition in the range of 55.18% - 83.25% which confirmed their COX-2 inhibitory properties. Moreover, *in vivo* antibacterial activity against *S.aureus* 

and E.coli infections confirmed their potent antibacterial action with  $ED_{50}$  values in the range of  $302.1 - 413.1 \,\mu$ mol/kg against S.aureus and  $272.7 - 419.2 \,\mu$ mol/kg against E.coli. Furthermore,



Figure 6. In the left side: 2D-style of the docking solutions of the co-crystallised ligand inhibitor, sulfamethoxazole, and 5j-metabolite in the active site of DHPS (PBD code 3TZF) to visualise the interaction forces with the active site. In the right side: there are the corresponding 3D-style docking solutions of sulfamethoxazole, and 5j-metabolite to visualise the orientation of the docked compound in the active site. Amino acid residues presented by the protein sequence code of three letters and numbers, atoms assigned by colours, (blue nitrogen; red oxygen; yellow sulphur; grey carbon). Interaction forces in the 2D-style are categorised according to the description scheme associated with the figure while in the 3D-style, it appeared as brown dotted lines for the hydrogen bonding and green dotted lines for the hydrophobic interactions.

docking studies of the same four compounds into COX-2 active site emphasised their anti-inflammatory potential in addition to being harmonised to a great extent with their  $IC_{50}$  values and selectivity indices obtained from the *in vitro* assay. Besides, docking of **5j** metabolite into DHPS active site illustrated the inhibitory activity of the new candidates against bacteria. Finally, *in silico* prediction of the pharmacokinetic properties and toxicity profile as well as ligand efficiency metrics furnished extra support for the lead-like character of the target compounds. Compound **5j** achieved the target goal as potent *in vivo* dual COX-2/DHPS inhibitor. In general, the antibacterial characters together with anti-inflammatory properties of the newly synthesised compounds confirmed their ability for further optimisation process as potential antibacterial agents.

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