Synthesis and characterization of 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide derivatives as PDE-4 inhibitors for the treatment of anti-inflammatory, analgesic and antimicrobial activities

Vikash Srivastava, Prof. Surya Pal Singh

Abstract— we reveal a series of novel 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide derivatives as PDE4 inhibitor for the treatment of inflammatory, analgesic and antimicrobial diseases. All compounds were evaluated for their anti-inflammatory, analgesic (compared to the reference drug Indomethacin) and antimicrobial activities (compared to the reference drug Ampicillin and Fluconazole). Compounds 5e, 5f, and 5g were found to be the more active anti-inflammatory drugs revealing potency ranging from 1 - 1.01 compared to the reference drug indomethacin. In accumulation of docking study of these highly active 3 compounds against the active site of cyclooxygenase-2 enzyme (COX-2), among the established compounds. Compounds 5e, 5f, and 5g showed multiple activities; anti-inflammatory, analgesic and anti-bacterial activities.

Keywords: Anti-inflammatory, analgesic, anti-bacterial, 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide, PDE4 inhibitor

I. INTRODUCTION

PDE-4 inhibitors for the treatment of anti-inflammatory, analgesic and antimicrobial activities. The PDE4 family of enzymes are the utmost regular PDE in immune cell. They are principally in control for hydrolysing cAMP within both immune cells and cells in the central nervous system.

Fig-1: Phosphodiesterase-4 (PDE4)

Phosphodiesterase 4 (PDE4) an appropriate group of enzymes that catalyze the itemization of 3.50’-cAMP (cAMP) in numerous types of cells, comprising inflammatory cells, and is considered an essential player of the inflammatory cascade. Dermatologic / Rheumatologic is an accepted Apremilast for the treatment of inflammatory conditions, and shows efficacy in a wide range of immune-mediated inflammatory diseases. Prototype PDE4 inhibitors can have rolipram (including long-term memory-enhancing), neuroprotective and anti-inflammatory effects. As a consequence, PDE4 inhibitors have been investigated for the treatment of a variety of disorders, including clinical depression, anxiety disorders, schizophrenia, Parkinson’s disease, Alzheimer’s disease, multiple sclerosis, attention deficit- and hyperactivity such as central nervous system. Inflammatory conditions such Huntington’s disease, stroke, autism and chronic obstructive pulmonary disease (COPD), asthma, and rheumatoid arthritis.

PDE4D inhibition, along with PDE4A inhibition also appears to be responsible for the antidepressant effects of PDE4 inhibitors.

Similarly PDE4B inhibition appears to be required for the antipsychotic effects of PDE4 inhibitors, in line with this view PDE4B polymorphisms and altered gene expression in the central nervous system have been associated with schizophrenia and bipolar disorder in a postmortem study. PDE4 also regulates the D3/PKA/DARPP-32 signalling cascade in the frontal cortex, which may contribute to the antipsychotic and precognitive effects of PDE4 inhibitors. While PDE4 is articulated mainly in the m fronto cortex, a few different lines of evidence suggests the therapeutic utility.
Immune responses and acute inflammation in pulmonary airways are closely related to the onset and progression of COPD. Infiltration of natural cells into the native lung tissue is considered an important pathogen in COPD patients. Asthma is another inflammatory airway disease that does not cure, bronchial hyperactivity, mucus production, airway defect and remodelling, and inflammation of inflammatory cells, especially neutrophils. Certainly, COPD and asthma patients share similar clinical phenotypes, and asthma COPD is difficult to differentiate, especially in elderly patients when they live together. Over the past years, PDE4 inhibitors have strongly attracted the benefit of pharmacists in treating COPD and asthma. PDE4 inhibition conquers excessive airway inflammation and relaxes smooth muscle by improving CMP levels. Roughly roflumilast has been investigated as a patented anti-inflammatory to control airway inflammation. In vitro, roflumilast inhibits PDE4 activity (IC50 = 0.8 nm) with high selectivity from human neutrophils, thereby activating FMLP-induced leukotriene B4 (LTB4) and reactive oxygen species in human neutrophils and lipopolysaccharides, showed anti-inflammatory potential. LPS) TNF-α synthesis in monocytes, dentin cells and cytokines produced in anti-CD3 / CD28-stimulated CD4 + 13T cells. In addition, roflumilast significantly suppresses the production of inflammatory mediators in macrophages by stimulating the expression of hep oxygenase (HO-1) and inhibition of NF-κ, MAPK and JNKK activation. Studies show that mitophagy mediates pulmonary epithelial cell death. Roflumilast protected against CSE-induced cell death in Boff-2b cells, showing good performance in COPD treatment. In vivo potency of in airway inflammatory models, roflumilast has been shown to have bronchodilatory activities in spaw-challenged mice and guinea pigs. The dependent force of airway growth by eosinophilic inflammation developed by ovalbumin (OVA).

