ONE POT SYNTHESIS AND IN-VITRO ANTIBACTERIAL, ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF β-LACTAM HETEROCYCLES.

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ABSTRACT
We report here in the new and unreported yet the one pot synthesis by use of ethyl chloroformate for the formation of an active anhydride intermediate, is a stable compound during reaction, readily accessible, commercially available and inexpensive. The crucial step of the present approach was the reaction between a carboxylic acid and ethyl chloroformate, which gives entirely new products, which on in-situ condensation with C-3 substituted 7-ACAs gives new β-lactams with quality and yield. (Scheme I) The synthesized compounds 2.2 (A-E) were screened for their antimicrobial activity against four microorganisms: Escherichia coli (Gram negative), Staphylococcus typhi (Gram negative), Staphylococcus aureus (Gram positive) Bacillus megaterium (Gram positive), and they were found to exhibit good to moderate antimicrobial activity. The antifungal activity of these compounds was also tested against three different fungal species. None of them were active against the species tested. Novel 3-(acetoxymethyl-7-(2-(7-methyl-2-p-tolylimidazo[1,2-a]pyridine-3yl)acetamide-8-oxo-5thia-1-azabicyclo[4.2.0]oct-2-ene-2-carbaylic acid 2.2 (A-E) were subjected for pharmacological screening to evaluate analgesic and anti-inflammatory activity by using acetic acid writhing and Carrageenan Footpad Edema method respectively in mouse as an animal model. Novel 3-(acetoxyxymethyl-7-(2-(7-methyl-2-p-tolylimidazo [1, 2-a] pyridine-3yl)acetamide-8-oxo-5thia-1-azabicyclo [4.2.0] oct-2-ene-2-carbaylic acid 2.2 (A-E) were synthesized and screened for antibacterial, analgesic and anti-inflammatory activity.

Keywords: 6-Methyl-2-(4-methylphenyl) imidazol [1, 2-a]-pyridine-3-acetic acid, β-lactams, antimicrobial, analgesic, anti-inflammatory activity.

INTRODUCTION
The β-lactam heterocycles are still the most prescribed antibiotics used in medicine. They are considered as an important contribution of science to humanity. The most widely used antibiotics such as the penicillin, cephalosporin, carumonam, aztreonam, thienamycine and the nocardicins all contain β-lactam rings. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing β-lactams ring. Such biological activities include antimicrobial, anti-tubercular, carbonic anhydrase inhibitors, local anaesthetics, anti-inflammatory, anticonvulsant and hypoglycemic agents activity.
6-Methyl-2-(4-methylphenyl)imidazol[1,2-a]-pyridine-3-acetic acid derived from a 2 amino pyridine constitute another class of heterocyclic that possess antimicrobial and various other pharmacological activities like diuretic, antiulcer, antihistamine and anticancer properties.

To the best of our knowledge, no report has been cited in the literature on the coupling of 6-Methyl-2-(4-methylphenyl)imidazol[1,2-a]-pyridine-3-acetic acid and β-lactams heterocycles. Hence, with a view to assess the pharmacological profile of this class of compounds, here we plan to synthesize some new congeners of β-lactam heterocycles by incorporating the 6-Methyl-2-(4-methylphenyl)imidazol[1,2-a]-pyridine-3-acetic moieties in a single molecular framework. The present work deals with the synthesis of the title compounds starting from 6-Methyl-2-(4-methylphenyl) imidazol [1, 2-a]-pyridine-3-acetic acid β- lactams heterocycles.

**EXPERIMENTAL**

**Synthesis of compounds**

Melting points were taken on a precision melting point apparatus (DBK) instrument and are uncorrected. IR spectra were obtained in potassium bromide (KBr) disks on a Bruker IR spectrometer, and 1H NMR spectra were obtained on deuteriochloroform (CDCL3) and or DMSO-d6 solution on a Varian 400 MHz spectrometer. Mass spectra were recorded on a MicroMass spectrometer by Waters. The yields unless otherwise mentioned are for pure product. All the raw materials, reagents and solvents used were of commercial grade only.

