3

Materials and Methods

3.1 GENERAL INFORMATION

3.1.1 Reagents and Solvents: All reagents and solvents were obtained from Sigma-Aldrich was used as received unless otherwise stated. Dry solvent diethyl ether was predried over $CaCl_2$ 6 to 12hrs and dried over molecular sieve overnight.

3.1.2 Chromatography: Thin layer chromatography (TLC) was performed using glass plates coated with silica gel G to monitor and check the completion of each reaction. Ethyl acetate and petether and alcohol solvent mixtures were used as eluting solvents.

- Purification of the crude products was carried out using chromatographic columns packed with alumina.

In place where the preparation of a series of similar compounds is described and for the individual compound the quantity of the reactants as well as the reagents employed are indicated under each of them.

3.1.3 Melting Point: Melting points were determined using Biochem melting point apparatus, and are uncorrected.

3.1.4 IR-Spectroscopy: Spectra were recorded on a Fourier transform infrared spectrometer using KBr pellet technique on an **IR-AFFINITY-I**. Absorption frequencies are quoted in reciprocal centimeter.

3.1.5 ^{1}H-, ^{13}C-NMR:

Nuclear Magnetic Resonance (^{1}HNMR) spectra were determined by Bruker modern 600MHz and JEOL 600MHz NMR instrument in DMSO-d_6 and Trifluoroacetic acid-d_1 , with tetra methyl silane as internal reference. The 600 MHz NMR machine was utilized to measure two dimensionalspectra. Chemical shifts are reported in parts per million (ppm) relative to solvent peak or trimethylsilane (TMS). Coupling constants (*J*) are reported in Hertz (Hz). The multiplicities are written as: *s*=singlet, bs=broad

singlet, *d*=doublet, *t*=triplet, *m*=multiplet and their combinations, such as *dd*=doublet of a doublet.

3.1.6 Mass Spectrometry: The mass spectrum was recorded using Jeol GC-mate–II spectrophotometer.

3.2 PREPARATION OF ACONIC ACID

3.2.1 Preparation of Sodium aconite

Itaconic acid (260g) was powdered and stirred to a paste with water (340ml) in 5-litre beaker. Bromine (320g) was slowly added, keeping the temperature below 50˚C. When all but trace of bromine had disappeared, the solution was neutralized with sodium bicarbonate (336g). The mixture was then heated to 50˚ C on a water bath and treated with a suspension of anhydrous sodium carbonate (106g) in water (158ml) at 50˚C, added in small portions until the solution remained neutral. The mixture was cooled and allowed to remain at 0˚C for an hour. The crystalline sodium salt was collected by filtration, washed with a small amount of ice water and then with 95% alcohol and dried under vacuum **(N. R. Campbell and J. H. Hunt, 1947).**

3.2.2 Preparation of Aconic acid

Dry sodium aconate 100g was suspended in dry ether (300ml). Dry hydrogen chloride gas was then passed with stirring, until a gain in weight (30g) was obtained. The mixture was left over night; the solid (115g) was filtered off and extracted with ether in a soxhlet. Evaporation of the solvent gave the white crystals of aconic acid

3.2.3 2-OXO-2*H*-PYRIDO [1,2-*a*] PYRIMIDIN-3(4*H*)-YLIDENE ACETIC ACIDS

General Procedure

To a magnetically stirred solution of aconic acid (0.02mole) in alcohol, added drop wise the alcoholic solution of 2-amino pyridine (0.01mole) at room temperature. The precipitated solid was filtered, dried and was recrystallised from ethanol as colourless crystals **(Venugopal & Sundaram, 2016).**

2-oxo-2*H*-pyrido [1,2-*a*]pyrimidin-3(4*H*)-ylidene acetic acid (3a)

Aconic acid: 2.56g; 2-aminopyridine: 1.88g; Ethanol: 50ml melting point:221˚C, Yield - 4g (91%)

IR (g) $_{max:}$ 2954 cm^{-1} (-OH), 1741 cm^{-1} (-CO), 1670 cm^{-1} (-CO), 1637 cm^{-1}(-CN), 1271 cm^{-1}, 985 cm^{-1}

