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# Design, solvent free synthesis, and antimicrobial evaluation of 1,4 dihydropyridines

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ABSTRACT

Here in, we report the usage of cellulose sulfuric acid as a heterogeneous eco friendly catalyst for the synthesis of 1,4 dihydropyridines under solvent free conditions via Hantzsch three component reaction of an aldehyde, ethyl acetoacetate and ammonium acetate at 100 °C for 2–5 h. In silico studies were performed on twenty two possible 1,4 dihydropyridines (DHPs) analogues against $K^+$ channel receptor (KcsA). In order to validate in silico studies, thirteen compounds were synthesized and evaluated as antibacterials against twenty seven ESBL isolates of *Klebsiella pneumoniae* and *Escherichia coli*.



Bacterial resistance is emerging world wide as a threat to clinical therapeutics. β-lactamases production by bacterial species is one of the most important mechanisms of resistance to *penicillins* and *cephalosporins*. The mechanism of this resistance is the production of extended spectrum β-lactamases (ESBL). ESBLs are capable of hydrolysis and inactivating the β-lactam rings in the third generation antibiotics like *penicillins*, *cephalosporins* and *aztreonam*. Wide spread use of third generation antibiotics has led to mutations in these enzymes leading to the emergence of ESBLs. Although ESBL isolates were first discovered in the mid 1980's in Western Europe their occurrence is currently a worldwide problem ESBL isolates are very predominant in *Klebsiella pneumoniae* and *Escherichia coli*. Rise of drug resistance in many human pathogens necessitates the development of new drug therapeutic agents. Development of drugs for new targets is the need of the hour. In the present scenario synthesis of DHPs and their derivatives emerged as a hot area of research.

1,4 dihydropyridines (DHPs) class of drugs are well known for their calcium channel modulation,[1] recent studies have shown that they play an important role in $K^+$ and $Na^+$ channel modulation.[2] These compounds have a remarkable significance, because of their wide range of pharmacological and biological activity such as cardiovascular diseases including hypertension,[3] anti-inflammatory,[4] antiviral,[5] cytotoxicity,[6] anticonvulsants,[7] anti tuberculosis,[8] antithrombotic,[9] in the treatment of Alzheimer's diseases,[10] calcium agonists and antagonists,[11] more recently as enhancers of the vanilloid receptor 1 (TRPV1)[12] and screened as the human multidrug resistance protein[13].

DHPs were first synthesized by Hantzsch in 1882 via three component synthesis[14] of an aldehyde, ethyl acetoacetate and ammonium acetate in ethanol or acetic acid at 80 °C. This reaction was further fine tuned by the development of several synthetic strategies and methodologies including microwave irradiation,[15] ultrasounds,[16] ionic liquids,[17] phase transfer catalysts,[18] Brønsted bases,[19] solvent free synthesis,[20] Lewis acids,[21] Brønsted acids[22] and Lewis base[23] catalyzed solvent free synthesis of DHPs. Here in, we report a simple and practical method for the synthesis of DHPs by impressive Hantzsch protocol using catalytic amount (0.05 g) of cellulose sulfuric acid as a heterogeneous catalyst under solvent free conditions. More recently, in silico studies are being performed for rational design and synthesis of new analogues with improved pharmacological profile. Preliminary studies in our lab have shown that DHPs are very effective antimicrobial agents

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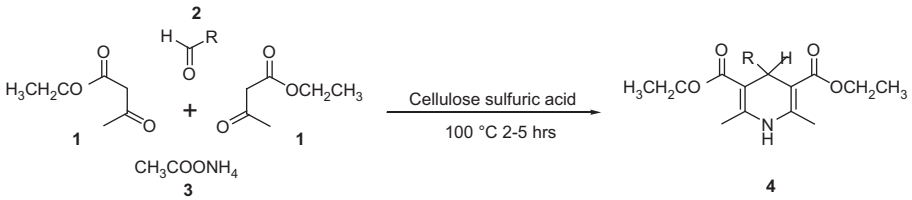


**Scheme 1.** Solvent free synthesis of 1,4 dihydropyridines catalyzed by 10 mol % of Cellulose sulfuric acid.

**Table 1**
Cellulose sulfuric acid catalyzed synthesis of DHPs in different solvents and under solvent free conditions at 100 °C

| Entry | Solvent | Catalyst mole (%) | Time (h) | Yield (%) |
|---|---|---|---|---|
| 1 | Ethanol | 10 | 10 | 78 |
| 2 | Methanol | 10 | 10 | 63 |
| 3 | Toluene | 10 | 13 | 32 |
| 4 | Acetontrile | 10 | 16 | Trace |
| 5 | Dioxan | 10 | 20 | Trace |
| 6 | THF | 10 | 17 | 55 |
| 7 | Solvent Free | 5 | 5 | 56 |
| 8 | Solvent Free | 10 | 5 | 80 |
| 9 | Solvent Free | 15 | 5 | 81 |
| 10 | Solvent Free | 20 | 5 | 78 |