II. Results and discussion:

Synthesis was commenced with the available commercial material diethyl 2-(ethoxymethylene) malonate(1) was first treated with hydrazine hydrate in methanol by using freshly prepared sodium methoxide at reflux condition provided Ethyl 3-oxo-2, 3-dihydro-1H-pyrazole-4-carboxylic acid (2) cyclized compound. This compound was confirmed by 1H NMR, at δ 9.22 showed the

Table 1: PDE family, substrate and tissue distribution, inhibitors type and their clinical applications as shown in below:

<table>
<thead>
<tr>
<th>PDE family</th>
<th>Substrate</th>
<th>Tissue distribution</th>
<th>Inhibitors</th>
<th>Clinical applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE1</td>
<td>cAMP</td>
<td>Brain, heart, muscles</td>
<td>PDE4-selective inhibitors</td>
<td>Treatment of inflammatory conditions</td>
</tr>
<tr>
<td>PDE2</td>
<td>cAMP</td>
<td>Brain, heart, muscles</td>
<td>PDE4-selective inhibitors</td>
<td>Treatment of inflammatory conditions</td>
</tr>
<tr>
<td>PDE3</td>
<td>cAMP</td>
<td>Brain, heart, muscles</td>
<td>PDE4-selective inhibitors</td>
<td>Treatment of inflammatory conditions</td>
</tr>
<tr>
<td>PDE4</td>
<td>cAMP</td>
<td>Brain, heart, muscles</td>
<td>PDE4-selective inhibitors</td>
<td>Treatment of inflammatory conditions</td>
</tr>
</tbody>
</table>

Fig 3: Effective therapeutic strategy for inflammatory conditions

Targeted inflammatory conditions include asthma, chronic obstructive pulmonary disease (COPD), psoriasis, atopic dermatitis (AD), inflammatory bowel disease (IBD, rheumatoid arthritis (RA), lupus, and neuroinflammation). With great efforts, roflumilast, apremilast and crisaborole have been approved respectively for the treatment of inflammatory airways or skin diseases. In addition, a novel PDE4 2 inhibitor in controlling inflammation has also been developed and has a satisfactory therapeutic potential. C technology and clinical descriptions of licensed PDE4 inhibitors or in the process. Involved in the inhibition of PDE4 in unpredictable tissues, nausea, emotion, astrological effects, and other adverse effects greatly inhibit clinical application.
characteristic broad singlet peak of pyrazole and at 4.22 quartet peak of CH$_3$- and triplet peak of CH$_2$-F. Furthermore, synthesis of intermediate (A) was two-step process; In first step, reduction of diethyl 2,2-dimethylmalonate was performed on treatment with Lithium aluminium hydride at 0 °C and this reaction mixture was quenched with Fisher work up method, gave reduced product 2, 2-dimethylpropane-1, 3-diol (7). At second step; protection of alcohol was achieved by using tosyl chloride in presence of pyridine exhibited potent acid are the aryl imino function as in compounds (Indocin®) showing inhibition potency ranging from 0.68% to 1.10% for the reference drug Indomethacin. It is worth mentioning that, the highly potent compounds were those comprising 3-fluoro-pyridyl-2-yl amine rings attached to different side of the acid are the aryl imino function as in compounds 5f-g, heteroaryl group attached, Among the moderate potent Methyl, hydroxy and chloro function with pyridine exhibited potent activity comparable to the reference drug Indomethacin (Indocin®) (83-49).