2-oxo-8-methyl-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidene acetic acid (3b) Aconic acid: 2.56g; 4-methyl-2-aminopyridine: 2.38g; Ethanol: 50ml Melting point : 233˚C, Yield - 4.4g (93%)

IR (g) $_{max:}$ 3014 cm^{-1} (-OH), 1749 cm^{-1} (-CO), 1668 cm^{-1} (-CO), 1640 cm^{-1}(-CN), 1269 cm^{-1}, 983 cm^{-1}

2-oxo-7-methyl-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidene acetic acid (3c) Aconic acid: 2.56g; 5-methyl-2-aminopyridine: 2.38g; Ethanol: 50ml Melting point : 233˚C, Yield - 4.1g (88%)

IR (g) $_{max:}$ 3012 cm^{-1} (-OH), 1752 cm^{-1} (-CO), 1671 cm^{-1} (-CO), 1642 cm^{-1}(-CN), 1271 cm^{-1}, 980 cm^{-1}

3.3 SYNTHESIS AND CHARACTERIZATION OF PYRIDO [1,2-*a*] PYRIMIDIN-2-ONES DERIVATIVES

General Procedure

To a stirred suspension of 10 mmoles of powdered Lewis acid catalyst and 5 ml of DMF warmed to about 50^0C with constant stirring. To this a solution of 204mg (1mmoles) of diene in 10 ml of DMF was added slowly within 10 min. Lewis acid catalyst rapidly dissolved to form a slightly yellow clear solution. At this stage dienophile 1mmole, was bubbled through the solution with stirring. The mixture was kept for refluxing in a water bath. The temperature of the water bath was maintained between 80^0C-90^OC by external heating. The resulting solution was cooled and treated with water, and the precipitate was filtered and collected and recrystallized using ethyl alcohol **(Inukai & Kasai, 1965).**

3-BUTOXY-2,3,4,5-TETRAHYDROPYRANO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-4-CARBOXYLIC ACID(5a)

Diene - 2-oxo-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidiene Acetic acid; Dienophile – Butyl vinyl ether ; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- *4½* hrs; Yield-55% ; Melting Point- 280-285˚*C*.

FTIR: 3327 cm^{-1}(OH)**,** 1686cm^{-1}(C=O), 1574 cm^{-1}(C=N), 1373cm^{-1}(O-H), 1294 cm^{-1}(C-O)

H^1 NMR: (600 MHz, DMSO-d_6) δ 10.2 (s, 1H,19-H), 7.75(t, J=12Hz, 1H,1-H), 8.38(t, J=12,6Hz, 1H,2-H), 7.87(d, J=6Hz, 1H,3-H), 7.05(t, J=12,6Hz, 1H,6-H), 5.08(s, 2H,10-H), 7.3(t, J=12,6Hz, 2H,12-H), 6.4(dd, J=12,12,6Hz, 1H,13-H), 2.3/2.6(t, J=6,6Hz, 1H,14-H), 1.15-1.03(m, 6H,15, 16,17-H), 0.87(t, J=6,6Hz, 3H,18-H).

C^{13}NMR :(150 MHz, DMSO-d6) δ 168, 152, 151, 147, 137, 132, 118, 116, 114, 111, 64, 54, 29, 20, 18, 13

MASS: *m/z* ratio 306.0340(M^+)

3-BUTOXY-9-METHYL-2, 3, 4, 5-TETRAHYDROPYRANO [2, 3-*d*] PYRIDO [1, 2-*a*] PYRIMIDINE-4-CARBOXYLIC ACID (5b)

Diene - 2-oxo-8-methyl-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidene acetic acid; Dienophile - Butyl vinyl ether; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 5 hrs; Yield-58% ; Melting Point-292-297˚C.