**Synthesis of the (ethyl carbonic) 2-(7-Methyl-2- p-tolyimidazo [1, 2-a] pyridin-3-yl) acetic anhydride (II)**

**General procedure:**

Added 6-Methyl-2-(4-methylphenyl) imidazol [1, 2-a]-pyridine-3-acetic acid, (5 gm 0.018mole) in 50 ml of dichloromethane. And applied ice salt cooling to bring temperature 0 to –5 °c. Then slowly added (2.21g, 0.02mole) ethyl chloroformate by keeping the reaction temperature below 5 °c, simultaneously add the triethyl amine solution (2.0 g, 0.02mole). Stirred the reaction for 60 min at the same temperature, the reaction mass was used as such for coupling with 7-ACA. Similarly by following the same procedure other derivatives of the series were prepared and used as such for coupling.

**Synthesis of 3-(acetoxymethyl-7-(2-(7-methyl-2-p-tolylimidazo [1, 2-a] pyridine-3 yl) acetamide-8-oxo-5thia-1-azabicyclo [4.2.0] oct-2-ene-2-carbaylic acid 2.2 (a) (in situ)**

**General procedure:**

To the (ethyl carbonic) 2-(7-Methyl-2- p-tolimidazo [1, 2-a] pyridin-3-yl) acetic anhydride (II) (1mole), added 7-amino-3-methyl-8-oxo-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylic acid, (1.1 mole). And maintained the reaction temperature 0-5 °c and pH between 7-7.5 using the triethyl amine solution. After completion of the reaction solvent was removed on rotavapour, added water and pH of the reaction mixture was brought to 2.5-3.0 using dilute Hydrochloric acid. The solid obtained was filtered and washed with water and dry in oven, at 40-45 °c, to get desired product. Similarly by following the same procedure other derivatives of the series were prepared. The physical data of the compounds are recorded in the Table 1.1.

**IR** (KBr, cm⁻¹): 3312 (OH stretching), 3198 (CH stretching), 2945 and 2927 (CH aliphatic asymmetric and symmetric stretching, respectively), 1780 (ester C=O stretching), 1588 (C=O stretching) and 1574 (CONH stretching).

**1H NMR in (DMSO-d₆):** δ 2.00 (s,3H,-CH₃); δ 2.30 (s,3H,-CH₃); δ 2.37 (s,3H,-CH₃); δ 3.54 (s,2H,-CH₂); δ 3.60-3.58(m,2H,-CH₂); δ 4.02-4.00 (m,2H,-CH₂); δ 5.10-5.06 (d, 1H, β-lactam ring protons); δ 5.60-5.56 (m, 1H, β-lactam ring protons); δ 7.35-7.30 (d 2H,Ar-H); δ 7.62-7.60(d 2H,Ar-H); δ 7.73-7.69 (d,2H,Ar-H); δ 8.60 (s,1H,Ar-H, ); δ 9-25(s, 1H, -OH proton exchanges with D₂O).**Mass (m/z):** 535 M⁺1.\n
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Synthesis of 7-(2-(7-methyl-2-p-tolylimidazo [1, 2-a] pyridine-3-yl) acetamide-8-oxo-3-vinyl-5thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid 2.2 (b) (in situ)

IR (KBr, cm\(^{-1}\)): 3312 (OH stretching), 3198 (CH stretching), 2945 and 2927 (CH aliphatic asymmetric and symmetric stretching, respectively), 1780 (ester C=O stretching), 1588 (C=O stretching) and 1574 (CONH stretching).

\(^1\)H NMR in (DMSO-d\(_6\)): δ 2.32 (s, 3H, -CH\(_3\)) ; δ 2.39 (s, 3H, -CH\(_3\)) ; δ 3.55 (m, 2H, -CH\(_2\)) ; δ 4.0 (m, 2H, -CH\(_2\)) δ 4.75-4.77 (dd, 1H, -CH\(_2\) vinyl) ; δ 4.99-4.98 (dd, 1H, =CH-); δ 5.23-5.56 (dd, 1H, CH\(_2\) vinyl -); 5.10-5.08 (d, 1H, β-lactam ring protons), 5.62-5.65 (m, 1H, β-lactam ring protons) δ 6.85-6.89 (d 2H, Ar-H); δ 7.22-7.18 (d 2H, Ar-H); δ 7.53-7.59 (d, 2H, Ar-H); δ 8.40 (s, 1H, Ar-H); δ 9.44 (s, 1H, -OH proton exchanges with D\(_2\)O). Mass (m/z): 489 M\(^+\).