As revealed from the results presented in Tables 1-3 that, compounds 5c, 5f and 5g exhibited the most potent analytic activity with potency ranging from 1 - 1.10 to the reference drug Indomethacin. It is to be noted that some functions are assumed to be exerted comparable to the reference drug Indomethacin (Indocin®) (83-49). For the tested compounds 5a-5j, the resulting inhibition zones were measured in mm diameter, Among the tested compounds, compounds 5a, 5c, 5d, 5e, 5f and 5g were found to be the most active. Inhibition zones (IZ) in mm diameter for compounds of scheme 1 compound (5a-j).

Table 1. Anti-inflammatory and analgesic results for compounds of Scheme 1 compound (5a-j).

<table>
<thead>
<tr>
<th>Comp No</th>
<th>structure</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>6h</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contr a</td>
<td></td>
<td>0.23 ± 0.03</td>
<td>0.26 ± 0.05</td>
<td>0.45 ± 0.01</td>
<td>0.54 ± 0.08</td>
<td>0.63 ± 0.04</td>
<td>0.78 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Induced a</td>
<td></td>
<td>0.22 ± 0.03</td>
<td>0.14 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>0.23 ± 0.04</td>
<td>0.22 ± 0.08</td>
<td>0.85 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td></td>
<td>0.27 ± 0.02</td>
<td>0.43 ± 0.05</td>
<td>0.23 ± 0.08</td>
<td>0.36 ± 0.05</td>
<td>0.22 ± 0.02</td>
<td>0.67 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>5b</td>
<td></td>
<td>0.22 ± 0.02</td>
<td>0.21 ± 0.04</td>
<td>0.25 ± 0.01</td>
<td>0.25 ± 0.05</td>
<td>0.13 ± 0.05</td>
<td>0.89 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>5c</td>
<td></td>
<td>0.23 ± 0.04</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.47 ± 0.07</td>
<td>0.21 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>5d</td>
<td></td>
<td>0.21 ± 0.02</td>
<td>0.21 ± 0.04</td>
<td>0.23 ± 0.06</td>
<td>0.23 ± 0.03</td>
<td>0.23 ± 0.02</td>
<td>0.69 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>5e</td>
<td></td>
<td>0.19 ± 0.03</td>
<td>0.34 ± 0.04</td>
<td>0.25 ± 0.04</td>
<td>0.25 ± 0.05</td>
<td>0.15 ± 0.06</td>
<td>1.04 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>5f</td>
<td></td>
<td>0.36 ± 0.04</td>
<td>0.43 ± 0.04</td>
<td>0.21 ± 0.02</td>
<td>0.22 ± 0.06</td>
<td>0.42 ± 0.04</td>
<td>1.04 ± 0.10</td>
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<tr>
<td>5g</td>
<td></td>
<td>0.24 ± 0.03</td>
<td>0.33 ± 0.03</td>
<td>0.40 ± 0.02</td>
<td>0.32 ± 0.08</td>
<td>0.76 ± 0.06</td>
<td>0.63 ± 0.20</td>
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<tr>
<td>5h</td>
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<td>0.22 ± 0.07</td>
<td>0.17 ± 0.03</td>
<td>0.46 ± 0.03</td>
<td>0.34 ± 0.04</td>
<td>0.23 ± 0.02</td>
<td>0.63 ± 0.33</td>
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<tr>
<td>5i</td>
<td></td>
<td>0.19 ± 0.06</td>
<td>0.14 ± 0.03</td>
<td>0.37 ± 0.01</td>
<td>0.41 ± 0.07</td>
<td>0.23 ± 0.06</td>
<td>0.69 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>5j</td>
<td></td>
<td>0.42 ± 0.01</td>
<td>0.24 ± 0.02</td>
<td>0.41 ± 0.01</td>
<td>0.33 ± 0.03</td>
<td>0.23 ± 0.02</td>
<td>0.97 ± 0.24</td>
<td></td>
</tr>
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</table>