FTIR: 3330 cm^{-1}(OH)**,** 1683cm^{-1}(C=O), 1636 cm^{-1}(C=N), 1370cm^{-1}(O-H), 1294 cm^{-1}(C-O)

H^1 NMR: (600 MHz, DMSO-d*6*) δ 10.15 (s, 1H,19-H), 7.75(m, 2H,1,6-H), 6.8(s, 1H,3-H), 5.05(s, 2H,10-H), 6.29(d, J=30Hz, 2H,12-H), 6.73(dd, J=12,12,30Hz, 1H,13-H), 4.04/3.99(t, J=6,6Hz, 1H,14-H), 1.53-0.90(m, 6H,15, 16,17-H), 0.73(t, J=6,6Hz, 3H,18-H), 2.29(s,3H,20-H)

C^{13}NMR:(150MHz,DMSO-d*6*)δ
167,152,151,149,147,132,119,118,114,111,64,54,29,20,20,18,13

MASS: *m/z* ratio 319.1080(M^+)

3-BUTOXY-8-METHYL-2, 3, 4, 5-TETRAHYDROPYRANO [2, 3-*d*] PYRIDO [1, 2-*a*] PYRIMIDINE-4-CARBOXYLIC ACID (5c)

Diene - 2-oxo-7-methyl-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidene acetic acid; Dienophile - Butyl vinyl ether; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 5½ hrs; Yield-59% ; Melting Point-298-300˚C.

FTIR: 3431 cm^{-1}(OH)**,** 1691cm^{-1}(C=O), 1562cm^{-1}(C=N), 1378cm^{-1}(O-H), 1293 cm^{-1}(C-O)

H^1 NMR: (600 MHz, DMSO-d_6) δ 10.06 (s, 1H,19-H), 8.1(m, 2H,2,3-H), 7.3(s, 1H,6-H), 4.99(s, 2H,10-H), 7.5(d, J=30Hz, 2H,12-H), 6.8(d, J=12,30Hz, 1H,13-H), 5.9(d, J=12Hz, 1H,14-H), 1.45-1.62(m, 6H,15, 16,17-H), 0.84(t, J=6,6Hz, 3H,18-H), 2.21(s,3H, 20-H)

C^{13}NMR:(150MHz,DMSO-d_6)δ
168,150,149,147,138,132,128,126,114,111,54,30,29,20,18,17,13

MASS: *m/z* ratio 319.0943(M^+)

3a-METHYL-2-OXO-1-PHENYL-1,2,3,3*a*,4,5-HEXAHYDROPYRAZOLO[1,5-*b*]PYRIDO[1',2':1,2]PYRIMIDO[5,4-*e*][1,2]OXAZINE-4-CARBOXYLIC ACID (7a)

Diene - 2-oxo-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidiene Acetic acid; Dienophile - 1-phenyl-3-methyl-pyrazolone; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 4 hrs; Yield- 61%; Melting Point-284-294˚C.

FTIR: 3417 cm^{-1}(OH)**,** 1598 cm^{-1}(C=O), 1499cm^{-1}(C=N), 1368cm^{-1}(O-H), 1273 cm^{-1}(C-O)

H^1 NMR: (600 MHz, DMSO-d_6) δ 10.42 (s, 1H,25-H), 7.5(t, J=12,6Hz, 1H,1-H), 7.3(t, J=12,12Hz, 1H,2-H), 7.8(d, J=12Hz, 1H,3-H), 7.9(d, J=6Hz, 1H,6-H), 2.86(s, 2H,10-H),2.83/2.73(s, 1H,14-H), 2.3(s, 2H,17-H), 2.1(s, 3H,18-H), 7.7(d, J=12Hz, 2H,20,24-H), 7.4(t, J=12Hz, 2H,21,23-H), 7.2(t, J=12Hz, 1H,22-H)

C^{13}NMR :(150MHz, DMSO-d_6) δ 174, 149, 145, 144, 137, 136, 128.7, 129, 128, 127, 125, 120, 101, 36, 32, 27, 11

MASS: *m/z* ratio 379.8493(M^+)

9-DIMETHYL-2-OXO-1-PHENYL-1,2,3,3*a*,4,5-HEXAHYDROPYRAZOLO[1,5-*b*]PYRIDO[1',2':1,2]PYRIMIDO[5,4-*e*][1,2]OXAZINE-4-CARBOXYLIC ACID (7b)

Diene - 2-oxo-8-methyl-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidene acetic acid; Dienophile - 1-phenyl-3-methyl-pyrazolone; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 5½hrs; Yield-63% ; Melting Point-above 300˚C.

