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Zinc Montmorillonite – A Harmonious Clay Catalyst for the Synthesis of Hantzsch 1,4-Dihydropyridine Antimicrobial Agents

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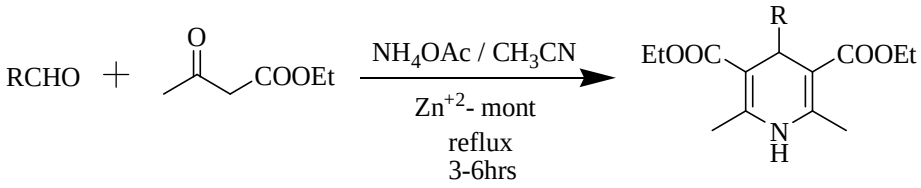
ABSTRACT

A series of 1,4-dihydropyridines were accomplished by zinc-montmorillonite catalysed one-pot synthesis using a reaction between aldehydes, ammonium acetate and ethyl aceto acetate in ethanol - dimethyl formamide (3:1) under reflux conditions. Out of the ten analogs, methoxy, methyl, isopropyl and 4-nitro substituted analogues have exhibited higher antibacterial activity and antifungal activity. The binding energies measured using molecular docking studies, too, found in agreement with activity conforming the invitro studies. The interaction between the protein and the compounds proposed in this study are useful for understanding the potential mechanism of inhibitor binding.

Graphical Abstract:



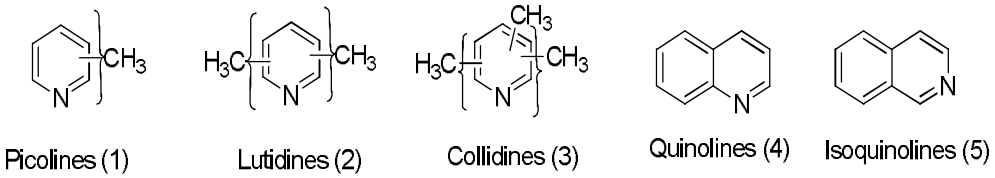
R= 4-OCH_3-Ph-, 4-F-Ph, 4-Cl-Ph, 4-Me-Ph, 4-^{i}Pr-Ph, 4-^{t}Bu-Ph, 4-NO_2-Ph, 3-NO_2-Ph, 4-OH-Ph, 2-Naphthyl

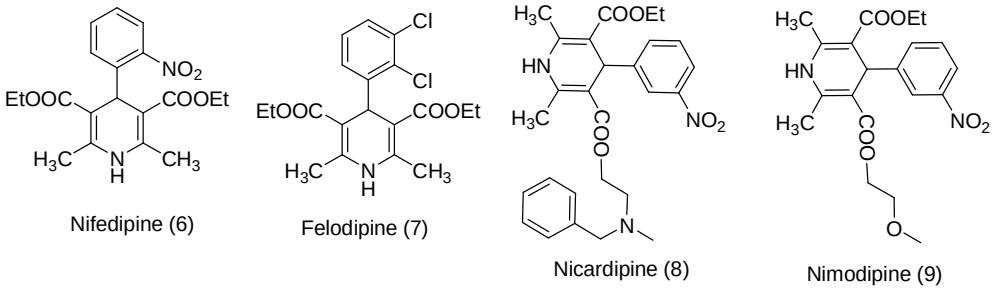
Keywords: Multi component reactions (MCRs), 1,4-dihydropyridines, zinc montmorillonite, Ethanol-DMF (3:1), Antimicrobial activity.