a, b: Significantly different from control value and reference value at P < 0.05. S.D. = Standard deviation.

The results revealed that, compounds 5e, 5f, and 5g exerted highly potent anti-inflammatory effect, comparable to that of indomethacin (Indocin®) at 6 h interval post carrageenan showing inhibition potency ranging from 1.01% - 1.05%. While, compounds 5b, 7b, 5d, 5i, and 5j exerted moderate anti-inflammatory activity at 6h interval post carrageenan, comparable with that of indomethacin (Indocin®) showing inhibition potency ranging from 0.68% - 1%. In addition to, compounds 5h and 5a, which showed weak anti-inflammatory activity at 6 h interval less than indomethacin showing inhibition potency ranging from 0.36% - 0.67%. It is worth mentioning that, the highly potent compounds were those comprising 3-fluoro-pyridyl-2-yl amine rings attached to different side of the acid are the aryl imino function as in compounds 5f-g, heteroaryl group attached, Among the moderate potent Methyl, hydroxy and chloro function with pyridine exhibited potent activity comparable to the reference drug Indomethacin (Indocin®) (83-49).
Table 2. For the tested compounds 5a-5j, the resulting inhibition zones were measured in mm diameter.

<table>
<thead>
<tr>
<th>Compound no</th>
<th>S. aureus</th>
<th>E. Coli</th>
<th>Ps. aeruginosa</th>
<th>C. albicans</th>
</tr>
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<tbody>
<tr>
<td>5a</td>
<td>12</td>
<td>9</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>5b</td>
<td>11</td>
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<td>15</td>
<td>17</td>
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<td>5c</td>
<td>9</td>
<td>28</td>
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<td>5d</td>
<td>15</td>
<td>18</td>
<td>29</td>
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<tr>
<td>5e</td>
<td>11</td>
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<td>32</td>
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<td>5f</td>
<td>9</td>
<td>12</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td>5g</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td>9</td>
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<tr>
<td>5h</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>15</td>
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<tr>
<td>5i</td>
<td>10</td>
<td>19</td>
<td>30</td>
<td>10</td>
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<tr>
<td>5j</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Amphicillin</td>
<td></td>
<td>22</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td></td>
<td>-</td>
<td>32</td>
</tr>
</tbody>
</table>

Apart for these microbial activity individual activity as follows Compound 5a; 4-bromo-3 methyl aniline substituted carboxamide showed highly active against Ps. Aeruginosa bacteria but slightly poor active against S. aureus and E. Coli as compared to Ampicillin and also poor active against C.albicans bacteria as compared to standard Fluconazole compound 5b; 3-hydroxyprpyridin-2-ylamine carboxamide derivative and compound 5j; 2-chloropyridin-4-ylamine highly active against C.albicans as compared to the standard Fluconazole.[60]. Compound 5e; 5-bromopyrazin-2-ylamine is highly or better active against E.coli related to standard Ampicillin. Compound 5d; 3-hydroxy-6-methylpyridin-2-ylamine, Compound 5e; 5-fluoropyridin-2-ylamine Compound 5f; benzo[d]thiazol-6-yl; Compound 5g; 3-methylishotiazol-5-ylamine; Compound 5i 4, 6-dimethylpyridin-2-ylmine highly active against Ps. Aeruginosa as compared with ampicillin and 5d found good activity against C.albicans bacteria as compared to standard Fluconazole but 5e poor active. But compound 5b; 3-hydroxyprpyridin-2-ylamine and compound 5h; 4-bromo-3-methylphenyl amine are very poor active against all bacterial stain with compared to standard.[61].