**Table 2**
Cellulose sulfuric acid catalyzed synthesis of DHPs at 80 °C under solvent free conditions

| Entry | R | Product | Yield (%) | Mp reference | Mp found |
|---|---|---|---|---|---|
| 1 | 2-Cl-C6H$_4$ | **4a** | 87 | 83–85[15a] | 120–125 |
| 2 | 3-NO$_2$-C$_6$H$_4$ | **4b** | 90 | 164–165[15a] | 144–154 |
| 3 | 4-OH-C$_6$H$_4$ | **4c** | 91 | 229–231[15a] | 205–210 |
| 4 | 4-OMe-C$_6$H$_4$ | **4d** | 80 | 158–160[15a] | 132–140 |
| 5 | 4-Cl-C$_6$H$_4$ | **4e** | 78 | 145–146[15a] | 129–135 |
| 6 | 3-OH-C$_6$H$_4$ | **4f** | 85 | 180–182[19e] | 105–110 |
| 7 | 2-OH-C$_6$H$_4$ | **4g** | 78 | — | 94–99 |
| 8 | 4-OH-3OMe-C$_6$H$_3$ | **4h** | 85 | 156–158[15a] | 150–151 |
| 9 | 3,4(OMe)$_2$C$_6$H$_3$ | **4i** | 90 | 163–165[21d] | 160–162 |
| 10 | 3,4,5(OMe)$_3$C$_6$H$_2$ | **4j** | 85 | — | 125–127 |
| 11 | C$_6$H$_6$ | **4k** | 90 | 156–158[15a] | 142–150 |
| 12 | H | **4l** | 92 | — | 168–174 |
| 13 | 2,5(OMe)$_2$C$_6$H$_3$ | **4m** | 80 | — | 151–153 |

especially against drug resistant isolates of bacteria (ESBL). We reasoned that DHPs could also be effective against ion channels. Hence, we have attempted to study the effect of DHPs on $K^+$ channel. In the present study, twenty two possible DHP analogues were docked against $K^+$ channel receptor (KcsA). In silico predictions have been carried out and analyzed. Based on the docking studies various DHP analogues were synthesized. To validate our in silico studies, we have performed in vitro bioassays of the synthesized analogues against ten bacterial and twenty seven extended spectrum beta lactamase (ESBL) bacterial isolates.

In the present work, Hantzsch-type cyclocondensation reaction was studied for the preparation of 4-aryl-1,4-dihydropyridines (**4a**–**4m**), these were synthesized by the one pot condensation of aromatic aldehyde (0.01 moles), ethyl acetoacetate (0.025 moles) and ammonium acetate (0.02 moles) utilizing the catalyst cellulose sulfuric acid Scheme 1. In an initial attempt the reaction was performed with 10 mol % of catalyst at rt in different solvents viz. ethanol, methanol, *t*-butanol, dioxan, acetonitrile and under solvent free conditions. After 10 h only 30% of the product was obtained under solvent free conditions. When the reaction temperature was increased to 100 °C better results were observed with low reaction times Table 1. To optimize the amount of the catalyst we have carried out the reaction with various mol % of the catalysts. However there is no recognizable change in either % of yield or the reaction time by the increased amounts in catalysts over 0.05 g of cellulose sulfuric acid. For the scope and limitations of the catalyst performance the reaction was carried out with various substituted aromatic aldehydes in the optimized conditions Table 2.