Synthesis of 3-methyl-7-(3-(6-methyl-2-p-tolylimidazo [1, 2-a] pyridine-3-yl) acetamide-8-oxo-3-vinyl-5thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid 2.2 (b) (in situ)

IR (KBr, cm\(^{-1}\)): 3298 (OH stretching), 3172 (CH stretching), 2923 and 2911 (CH aliphatic asymmetric and symmetric stretching, respectively), 1762 (ester C=O stretching), 1554 (C=O stretching) and 1522 (CONH stretching).

\(^1\)H NMR in (DMSO-d\(_6\)): δ 2.04 (s, 3H, -CH\(_3\)) δ 2.37 (s, 3H, -CH\(_3\)) ; δ 2.41 (s, 3H, -CH\(_3\)) ; δ 3.59 (m, 2H, -CH\(_2\)) ; δ 4.11 (m, 2H, -CH\(_2\)) ; 5.05-5.06 (d, 1H, β-lactam ring protons), 5.63-5.60 (m, 1H, β-lactam ring protons) δ 6.75-6.79 (d 2H, Ar-H); δ 7.10-7.14 (d 2H, Ar-H); δ 7.45-7.53 (d, 2H, Ar-H); δ 8.33 (s, 1H, Ar-H); δ 9-31 (s, 1H, -OH proton exchanges with D\(_2\)O).

Mass (m/z): 477 M\(^+\).

Synthesis of benzhydryl-7-(3-(6-methyl-2-p-tolylimidazo [1, 2-a] pyridine-3-yl) acetamide-8-oxo-3-vinyl-5thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate 2.2 (d) (in situ)

IR (KBr, cm\(^{-1}\)): 3110 (CH stretching), 3083 and 3010 (CH aliphatic asymmetric and symmetric stretching, respectively), 1798 (ester C=O stretching), 1593 (C=O stretching) and 1587 (CONH stretching).

\(^1\)H NMR in (DMSO-d\(_6\)): δ 2.35(s,3H,-CH\(_3\)); δ 3.01 (m, 2H, -CH\(_2\)) ; δ 3.87 (m, 2H, -CH\(_2\)) δ 3.94 (m, 2H, -CH\(_2\)) δ 4.64-4.66 (dd, 1H, =CH-); δ 4.89-4.94 (dd, 1H, =CH-); δ 5.20-5.18 (dd, 1H, CH\(_2\) vinyl -); δ 5.33-5.35 (dd, 1H, =OC(6H)\(_5\)); 5.14-5.10 (d, 1H, β-lactam ring protons), 5.67-5.64 (m, 1H, β-lactam ring protons) δ 7.31-7.40 (10H, Ar-H); δ 7.67-7.64 (4H, Ar-H); δ 7.83-7.859 (3H, Ar-H); δ 8.30 (s, 1H, Ar-H); δ 9-39 (s, 1H, -OH proton exchanges with D\(_2\)O). Mass (m/z): 655 M\(^+\).

RESULTS AND DISCUSSION

Chemistry

The synthetic route of compounds is shown in Scheme 1. Reaction of 6-Methyl-2-(4-methylphenyl) imidazol [1, 2-a]-pyridine-3-acetic acid and ethylchloroformate using triethyl amine as base yielded the active anhydride intermediate, (II) i.e. (ethyl carbonic) 2-(7-Methyl-2-p-tolylimidazo [1, 2-a] pyridin-3-yl) acetic anhydride. in-situ condensation with C-3 substituted 7-ACAs gives new β-lactams compounds

All the compounds were synthesized in good yield; the physical data of all novel synthesized compounds are recorded in the Table 1.
Table-1: Physical data of the new synthesized substituted 3-(acetoxymethyl-7-(2-(7-methyl-2-p-tolylimidazo [1, 2-a] pyridine-3yl) acetamide-8-oxo-5thia-1-azabicyclo [4.2.0] oct-2-ene-2-carbaylic acid 2.2 (A-E)