Computer Aided Docking, the most active twenty compounds as anti-inflammatory agents 5e, 5i, and 5g were subjected to docking using Molecular Operating Environment (MOE) program on the 3D structure of the cyclooxygenase-2 enzyme (COX-2) in a trial to predict their mode of action as anti-inflammatory drug.[196].

(COX-2) Docking on the Active Site of Cyclo-oxygenase-2 Enzyme:
Diclofenac interacted as hydrogen bond acceptor via four hydrogen bonds via both the oxygen atoms of carbonyl group with the amino acid residues Tyr 385 (2.73 Å) and Ser 530 (2.65 Å, 2.91 Å and 3.04 Å) as shown in Figure 1.

Docking of Compound 5e into COX-2;Active site revealed that several molecular interactions were considered to be responsible for the observed affinity, as the N of pyridine moiety acted as a hydrogen bond acceptor with the side chain residue; His 90 (2.25 Å) with a strength of 81.3%. In addition to a hydrogen bond interaction between the hydrogens of the amino group which acted as a hydrogen bond donor with the side chain residue Tyr 355 (2.61 Å) with a strength of 5.3%. Besides to, hydrophobic interactions involving the following amino acid residues; His 90, Met 113, Val 116, Leu 117, Arg 120, Val 349, Leu 352, Ser 353, Tyr 355, Leu 359, Leu 384, Tyr 385, Trp 387, Phe 518, Met 522, Val 523, Gly 526, Ala 527, Ser 530 and Leu 531.

Docking of Compound 5f into COX-2;Active site illustrated the presence of several interactions of the thiazole group with different amino acid residues as it acted as a hydrogen bond acceptor with the side chain residues; His 90, Tyr 355 and Arg 513 (3.5 Å, 2.43 Å and 3.16 Å; respectively) at a strength of 2.1%, 90.6% and 13.4%; respectively. This beside hydrophobic interactions among the thiazole moiety and the following amino acid residues; His 90, Val 116, Leu 117, Arg 120, Glu 192, Val 349, Leu 352, Ser 353, Tyr 355, Leu 359, Tyr 385, Trp 387, Arg 513, Ala 516, Ile 517, Phe 518, Val 523, Gly 526, Ala 527, Ser 530 and Leu 531.

Docking of Compound 5g into COX-2; Active site revealed the presence of four hydrogen bonds and iso(thiazole interactions. In which the amino group acted as a hydrogen acceptor via three hydrogen bonds with the amino acid residues His 90, Tyr 355 and Arg 513 (2.25 Å, 3.32 Å and 3.43 Å; respectively) with a strength of 3.5%, 9.1% and 43.2%; respectively. While nitrogen atom acted as a hydrogen bond acceptor with the amino acid residue His 90 (3.41 Å) with strength of 2.2%. and the following amino acid residues: Pro 86, Val 89, His 90, Arg 120, Val 349, Leu 352, Tyr 355, Arg 513, Ala 516, Phe 518, Val 523, Glu 524, Gly 526, Ala 527 and Ser 530.

III. Experimental Procedure:
Material and Methods: The melting points of compounds were determined by open tube capillary method using Digital Melting Point apparatus (model-B-APC-3), in Celsius scale and uncorrected. Purity of the compound was verified by pre-coated TLC plates (E-Merk Kieselgel 60 F254). 1H NMR, 13C NMR spectra are recorded on Varian 400 MHz spectrometer using DMSO-d6 as
solvent and tetra-methylsilane (TMS) as internal standard. Mass spectra are recorded on Agilent triple quadruple mass spectrometer equipped with turboion spray interface at 375 C. All the organic extracts are dried over sodium sulfate after work up. Unless or else mentioned all the solvents and reagents used are of commercial grade.