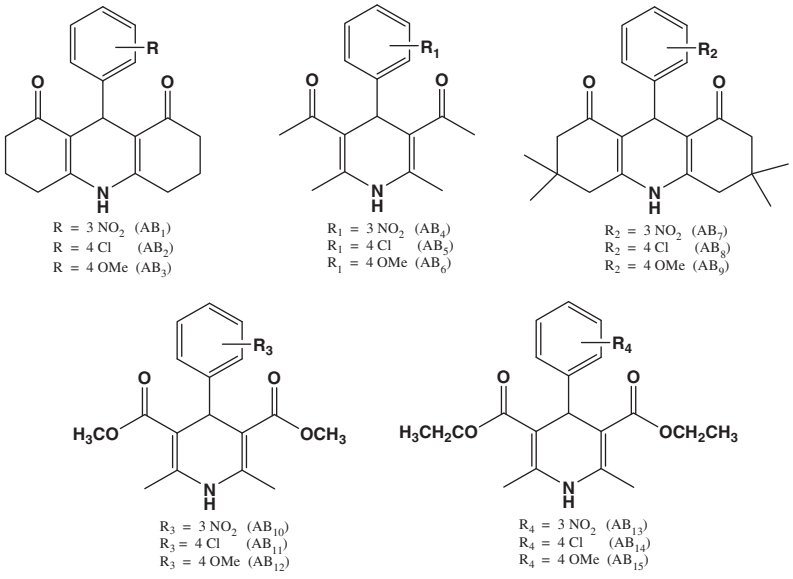


**Figure 1.** Mole docked DHP analogues ($AB_1$–$AB_{12}$) and compounds **4b**, **4d** and **4e**.

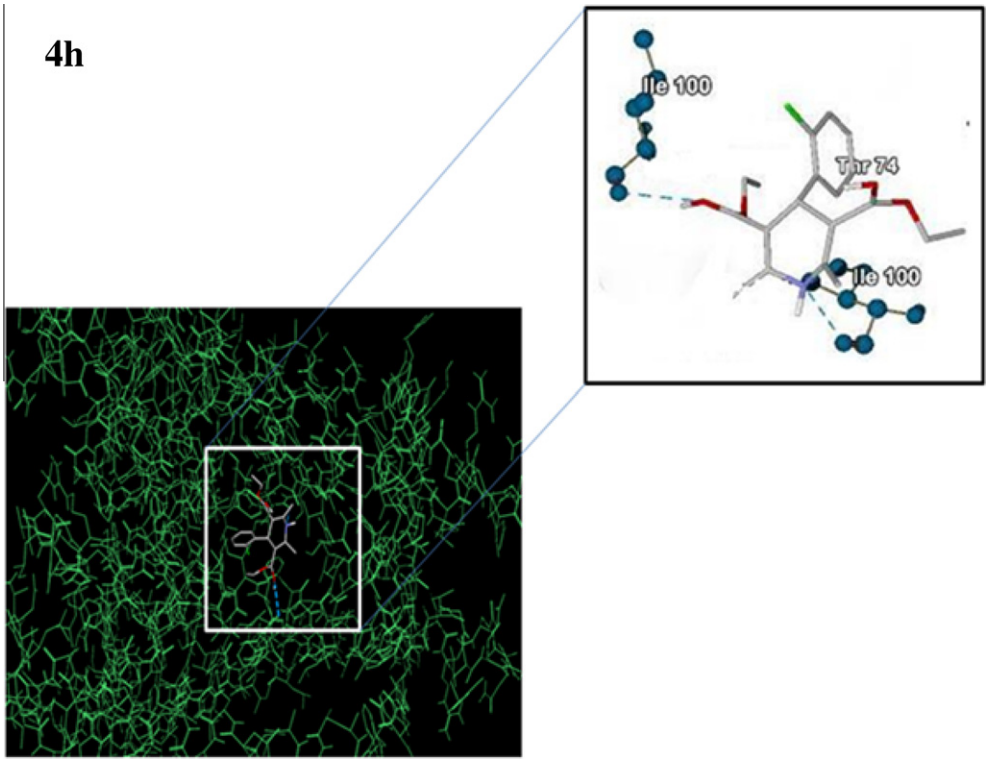


**Figure 2.1.** Docked structure of **4h** in model of $K^+$ Channel. *DHP is displayed as grey sticks; hydrogen bonds are represented with blue dashed lines.

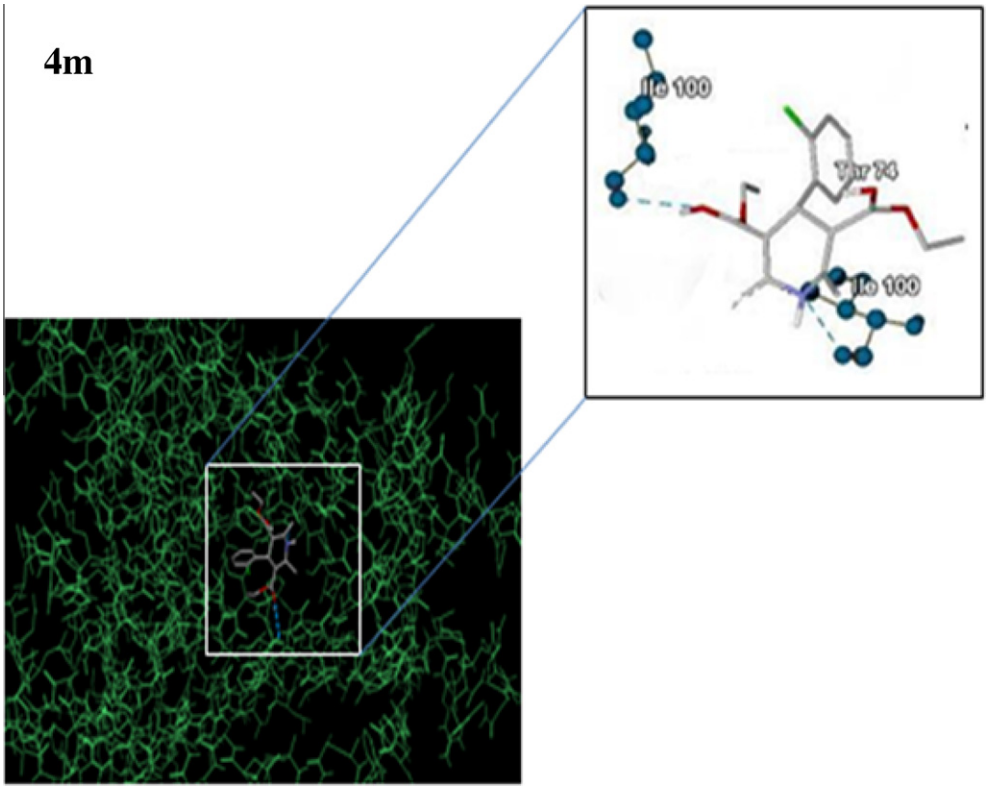


**Figure 2.2.** Docked structure of **4m** in model of $K^+$ channel. *DHP is displayed as grey sticks; hydrogen bonds are represented with blue dashed lines.