FTIR: 3412 cm^{-1}(OH)**,** 1605℃ cm^{-1}(C=O), 1498cm^{-1}(C=N), 1408cm^{-1}(O-H), 1270 cm^{-1}(C-O)

H^1 NMR: (600 MHz, DMSO-d6) δ 9.02 (s, 1H,25-H), 7.8(d, J=12Hz, 1H,1-H), 7.1(s, 1H,3-H), 7.5(d, J=12Hz, 1H,6-H), 2.8(s, 2H,10-H),2.72/2.78(s, 1H,14-H), 2.3(s, 2H,17-H), 2.1(s,

3H,18-H), 7.68(d, J=12, 2H,20,24-H), 7.4(t, J=12,6Hz, 2H,21,23-H), 7.2(t, J=12,6Hz, 1H,22-H), 1.9 (s, 3H,26-H)

C^{13}NMR :(150MHz, DMSO-d_6) δ163, 146, 137, 129.5, 129.4, 129, 126, 125.7, 125.2, 125, 121, 120.5, 120, 118, 36, 34, 28, 17, 11

MASS: *m/z* ratio 392.1080(M^+)

8-DIMETHYL-2-OXO-1-PHENYL-1,2,3,3*a*,4,5-HEXAHYDROPYRAZOLO[1,5-*b*]PYRIDO[1',2':1,2]PYRIMIDO[5,4-*e*][1,2]OXAZINE-4-CARBOXYLIC ACID (7c)

Diene - 2-oxo-7-methyl-2*H*-pyrido [1, 2-*a*] pyrimidin-3(4*H*)-ylidene acetic acid

Dienophile - 1-phenyl-3-methyl-pyrazolone; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 6hrs; Yield-68% ; Melting Point- above300 ˚C

FTIR: 3441cm^{-1}(OH) **,** 1606 cm^{-1}(C=O), 1487cm^{-1}(C=N), 1411cm^{-1}(O-H), 1274 cm^{-1}(C-O)

H^1 NMR: (600 MHz, DMSO-d6) δ 9.64 (s, 1H,25-H), 7.6(d, J=6Hz, 1H,2-H), 7.8(d, J=6Hz, 1H,3-H), 7.29(s, 1H,6-H), 2.87(s, 2H,10-H),2.72/2.78(s, 1H,14-H), 2.3(s, 2H,17-H), 2.1(s, 3H,18-H), 7.66(d, J=6, 2H,20,24-H), 7.4(t, J=6,6Hz, 2H,21,23-H), 7.2(t, J=6,6Hz, 1H,22-H), 1.9 (s, 3H,26-H)

C^{13}NMR :(150MHz, DMSO-d_6)δ 163, 146, 137, 129.41, 129.5, 129, 128, 125.5, 121.5, 121, 120, 119, 118, 109, 36, 34, 31, 14, 11

MASS: *m/z* ratio 393.5846(M^+)

13*a*-METHYL-1,2,3,4,4*a*,12,13,13*a*-OCTAHYDROCHROMENO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID (9a)

Diene - 2-oxo-2*H*-pyrido[1,2-a]pyrimidin-3(4*H*)-ylidiene Acetic acid; Dienophile – 1-methyl-1-cyclohexene ; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 6 hrs; Yield-57% ; Melting Point- 257-258^{0}C.

FTIR: 3394cm^{-1}(OH)**,** 1651 cm^{-1}(C=O), 1496cm^{-1}(C=N), 1388cm^{-1}(O-H), 1249 cm^{-1}(C-O)

H^1 NMR: (600 MHz, Trifluoroacetic acid-d_1)) δ 12.6 (s, 1H,19-H), 9.5(t, J=6,6Hz, 1H,1-H), 10.5(t, J=6,6Hz, 1H,2-H), 10.6(d, J=6Hz, 1H,3-H), 9.6(d, J=6Hz, 1H,6-H), 6.1(s, 2H,10-

H),6.3(t, J=18,6Hz, 1H,12-H), 5.9(s, 1H,14-H), 5.5(q, J=6,6,12Hz, 2H,15-H), 5.0(q, J=6,6,6,12Hz, 2H,16-H), 5.4(q, J=6,6,12,6Hz, 2H,17-H), 5.6(t, J=6,6Hz, 2H,18-H), 4.8/4.92 (s, 3H,20-H)

C^{13}NMR :(150MHz, Trifluoroaceticacid-d_1)) δ 183, 179, 161, 152, 151, 148, 141, 124, 121, 39, 37, 36, 35, 33, 30, 29, 21

MASS: *m/z* ratio 301.6421(M^+)

8,13*a*-DIMETHYL-1,2,3,4,4*a*,12,13,13*a*-OCTAHYDROCHROMENO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID (9b)

Diene - 2-oxo-8-methyl-2*H*-pyrido[1,2-a]pyrimidin-3(4*H*)-ylidene acetic acid; Dienophile - 1-methyl-1-cyclohexene; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 7 hrs; Yield-59% ; Melting Point-278-290^{0}C.