**Ethyl 3-oxo-2, 3-dihydro-1H-pyrazole-4-carboxylate**

To a stirred solution of diethyl 2-(ethoxymethylene) malonate (5.0g, 23.12mmol) in absolute ethanol (32 mL) was added sodium methoxide (2eq) and followed by addition of hydrazine hydrate (1.63g, 23.12 mmol) at 0 °C. The reaction mixture was refluxed for 2h then concentrated under reduced pressure. The residue was diluted with water and acified up to pH=2 by concentrated Hydrochloric acid and extracted with EtOAc. Combined organic extract were washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Residue was triturated with 10% EtOAc in hexane to afford as Ethyl 3-oxo-2, 3-dihydro-1H-pyrazole-4-carboxylate (3.2g, 88.9%).

**Chemical Formula:** C₇H₉NO₃

**Elemental Analysis calc:** C, 55.09; H, 6.16; N, 14.28; O, 24.46.

**Elemental Analysis found:** C, 55.16; H, 6.17; N, 14.32; O, 24.32.

**H NMR (DMSO-d₆, 400 MHz) δ 7.93s (1H), 7.42-7.45 (m) 5H (4H), 4.26-4.29 (m) 2H, 3.80s(2H), 3.59s(2H), 2.130(m, 3H), 0.94s(6H)

**13C NMR (DMSO-d₆, 400 MHz) δ 141.1, 121.1, 29.6, 60.2, 66.8, 87.0, 96.0, 136.6, 154.2, 162.4.

**HPLC purity:** 99.32% (λ =220 nm)

**Chemical Formula:** C₁₂H₁₁NO₄

**Elemental Analysis calc:** C, 58.91; H: 7.19; N: 12.49; O: 21.40.

**Elemental Analysis found:** C, 58.19; H: 7.49; N: 12.39; O: 21.20.

**H NMR (DMSO-d₆, 400 MHz) δ 7.93s (1H), 7.42-7.45 (m) 5H (4H), 4.26-4.29 (m) 2H, 3.80s(2H), 3.59s(2H), 2.130(m, 3H), 0.94s(6H)

**13C NMR (DMSO-d₆, 400 MHz) δ 141.1, 121.1, 29.6, 60.2, 66.8, 87.0, 96.0, 136.6, 154.2, 162.4.

**HPLC purity:** 99.32% (λ =220 nm)

**Chemical Formula:** C₁₀H₉NO₃

**Elemental Analysis calc:** C, 56.66; H, 11.56; O, 30.82.

**Elemental Analysis found:** C, 56.62; H, 11.54; O, 30.82.

**H NMR (DMSO-d₆, 400 MHz) δ 4.42(brs, 2H), 3.39(m, J = 8.2 Hz, 4H). 0.89s(6H)

**GC-MS (m/z): 104.0**

**2, 2-dimethylpropan-1, 3-diol bis (4-methylbenzenesulfonyl):** To a stirred solution of compound 2, 2-dimethylpropan-1, 3-diol (5.0g, 44.5 mmol) in dichloromethane (50mL) was added triethylamine (18.29 mL, 124.9 mmol) followed by addition of tosyl chloride (18.6g, 98.16 mmol) in portion wise. The reaction mixture was allowed to cooled, water was added and extracted with EtOAc. Combined organic extract were washed with water and neutralized with IN HCl and solid was precipitated and filtered through glass sintered and dried well to afford as white solid compound 2, 2-dimethylpropan-1, 3-diol bis (4-methylbenzenesulfonyl)(10.2, 54.4%).