Though DHPs are potent $Ca^{+2}$ channel blockers, their role in voltage $K^+$ channels has also been determined. In the present study our interest is to find out how DHPs interact with $K^+$ channel (KcsA) which is a homotetrameric protein, its architecture describes the pore region of $K^+$ and other channels. The 3D crystal structure coordinate of $K^+$ channel receptor (KcsA) of *Streptomyces*

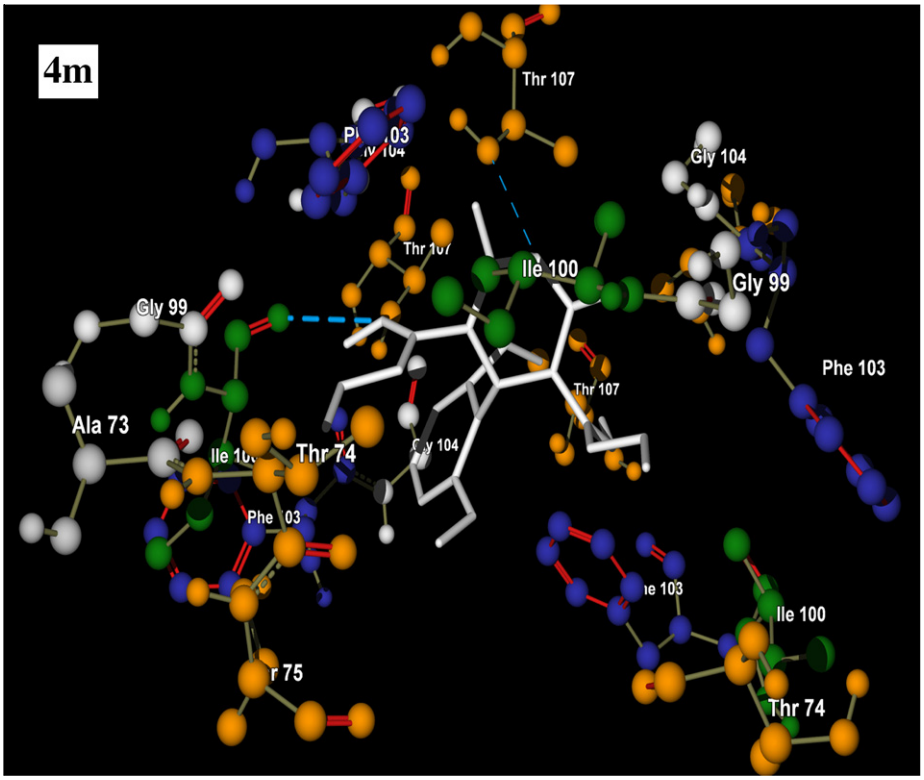


**Figure 3.1.** Hydrogen bonding interactions of the **4m** with ball and stick model of protein. DHP is displayed as white color sticks; hydrogen bonds are represented with blue dashed lines.

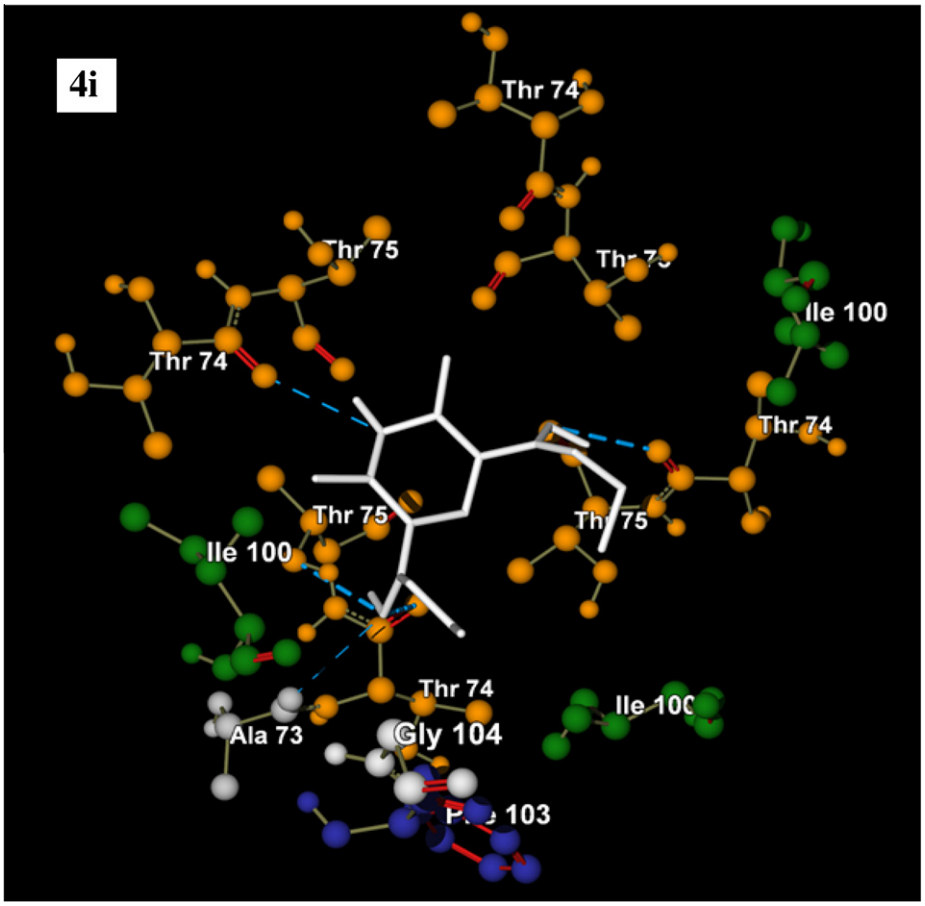


**Figure 3.2.** Hydrogen bonding interactions of the **4i** with ball and stick model of protein. DHP is displayed as white color sticks; hydrogen bonds are represented with blue dashed lines.