FTIR: 3333cm^{-1}(OH)**,** 1696 cm^{-1}(C=O), 1559cm^{-1}(C=N), 1390cm^{-1}(O-H), 1291 cm^{-1}(C-O)

H^1 NMR: (600 MHz, Trifluoroacetic acid-d) δ 12.4 (s, 1H,19-H), 10.26(d, J=12Hz, 1H,1-H), 9.45(s, 1H,3-H), 10.21(d, J=12Hz, 1H,6-H), 6.1(s, 2H,10-H),6.3(t, J=12,6Hz, 1H,12-H), 5.9(s, 1H,14-H), 5.5(dd, J=6,6,6Hz, 2H,15-H), 5.28(m, 6H,16,17,18-H), 4.8/4.92(s, 3H,20-H), 5.03(s, 3H,21-H),

C^{13}NMR :(150MHz, Trifluoroaceticacid-d_1) δ 183, 179, 161, 152, 150, 144, 131, 127, 124, 120, 38, 37, 36, 33, 30, 24.3, 24, 23, 21

MASS: *m/z* ratio 315.6425(M^+)

9,13*a*-DIMETHYL-1,2,3,4,4*a*,12,13,13*a*-OCTAHYDROCHROMENO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID (9c)

Diene - 2-oxo-7-methyl-2*H*-pyrido [1, 2-*a*] pyrimidin-3(4*H*)-ylidene acetic acid

Dienophile - 1-methyl-1-cyclohexene; Lewis acid Catalyst- Indium(III)chlorid*e;* Color- Reddish brown; Time- 7½ hrs; Yield-58% ; Melting Point-272-286 ^{0}C.

FTIR: 3354cm^{-1}(OH)**,** 1697 cm^{-1}(C=O), 1567cm^{-1}(C=N), 1480cm^{-1}(O-H), 1296 cm^{-1}(C-O)

H^1 NMR: (600 MHz, Trifluoroacetic acid-d_1) δ 12.6 (s, 1H,19-H), 10.6(d, J=6Hz, 1H,2-H), 10.5(d, J=6Hz,1H,3-H), 10.1(s,1H,6-H), 6.1(s, 2H,10-H),6.3(t, J=12,6Hz, 1H,12-H), 5.9(s, 1H,14-H), 5.5(dd, J=6,6,6Hz, 2H,15-H), 5.0(m, 4H,16,17-H), 5.4(t, J=6,6Hz,2H,18-H), 4.8/4.92(s, 3H,20-H), 4.88(s, 3H,21-H),

C^{13}NMR :(150MHz, Trifluoroaceticacid-d_1) δ 183, 155, 152, 150, 147, 134, 130, 127, 124, 37, 36, 35, 33, 30, 19, 18, 17

MASS: m/z ratio 315.5496(M^+)

3,4,4*a*,12,13,13*a*-HEXAHYDRO-2*H*-PYRANO[2',3':5,6]PYRANO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID (11a)

Diene - 2-oxo-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidiene Acetic acid; Dienophile – 3,4-Dihydro-2*H*-pyran ; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 4½hrs; Yield-62% ; Melting Point-260-270 ^{0}C.