**Chemical Formula:** C₁₉H₂₄O₄S₂

**Elemental Analysis calc:** C, 55.32; H, 5.86; O, 23.77; S, 15.54.

**Elemental Analysis found:** C, 55.32; H, 5.86; O, 23.77; S, 15.54.

**H NMR (DMSO-d₆, 400 MHz) δ 7.75-7.78 (m) 4H, 7.42-7.47 (m) 4H, 3.39(t, J = 8.2Hz, 4H), 2.43(s, 6H), 0.89(s, 6H)

**GC-MS (m/z): 412.**

**Ethyl 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide:**

To a stirred solution of compound Ethyl 3-oxo-2, 3-dihydro-1H-pyrazole-4-carboxylate (2.0 g, 12.8mmol) in N, N-dimethylformamide (20mL) was added potassium carbonate (4.4g, 32.0 mmol) and stirred for 15 min then added 2, 2-dimethylpropan-1, 3-diol bis (4-methylbenzenesulfonyl)(5.4g, 12.8 mmol). The reaction mixture was heated at 100 °C for 12h. Reaction mixture was allow to cooled, water was added and extracted with EtOAc. Combined organic extract were washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (100-200 mesh size silica gel and 20-30% EtOAc in hexane as an eluent) to afford as yellow solid compound Ethyl 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide (1.9g, 63.97%).
Synthesis and characterization of 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide derivatives as PDE-4 inhibitors for the treatment of anti-inflammatory, analgesic and antimicrobial activities

5f) N-(benzo[d]thiazol-6-yl)-6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide as white solid; Yield=65.24%.

5g) 6, 6-dimethyl-N-(3-methylisothiazol-5-yl)-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide as off white solid; Yield=26.24%.

5h) N-(4-bromo-3-methylphenyl)-6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide as yellow solid; Yield=40.26%.

Chemical Formula: C\textsubscript{14}H\textsubscript{14}BrN\textsubscript{3}O\textsubscript{2}

Elemental Analysis calc.: C, 52.76; H, 4.98; Br, 21.94; N, 11.54; O, 8.78; found: C, 52.71; H, 4.96; Br, 21.27; N, 11.91; O, 11.54.

HPLC purity: 97.56% (λ = 220 nm)

1H NMR (DMSO-d\textsubscript{6}, 400 MHz) δ 8.10(brs, 1H), 7.93(s, 1H), 7.26(s, 1H), 3.80(s, 2H), 3.56(s, 2H), 2.46(s, 3H), 0.94(s, 6H).

13C NMR (DMSO-d\textsubscript{6}, 400 MHz) δ 121.2, 23.8, 29.3, 63.4, 83.4, 111.2, 111.8, 116.2, 126.3, 139.6, 147.3, 148.2, 154.4, 164.7.

MS (ESI+) for m/z=293

5i) N-(4-(5H-dihydro-1H-oxazolo[5,1-b][1,3]oxazine-3-carboxamide-7-yl)pyridin-2-yl)-6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide as off white solid; Yield=29.6%.

Chemical Formula: C\textsubscript{19}H\textsubscript{17}BrN\textsubscript{3}O\textsubscript{2}

Elemental Analysis calc.: C, 57.92; H, 5.21; F, 6.54; N, 19.30; O, 11.02; Elemental Analysis found: C, 51.82; H, 4.62; F, 19.54; N, 17.81; O, 6.60.

HPLC purity: 99.67% (λ = 220 nm)
The synthesized new compounds were evaluated for their anti-inflammatory, analgesic (associated to the reference drug Indomethacin) and antimicrobial activities (associated to the reference drug Ampicillin and Fluconazole). Compounds 5e, 5f and 5g were found to be the more active anti-inflammatory drugs revealing potency ranging from 1 - 101 compared to the reference drug indomethacin. In accumulation of docking study of these highly active ten compounds against the active site of cyclooxygenase-2 enzyme (COX-2), among the established compounds, compounds 5e, 5f, and 5g showed multiple activities; anti-inflammatory, analgesic and anti-bacterial activities.

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VI. REFERENCES


First Author Vikash Srivastava, M.Sc (from BHU Varanasi), PhD, publication in BMCL, Journal of heterocyclic chemistry, IISR, etc.

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