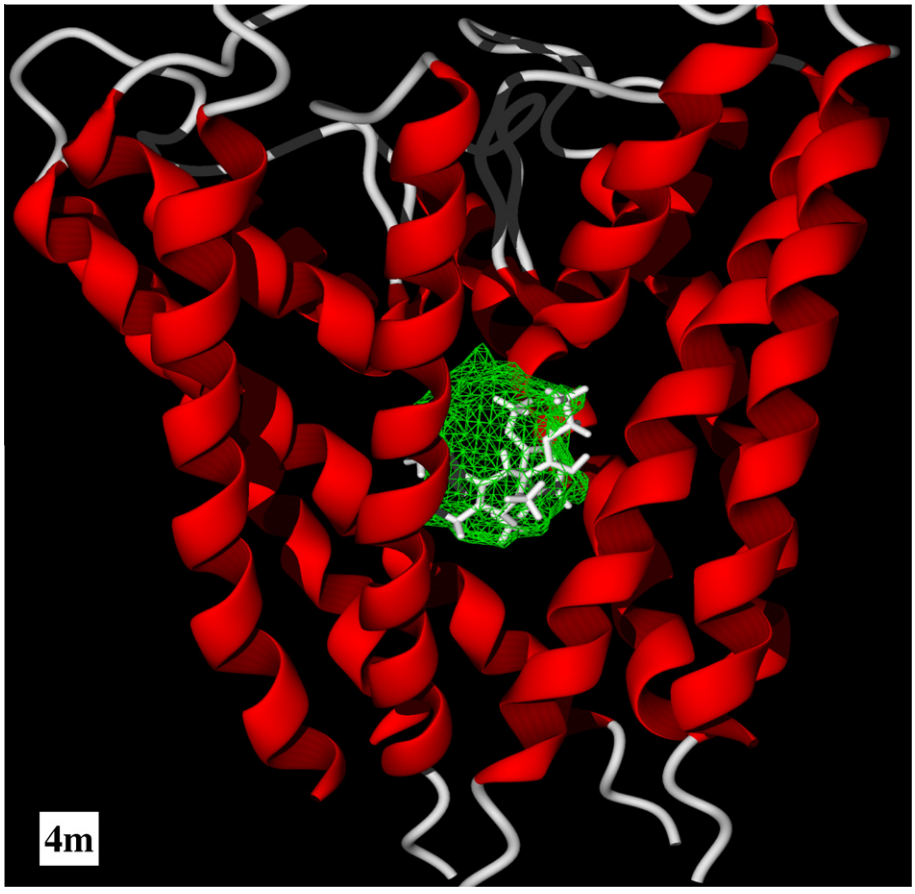


**Figure 4.1.** Binding conformation of **4m** and space occupied (green wireframe around ligand) in the 1BL8 binding pocket.

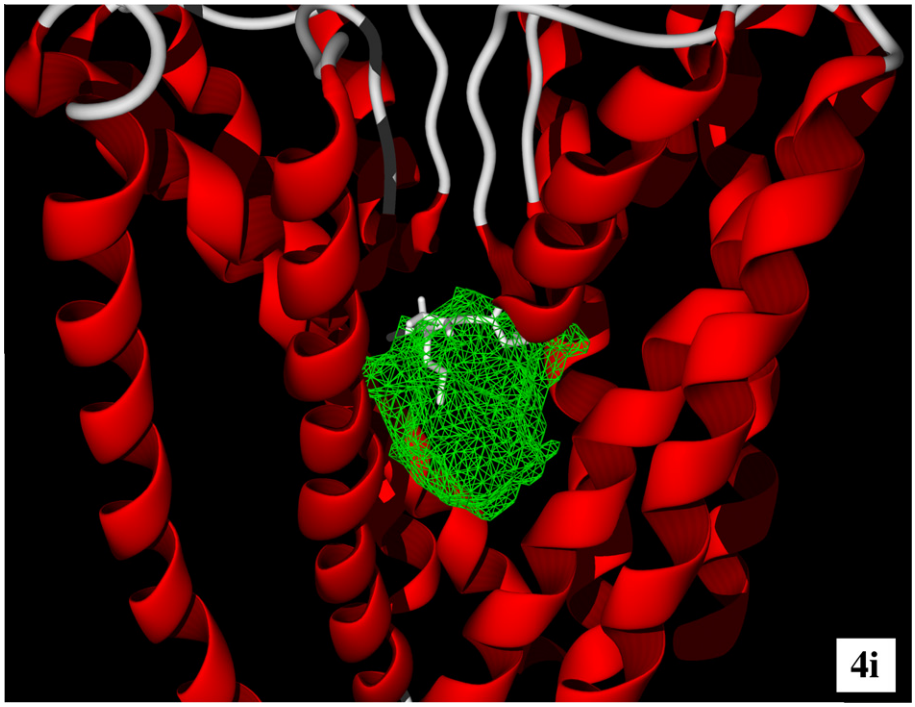


**Figure 4.2.** Binding conformation of **4i** and space occupied (green wireframe around ligand) in the 1BL8 binding pocket.