FTIR: 3426cm^{-1}(OH)**,** 1699 cm^{-1}(C=O), 1581cm^{-1}(C=N), 1378cm^{-1}(O-H), 1295 cm^{-1}(C-O)

H^1 NMR: (600 MHz, Trifluoroacetic acid-d_1) δ 12.4 (s, 1H,19-H), 10.1(t, J=6,6Hz, 1H,1-H), 10.3(t, J=6,6Hz,1H,2-H), 10(d, J=6Hz ,1H,3-H), 9.8(d, J=6Hz ,1H,6-H), 5.1(s, 2H,10-H),6.1(m,1H,12-H), 5.9/5.5(t, 10(d, J=6,6Hz,1H,13-H), 5.8(d, J=6Hz, 1H,14-H), 5.4(m, 6H,15,16,17-H)

C^{13}NMR :(150MHz, Trifluoroaceticacid-d_1) δ 176, 167.7, 157.6, 138.1, 138, 129.4, 120.7, 112.4, 104, 63.4, 44.7, 35.3, 28.2, 25.2

MASS: *m/z* ratio 290.26(M^+)

8-METHYL-3,4,4*a*,12,13,13*a*-HEXAHYDRO-2*H*-PYRANO[2',3':5,6]PYRANO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID (11b)

Diene - 2-oxo-8-methyl-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidene acetic acid; Dienophile - 3,4-Dihydro-2*H*-pyran; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 6 hrs; Yield-61% ; Melting Point- 290-300 ^{0}C.

FTIR: 3423cm^{-1}(OH)**,** 1610 cm^{-1}(C=O), 1565cm^{-1}(C=N), 1390cm^{-1}(O-H), 1270 cm^{-1}(C-O)

H^1 NMR: (600 MHz, Trifluoroacetic acid-d_1) δ 12.46 (s, 1H,19-H), 10.2(d, J=12Hz, 1H,1-H), 9.4(d,J=12Hz,1H,6-H),9.21(s,1H,3-H),5.8(s,2H,10-H),5.39(dd,J=12,12,6Hz,1H,12-H),6.4(t, J=12,12Hz,1H,13-H), 6.1/4.9(d, J=12Hz,1H,14-H), 5.3(m, 6H,15,16,17-H), 4.79(s,3H,20-H),

C^{13}NMR :(150MHz, Trifluoroaceticacid-d_1) δ 183, 173, 162, 152, 150, 136, 130, 94, 45, 39, 37, 36, 33, 24, 23, 21

MASS: *m/z* ratio 303.2465(M^+)

9-METHYL-3,4,4*a*,12,13,13*a*-HEXAHYDRO-2*H*-PYRANO[2',3':5,6]PYRANO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID (11c)

Diene - 2-oxo-7-methyl-2*H*-pyrido [1, 2-*a*] pyrimidin-3(4*H*)-ylidene acetic acid

Dienophile - 3,4-Dihydro-2H-pyran; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 6½hrs; Yield-65% ; Melting Point-288-296^0*C.*

FTIR: 3370cm^{-1}(OH)**,** 1691 cm^{-1}(C=O), 1573cm^{-1}(C=N), 1378cm^{-1}(O-H), 1295 cm^{-1}(C-O)

H^1 NMR: (600MHz, Trifluoroacetic acid-d_1) δ 12.46 (s, 1H,19-H), 10.4(d, J=12Hz, 1H,2-H), 10.32(d,J=12Hz,1H,3-H),10.27(s,1H,6-H),5.8(s,2H,10-H),5.5(q,J=12,18,6Hz,1H,12-H),6.4(t, J=12,18Hz,1H,13-H), 6.3/4.9(d, J=12Hz,1H,14-H), 5.4(dd, 2H,15-H), 5.2-4.9(m, 4H,16,17-H), 4.8(s,3H,20-H)

C^{13}NMR :(150MHz, Trifluoroaceticacid-d_1) δ 183, 181, 162, 161, 152.4, 152, 150, 134, 78, 70, 58, 36, 25, 22, 21, 18.8

MASS: *m/z* ratio 303.8425(M^+)

13,13*a*-DIMETHYL-4*a*-MORPHOLINO-1,2,3,4,4*a*,12,13,13*a*-OCTAHYDROCHROMENO [2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID(13a)

Diene - 2-oxo-2*H*-pyrido[1,2-a]pyrimidin-3(4*H*)-ylidiene Acetic acid; Dienophile – 1-(cyclohex-1-en-1-yl) pyrrolidine; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 8 hrs; Yield- 25%; Melting Point- above 340^{0}C.