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Entry</th>
<th>R</th>
<th>R1</th>
<th>Molecular formula</th>
<th>Yield</th>
<th>Melting point (°C)</th>
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<tbody>
<tr>
<td>1.</td>
<td>2.2A</td>
<td>H</td>
<td>CH₂OCOCH₃</td>
<td>C₂₇H₂₆N₄O₆S</td>
<td>77%</td>
<td>238 Decomposed</td>
</tr>
<tr>
<td>2.</td>
<td>2.2B</td>
<td>H</td>
<td>CH₂=CH₂</td>
<td>C₂₆H₂₃N₄O₆S</td>
<td>80%</td>
<td>240</td>
</tr>
<tr>
<td>3.</td>
<td>2.2C</td>
<td>H</td>
<td>CH₃</td>
<td>C₂₅H₂₂N₄O₆S</td>
<td>76%</td>
<td>198-204</td>
</tr>
<tr>
<td>4.</td>
<td>2.2D</td>
<td>-CH-(C₆H₅)₂</td>
<td>CH₂=CH₂</td>
<td>C₂₉H₃₀N₄O₆S</td>
<td>79%</td>
<td>156</td>
</tr>
<tr>
<td>5.</td>
<td>2.2E</td>
<td>H</td>
<td>CH₂-CH=CH₂</td>
<td>C₂₇H₂₆N₅O₆S</td>
<td>75%</td>
<td>99-103</td>
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Antimicrobial activity
All the synthesised compounds 2.2 a-f were screened for their antibacterial activity against against four micro-organisms: Escherichia coli (Gram negative), Staphylococcus typhi (Gram negative), Staphylococcus aureus (Gram positive) by Agar dilution method using streptomycin as standard and Dimethyl sulphoxide as solvent. The activity of the above compounds tested in different bacteria found to be inferior with the standard drug Streptomycin tested at 100 µg/mL concentrations. Results are given in Table 2 as µg/mL.

Analgesic Activity
Swiss mice (both sexes, 20—30 g) obtained from the serum institute, Pune were used. Animals were housed in cages with free access to food and water. All animals were kept under a 12 hr: 12 hr light–dark cycle. Animals were treated with the extract 1hr before the experiments. Controls received vehicle at the same volume as the treated groups.
Writhing test in mice: Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect analgesic activity although some psychoactive agents also show activity. An irritating agent such as Pentazocin or acetic acid is injected intraperitoneally to mice and the stretching reaction is evaluated. The reaction is not specific for the irritant.

Table-2: Antimicrobial activity of substituted 3-(acetoxymethyl-7-(2-(7-methyl-2-p-tolylimidazo[1,2-a]pyridine-3yl)acetamide)-8-oxo-5thia-1-azabicyclo[4.2.0]oct-2-ene-2-carbaylic acid 2.1 (A-E)

<table>
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<th>Entry</th>
<th>R</th>
<th>R_1</th>
<th>E. coli</th>
<th>S. typhi</th>
<th>S. aureus</th>
<th>B. megaterium</th>
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<tr>
<td>2.2A</td>
<td>H</td>
<td>CH_3OCOCH_3</td>
<td>20</td>
<td>18</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>2.2B</td>
<td>H</td>
<td>CH_2=CH_2</td>
<td>17</td>
<td>15</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>2.2C</td>
<td>H</td>
<td>CH_3</td>
<td>14</td>
<td>13</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>2.2D</td>
<td>CH_2=CH_2</td>
<td>-CH-(C6H5)_2</td>
<td>13</td>
<td>13</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>2.2E</td>
<td>H</td>
<td>CH_2-CH=CH_2</td>
<td>15</td>
<td>14</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Std</td>
<td></td>
<td>Streptomycin</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td>24</td>
</tr>
</tbody>
</table>

Mice of either sex with a weight between 20 and 25 g are used. Pentazocin in a concentration of 0.02% is suspended in a 1% suspension of carboxy methylcellulose.