*lividans* (**RCSB,** PDB entry code 1BL8) was obtained from RCSB, PDB data base (www.rcsb.org). The protein is validated using Procheck[24] and what if sever.[25] The core structure of all ligands was predicted and sketched using ISIS draw software. They were

**Table 3**
Mol dock scores with $K^+$ channel and physical properties of the synthesized and predicted DHP analogues

| Compound | Mol dock score (Kcal/mol) | Molecular weight (Daltons) | Log P | H-Bond acceptor | H-bond donor | Molar Refractivity ($cm^3$) |
|---|---|---|---|---|---|---|
| **4a** | −133.454 | 363 | 4.89 ± 0.58 | 5 | 1 | 95.52 ± 0.3 |
| **4b** | −133.24 | 374 | 4.03 ± 0.58 | 6 | 1 | 97.17 ± 0.3 |
| **4c** | −130.858 | 345 | 3.56 ± 0.58 | 6 | 2 | 92.50 ± 0.3 |
| **4d** | −134.333 | 359 | 4.21 ± 0.58 | 6 | 1 | 97.30 ± 0.3 |
| **4e** | −124.96 | 363 | 4.89 ± 0.58 | 5 | 1 | 95.52 ± 0.3 |
| **4f** | −133.874 | 345 | 3.56 ± 0.58 | 6 | 2 | 92.50 ± 0.3 |
| **4g** | −134.437 | 345 | 3.56 ± 0.58 | 6 | 2 | 92.50 ± 0.3 |
| **4h** | −138.143 | 375 | 3.27 ± 0.59 | 7 | 2 | 99.18 ± 0.3 |
| **4i** | −129.574 | 329 | 4.30 ± 0.58 | 5 | 1 | 90.62 ± 0.3 |
| **4j** | −138.785 | 389 | 4.04 ± 0.59 | 7 | 1 | 103.98 ± 0.3 |
| **4k** | −105.734 | 253 | 2.51 ± 0.57 | 8 | 1 | 66.18 ± 0.3 |
| **4l** | −98.437 | 419 | 3.74 ± 0.64 | 8 | 1 | 66.18 ± 0.3 |
| **4m** | −152.478 | 389 | 4.18 ± 0.59 | 7 | 1 | 103.98 ± 0.3 |
| $AB_1$ | −105.73 | — | — | — | — | — |
| $AB_2$ | −91.91 | — | — | — | — | — |
| $AB_3$ | −98.16 | — | — | — | — | — |
| $AB_4$ | −111.31 | — | — | — | — | — |
| $AB_5$ | −109.59 | — | — | — | — | — |
| $AB_6$ | −112.50 | — | — | — | — | — |
| $AB_7$ | −106.81 | — | — | — | — | — |
| $AB_8$ | −100.64 | — | — | — | — | — |
| $AB_9$ | −114.99 | — | — | — | — | — |
| $AB_{10}$ | −113.95 | — | — | — | — | — |
| $AB_{11}$ | −115.65 | — | — | — | — | — |
| $AB_{12}$ | −117.529 | — | — | — | — | — |

— = Not calculated.

**Table 4**
Minimum inhibitory concentration in (**4a**–**m**) (mg/ml)

| Micro organisms | 4a | 4b | 4c | 4d | 4e | 4f | 4g | 4h | 4i | 4j | 4k | 4l | 4m | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* (ATCC 25922) | 0.25 | 2.5 | 2.5 | 1.25 | 0.125 | 0.5 | 0.25 | 0.125 | 2.5 | 2.5 | 2.5 | 1.25 | 0.5 | 0.2 |
| *Citrobacteri koseri* (ATCC 27028) | 0.25 | 2.5 | 2.5 | 1.25 | 0.125 | 0.5 | 0.125 | 0.125 | 2.5 | 2.5 | 2.5 | 1.25 | 0.25 | 0.025 |
| *Klebsiella pneumoniae* (ATCC 7761) | 0.25 | 2.5 | 2.5 | 1.25 | 0.125 | 0.5 | 0.125 | 0.125 | 2.5 | 2.5 | 1.25 | 1.25 | 0.25 | 0.5 |
| *Salmonella typhi* (ATCC 700931) | 0.25 | 1.25 | 5 | 5 | 0.5 | 0.5 | 0.5 | 0.125 | 1.25 | 1.25 | 2.5 | 2.5 | 0.5 | 0.005 |
| *Shigella dysentriae* (ATCC 32412) | 0.25 | 2.5 | 5 | 2.5 | 0.5 | 0.125 | 0.25 | 0.125 | 1.25 | 1.25 | 1.25 | 1.25 | 0.5 | 0.05 |
| *Pseudomonas aeruginosa* (ATCC 27853) | 0.25 | 5 | 5 | 5 | 0.5 | 1.25 | 0.25 | 0.125 | 1.25 | 2.5 | 1.25 | 1.25 | 2.5 | 0.002 |
| *Staphylococcus aureus* (ATCC 25923) | 0.125 | 1.25 | 2.5 | 2.5 | 0.125 | 1.25 | 0.25 | 0.125 | 1.25 | 1.25 | 1.25 | 1.25 | 0.25 | 0.010 |
| *Enterococcus faecalis* (ATCC 51299) | 0.125 | 2.5 | 2.5 | 2.5 | 0.125 | 1.25 | 0.25 | 0.125 | 1.25 | 1.25 | 2.5 | 1.25 | 0.25 | 0.015 |
| *Bacillus subtilis* (ATCC 9372) | 0.25 | 2.5 | 2.5 | 1.25 | 0.5 | 1.25 | 0.25 | 0.125 | 0.125 | 1.25 | 2.5 | 1.25 | 2.5 | 0.025 |
| *Streptococcus pyogenes* (ATCC 19615) | 0.125 | 2.5 | 2.5 | 2.5 | 0.125 | 1.25 | 0.25 | 0.125 | 1.25 | 2.5 | 2.5 | 1.25 | 0.25 | 0.00006 |

[A]A = Ampicillin.

geometrically optimized and energy minimized on to 3D structure using Dundee PRODRG-2[26] server and retrieved in PDB format for docking. The docking studies of all the synthesized compounds were carried out in Molegro Virtual Docker.[27]The structures of docked compounds ($AB_1$–$AB_{12}$) and **4b**, **4d** & **4e** are shown in Figure 1.