FTIR: 3379cm^{-1}(OH)**,** 1760 cm^{-1}(C=O), 1586cm^{-1}(C=N), 1378cm^{-1}(O-H), 1240 cm^{-1}(C-O)

8,13,13*a*-TRIMETHYL-4*a*-MORPHOLINO-1,2,3,4,4*a*,12,13,13*a*-OCTAHYDROCHROMENO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID(13b)

Diene - 2-oxo-8-methyl-2*H*-pyrido[1,2-*a*]pyrimidin-3(4*H*)-ylidene acetic acid; Dienophile -1-(cyclohex-1-en-1-yl) pyrrolidine; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 10hrs; Yield-28% ; Melting Point- above 340^0C

FTIR: 3379cm^{-1}(OH)**,** 1760 cm^{-1}(C=O), 1586cm^{-1}(C=N), 1378cm^{-1}(O-H), 1240 cm^{-1}(C-O)

9,13,13*a*-TRIMETHYL-4*a*-MORPHOLINO-1,2,3,4,4*a*,12,13,13*a*-OCTAHYDROCHROMENO[2,3-*d*]PYRIDO[1,2-*a*]PYRIMIDINE-13-CARBOXYLIC ACID (13c)

Diene - 2-oxo-7-methyl-2*H*-pyrido [1, 2-*a*] pyrimidin-3(4*H*)-ylidene acetic acid

Dienophile - 1-(cyclohex-1-en-1-yl) pyrrolidine; Lewis acid Catalyst- Indium(III)chloride; Color- Reddish brown; Time- 9 hrs; Yield-30% ; Melting Point- above 340^0C.

FTIR: 3379cm^{-1}(OH)**,** 1760 cm^{-1}(C=O), 1586cm^{-1}(C=N), 1378cm^{-1}(O-H), 1240 cm^{-1}(C-O)

H^1 NMR: : (600MHz, Trifluoroacetic acid-d_1) δ 12.4 (s, 1H,26-H), 10.6(d, J=12Hz, 1H,2-H), 10.5(d,J=12Hz,1H,3-H),9.2(s,1H,6-H),5.8(s,2H,10-H),6.5(q,J=12Hz,1H,13-H),6.3(d, J=12Hz,1H,14-H), 5.2(t, J=6Hz,2H,15-H), 5.6(q, 2H,16-H), 5.7(q, 2H,17-H), 6.2(q,2H,18-H), 6.7(t,2H,20-H), 6.9(t,4H,21,23-H), 6.6(t,2H,24-H), 4.8(s,3H,25-H)

MASS: *m/z* ratio 414.2413(M^+)

3.4 COMPUTATIONAL STUDIES

Gaussian 16W software package was used to perform DFT calculations. The frequency optimization was performed utilising density functional theory [DFT] with the B3LYP functional and a differentiated basis set based on atom type. Particularly, 6-311+G(d,p) was utilised for H, C, N, and O, which has been proven to produce relatively accurate energetics for cycloadditions, while effective core potential basis set DEF2TZVP was used for indium chloride. Using the Integral equation formalism polarizable Continuum Model (IEFPCM) solvent model, the role of DMF solvent was included during optimizations **(Narayan *et al.*, 2011, Bielecki & Lipiec, 2016 and Tirado-Rives & Jorgensen, 2008).**

3.5 ANTIBACTERIAL SUSCEPTIBILITY TESTING

The *in-vitro* antibacterial activity screened for the synthesized compounds against *Staphylococcus aureus* and *Escherichia coli* bacterial strains by disc diffusion method against the standard drug **Kanamycin**.

3.6 MOLECULAR DOCKING

Docking calculations were performed using the software Autodock vina 1.5.6, and the binding energy of the protein—synthesized adducts was determined. Using the graphical interface programme 'MGL tools,' the ligand was then converted into PDBQT. The protein data bank coordinate file with the names 2W9S and 1MBT was used as an antibacterial receptor molecule. To run docking simulations, the grid box was modified with 'MGL tools.' The best docked conformation and binding affinity for drugs with proteins were determined using Autodock Vina **(Forli *et al.*, 2016).**

3.7 LIPINSKI RULE OF FIVE (ADME)

ADME parameters and molecular properties were computed for both the standard drug and test compounds an online portal called SwissADME. **(Dumpati et al., 2018 and Prasad *et al.*, 2022).**