An aliquot of 0.25 ml of this suspension is injected intra peritoneally. Groups of 6 animals are used for controls and treated mice. Preferably, two groups of 6 mice are used as controls. Test animals are administered the drug or the standard at various pre-treatment times prior to Pentazocin administration. The mice are placed individually into glass beakers and five min are allowed to elapse. The mice are then observed for a period of ten min and the number of writhes is recorded for each animal. For scoring purposes, a writh is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The formula for computing percent inhibition is: average writhes in the control group minus writhes in the drug group divided by writhes in the control group times 100%. The time period with the greatest percent of inhibition is considered the peak time. A dose range is reserved for interesting compounds or those which inhibit writhing more than 70%. Compounds with less than 70% inhibition are considered to have minimal activity. All result shown graphically (Fig.1 and 2).

All data analyzed by one way ANOVA followed by Dunnets test (*p<0.05, **p<0.01, ***p<0.001)

Hot plate method: The paws of mice and mice are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses.

The method originally described by Woolfe and Mac Donald (1944) has been modified by several investigators. The following modification has been proven to be suitable: Groups of 10 mice of either sex with an initial weight of 18 to 22 g are used for each dose. The hot plate which is commercially available, consists of an electrically heated surface. The temperature is
controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch.

The latency is recorded before and after 20, 60 and 90 min following oral or subcutaneous administration of the standard or the test compound. All result shown graphically (Fig.3 and 4).

**Anti-inflammatory activity**

Carrageenan induced rat paw edema: Male Wistar rats (120 - 170 g) kept at the laboratory Animal home. The animals were maintained under standard environmental conditions and had free access to standard diet and water. Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay. Groups of 6 rats were given a dose of 30, 60 and 120 mg/kg .p.o .Diclofenac sodium was

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**Figure 1: Acetic acid Induced Writhing**

All data analyzed by one way ANOVA followed by Dunnetts test (*p<0.05, **p<0.01, ***p<0.001)

**Figure 2: Acetic acid Induced Writhing**

All data analyzed by one way ANOVA followed by Dunnetts test (*p<0.05, **p<0.01, ***p<0.001)
used as the standard drug. (10 mg/kg.p.o). After 1h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for 2, 3, 4 and 24 hours. Anti-inflammatory activity was measured as the change in paw volume of the test and standard drug treated animals. All result shown graphically (Fig.5 and 6).

Fig.-3: Hot plate test
All data analyzed by one way ANOVA followed by Dunnet's test (*p<0.05, **p<0.01, ***p<0.001)

Fig.-4: Hot plate test
All data analyzed by one way ANOVA followed by Dunnet's test (*p<0.05, **p<0.01, ***p<0.001)
Biological activity

As the contribution in this field we described here the use of ethyl chloroformate for the formation of an active anhydride intermediate, is a stable compounds during reaction, readily accessible, commercially available and inexpensive. The crucial step of the present approach was the reaction between a carboxylic acid and ethyl chloroformate, which gives entirely new products, which on in-situ condensation with C-3 substituted 7-ACAs gives new β-lactams with quality and yield.

The all novel synthesized compounds were found to exhibit good to moderate antimicrobial activity. The antifungal activity of these compounds was also tested against three different fungal species. None of them were active against the species tested.
From the present investigation it can be deduced that the series of compounds have analgesic activity in the following order 2.4D>2.3C>2.5E>2.2B >2.1A. In the acetic acid induced writhing the drugs demonstrated a dose dependent activity by inhibiting the no. of writhing. showing that the drug has peripheral analgesic activity. However in the hot plate test the drugs demonstrated slight inhibition of analgesia as the latency of paw licking was not increased significantly. In the carrageenan induced rat paw edema model, the drugs inhibited the rise in paw volume in a dose dependent manner, depicting the anti inflammatory activity. These observations provide sufficient proof that the series of compounds may act by inhibition of synthesis of prostaglandins by inhibiting the cyclo oxygenase pathway.

ACKNOWLEDGMENTS

Author is greatly thankful to the management of Maulana Azad Collage Aurangabad for technical support provided for carrying out this research work.

REFERENCES


[RJC-765/2011]