Protein ligand interactions were carried out in Molegro virtual docker (4.3.0).[28] Ligand free receptor proteins were imported. The missing bond orders, hybridization and charges were assigned to the protein which is implemented in the program itself. Potential binding sites were detected using cavity detection algorithm and docking was performed with the twenty two predicted ligands. The cavity docking procedure was carried with an energy grid resolution of 0.30 A° taking the default parameters. The algorithm Mol Dock SE includes total number of runs as 10, population size of 50 and the Maximum Iterations-1500. The output was visualised in Molegro Virtual docker. The docked structures of the compounds 4 h and 4 m in model of $K^+$ channel are shown in Figures 2.1 and 2.2. Hydrogen bonding interactions of the DHP with ball and stick model of protein are shown in Figs. 3.1 and 3.2. Binding conformation of DHP and space occupied (green wireframe around ligand) in the 1BL8 binding pocket involved in its recognition are shown in Figs. 4.1 and 4.2.

In order to find out the higher docked score DHPs, we docked different sets of DHP analogues. The mole binding affinity suggest that analogues from β-ketoester (ethyl acetoacetate) have a higher binding affinity to the potassium receptor (KcsA) than the other DHP analogues from β-diketones [1,3 cyclohexadione ($AB_1$–$AB_3$), acetyl acetone ($AB_4$–$AB_6$) and dimedone ($AB_7$–$AB_9$)] Fig. 1. Based on the in silico predictions we have synthesized thirteen DHP analogues with ethyl acetoacetate as one of the substrates. Out of the thirteen, synthesized compounds, **4m** exhibited high binding affinity (−152 Kcal/mole) followed by the compounds **4j**, **4h**, **4g**, **4d**, **4f**, **4a**, **4b**, **4c**, **4i**, **4e** and the compounds **4k** (−105 Kcal/mole) and **4l** (−98 Kcal/mole) exhibited low binding affinity. In compound **4l** there is no aromatic ring at 4th position, this suggests that the presence of aromatic ring enhances the ligand protein binding affinity interactions, which can be further enhanced by the presence of substituted aromatic rings with substituents like methoxy, hydroxy chlorine and nitro groups (**4m**, **4h**, **4i**, **4j**, **4f**, **4a**, **4b**, **4c**). DHPs with methoxy substituted aromatic ring at 4th position exhibits the highest binding affinity (**4m** and **4j**). A summary of the results are provided in Table 3.

All the synthesized compounds were evaluated against ten bacterial species. In addition, twenty seven clinically relevant bacterial isolates obtained from a certified diagnostic laboratory have also

**Table 5**
Minimum inhibitory concentration values in (μg/ml) against ESBL isolates

| Entry | Compound | *Escherichia coli* (15 isolates)[a] | *Klebsiella pneumoniae* (12 isolates)[a] |
|---|---|---|---|
| 1 | **4a** | 125–250 | 125–500 |
| 2 | **4b** | 125–250 | 125–250 |
| 3 | **4c** | 125–250 | 125–250 |
| 4 | **4d** | 62.5–125 | 125–250 |
| 5 | **4e** | 62.5–125 | 31.25–125 |
| 6 | **4f** | 125–250 | 62.5–250 |
| 7 | **4g** | 62.5–125 | 31.25–250 |
| 8 | **4h** | 31.25–62.5 | 31.25–62.5 |
| 9 | **4i** | 125–250 | 125–500 |
| 10 | **4j** | 250–500 | 250–500 |
| 11 | **4k** | 125–250 | 125–500 |
| 12 | **4l** | 125–250 | 62.5–250 |
| 13 | **4m** | 250–500 | 125–500 |

[a] Bacteria resistant to > 2000ug/ml of Ampicillin

been assayed. In vitro studies suggest that all the synthesized compounds possess antimicrobial activity which varies in different bacterial isolates. Among the compounds synthesized, **4m** has shown the greatest binding affinity to the receptor compared to the other compounds. This compound is also found to possess good antibacterial activity to most of the bacterial species studied. However, compounds **4h**, **4a**, **4g**, and **4f** are found to be the most effective bactericides. In silico studies validate these results as the mol binding affinity of analogues **4h**, **4a**, **4g** and **4f** are −138, −133, −134 and −133, respectively and have good affinity to the receptor. Compounds **4i**, **4b**, **4d**, and **4c** are less effective bactericides Table 4.

Drug resistance in bacteria has increased at an alarming rate.[29] Many bacterial strains have become resistant to all third generation antibiotics viz. Ampicillin, Tetracycline, Cotrimoxazole, Amoxicillin, Nalidixic acid and Fluoro quinolones. In the present study, the synthesized analogues have been bioassayed against 15 isolates of *Escherichia coli* and 12 isolates of *Klebsiella pneumoniae*. Our study suggests that all isolates of bacteria are sensitive to the synthesized analogues. All the bacterial species used in the present study are known to synthesize β-lactamase and could resist Ampicillin as high as 2000 μg/ml. The concentration of DHP analogues ranged from 31.25 μg/ml to 500 μg/ml. Thus, the compounds are known to be 4 to 12-fold more effective than Ampicillin. A summary of the results are provided in Table 5.

In Summary we have presented a simple practical methodology, for the solvent free synthesis of 1,4 dihydropyridines and Mol docked against K+ channel receptor (KcsA). Although the docking simulation suggested a good binding affinity of DHP to K+ channel receptor, in vitro studies did not validate in silico studies. The synthesized compounds were effective antimicrobial agents against ESBL isolates. The study suggests that the synthesized compounds may probably use a different target site other than β-lactam site. Whether K+ channels are involved in this mechanism needs to be further investigated. All the experimental work presented at end note. [31]

*4-(2-Hydroxy-phenyl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid diethyl ester (**4g**)*: mp 94–99 °C. IR (KBr) $cm^{-1}$: 3362, 3320, 2980, 2950, 1676, 1510, 1310, 1232, 1210, 1207, 772. $^1H$ NMR (200 MHz $CDCl_3$): δ 9.5 (s, 1H, OH) 7.80–7.70 (m, 2H), 7.32–7.20 (m, 2H), 6.12 (s, 1H), 5.49 (br s, 1H, NH), 4.22 (q, *J* = 7.2 Hz, 4H), 2.30 (s, 6H), 1.24 (t, *J* = 7.2 Hz, 6H); $^{13}C$ NMR (50 MHz $CDCl_3$) δ 168.45, 153.20, 144.8, 144.20, 136.43, 105.12, 104.20, 59.60, 19.20, 14.26 ppm; Anal. Calcd for $C_{19}H_{23}NO_5$ (%): C, 66.07; H, 6.71; N, 4.06. Found: C, 62.25; H, 6.03; N, 3.68.

*4-(3,4,5-Trimethoxy-phenyl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid diethyl ester (**4j**)*: mp 125–127 °C. IR (KBr) $cm^{-1}$: 3320, 2980, 2930, 1689, 1507, 1310, 1232, 1207, 782. $^1H$ NMR (200 MHz $CDCl_3$): δ 6.48 (s, 1H), 6.42 (s, 1H), 5.49 (br s, 1H, NH), 4.91(s, 1H), 4.20 (q, *J* = 7.2 Hz, 4H), 3.80 (s, 6H), 3.79 (s, 3H), 2.12 (s, 6H), 1.24 (t, *J* = 7.2, Hz, 6H); $^{13}C$ NMR (50 MHz $CDCl_3$): δ 168.45, 153.20, 144.8, 144.20, 136.43, 105.12, 104.20, 59.60, 56.44, 39.21, 19.20, 14.26 ppm; Anal. Calcd for $C_{22}H_{29}NO_7$ (%): C, 62.99; H, 6.97; N, 3.34. Found: C, 60.75; H, 6.03; N, 3.02.

*4-(2,5-Dimethoxy-phenyl)-2,6-dimethyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid diethyl ester (4m)*: mp 151–153 °C. IR (KBr) $cm^{-1}$: 3320, 2923, 2854, 1689, 1649, 1300, 1250, 1211, 787. $^1H$ NMR (200 MHz $CDCl_3$): δ 6.74 (m, 1H), 6.54 (m, 2H), 5.79 (br s, 1H, NH), 5.12 (s, 1H), 4.11 (q, *J* = 7.2, Hz, 4H), 3.82 (s, 3H), 3.81 (s, 3H), 2.14 (s, 6H), 1.24 (t, *J* = 7.2 Hz, 6H); $^{13}C$ NMR (50 MHz $CDCl_3$): δ 168.30, 153.25, 151.22, 144.23, 137.31, 117.68, 111.77, 110.14, 103.7, 59.37, 55.50. 38.81, 19.98, 14.26 ppm; Anal. Calcd for $C_{21}H_{27}NO_6$ (%): C, 64.77; H, 6.99; N, 3.60. Found: C, 63.75; H, 6.03; N, 3.02.

## References and notes


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31. Endnote (a) *Biology experimental*: One hundred micro liters suspension containing $10^8$ CFU/ml of bacteria was inoculated on the surface of Muller-Hinton agar medium (MHA) plates. The compound dissolved in 2% DMSO served as negative control and Amplicine was used as a standard reference to determine the sensitivity of each microbial species tested for antibacterial. The Minimum Inhibitory Concentrations (MIC) Table 4 of the compounds was estimated as per the guidelines of clinical laboratory standard[30]. Bacterial suspensions were prepared by suspending the cultures grown for 24 h in sterile normal saline. The turbidly of the bacterial suspension was adjusted to McFar land standard of 0.5 which is equivalent to $1.5 \times 10^8$ CFU/ml .Twofold serial dilution of each compound was prepared in MHA. The MIC of the compounds (**4a**–**m**) ranged between (0.125–5 mg/ml). **(b)** *General procedure for the synthesis of 1,4 Dihydropyridines:* aldehyde (0.01 moles), ethyl acetoacetate (0.025 moles) and ammonium acetate (0.02 moles) were refluxed at 100 °C under solvent free conditions for 2–5 h employing 0.05 g of cellulose sulfuric acid, the reaction was monitored by TLC. The reaction mixture was poured in to a ice cold water and extracted with ethyl acetate and dried over anhydrous magnesium sulfate. Purified through column chromatography and recrystalized in ethanol. Melting points were determined in open capillaries and all are uncorrected. IR spectra were recorded on a Shimadzu IR affinity-1. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker DRX 200 and 50 MHz respectively. NMR spectra were obtained on solutions in $CDCl_3$. Mass spectra were recorded on water XEVO QT of mass spectrometer.