INTRODUCTION

Heterocyclic compounds find unique position in the field of medicinal chemistry, in which, one nitrogen containing systems are simple and potential candidates such as picolines (1), lutidines (2), collidines (3), quinolines (4) and isoquinolines (5)..etc exhibit diverse properties. A partially saturated pyridine analogues- 1,4-Dihydropyridines (DHPs)- exhibited a range of biological activities stretching from anticonvulsant activity, [1] selective adenosine-A3 receptor antagonism, [2] radio protective activity, [3] sirtuin activation, inhibition, [4] to calcium channel blockers [5] such as Nifedipine (6), Felodipine (7), Nicardipine (8) and Nimodipine (9) to name a few. In this light these are found to be the most important compounds in the daily life and synthesis of such life saving drugs incorporated various strategies such as

Hantzsch synthesis, reduction of pyridines, addition to pyridines or cycloadditions etc. [6-8] involving the catalysts like lewis acids, [9] organocatalysts - ephedrine, L-pipecolic acid, L-proline and nanocatalysts CuO, [10] silica-supported perchloric acid ($HClO_4$-SiO_2) and $InCl_3$ [11].





A multi component reaction providing 1,4-Dihydropyridines (DHPs) involved either two component condensation of β-enamino esters with α,β-unsaturated aldehydes, a three-component reaction of a 1,3-dicarbonyl compound, primary amine and α, β-unsaturated aldehyde [12] or four-component synthesis of 1,4-DHPs [13]. Originally 1,4- DHPs were synthesized using the homo-condensation of the same dicarbonyl compound, but in the following years hetero-condensation was also employed in getting the molecules of interest. More recently, synthesis of 1,4-DHPs has been developed using cellulose sulfuric acid (in water) [14], ionic liquids [15], amino acids (with sugar aldehydes) [16]. But most of the above mentioned procedures suffer from serious issues like tedious working procedures, loss of compound during workup, poor yields and environmental hazardness. Added to this, enactment of stringent laws forced the chemists to develop methods that remain environ friendly simultaneously more productive.

Herein, we report environmental, economically benign, reusable and recyclable heterogeneous solid acid catalyst Zn^{+2}- montmorillonite for the synthesis of 1,4-DHPs. Actually interlamellar space in montmorillonite is sufficient for the adsorption of heavy metal ions to give inner sphere complexes following cation exchange mechanism using the Si–O and Al–O groups at the edges of clay particle [17-19]. The sandwiched metal cations can undergo exchange with cations from external solutions. In addition, organic molecules too can be covalently anchored to layer atoms. This provides tremendous scope for altering the properties of clays like acidity, pore size, surface area, polarity and other characteristics that govern their performance as catalysts [20, 21]. In this work, 1,4-Dihydropyridine ester derivatives were synthesized and evaluated for their antibacterial and antifungal studies using In Vitro and Insilico studies.

MATERIALS AND METHODS

Melting points were determined using a Buchi R-535 apparatus and are uncorrected. All reactions were monitored by thin-layer chromatography (TLC) using silica-coated plates and visualization under ultraviolet (UV) light. Yields refer to pure products isolated after column chromatography. ^{1}H NMR spectra were recorded on Varian FT 200-MHz (Gemini) and Bruker UXNMR FT 300-MHz (Avance) instruments in $CDCl_3$. Chemical shift values were reported in parts per million (δ) relative to tetramethylsilane (TMS) (δ 0.0) as an internal standard. Mass spectra(MS) were recorded under electron impact at 70 eV on an LC-MSD instrument (Agilent Technologies). Catalyst Zn^{+2} – montimorillonite was prepared according to the literature procedure [25].

General Procedure for synthesis of 1,4-dihydropyridines (3a-j): A mixture of aldehyde (1 mmol), ethyl acetoacetate (2 mmol), NH_4OAc (1.5 mmol), and Zn^{+2} – montimorillonite (0.1 mmol) was stirred and refluxed in ethanol-dimethyl formamide (3:1) for an appropriate time as mentioned in table 1. After completion of the reaction as indicated by TLC, solvent was removed and ethyl acetate was added to the residue. Ethyl acetate was washed with water and brine solution, dried, and concentrated. The crude residue was purified by column chromatography (EtOAc-hexane, ratio1:3) to afford the pure product. The structure of the products was confirmed by spectral data (IR, ^{1}H NMR, Mass).

Compound 3a: Mp 159˚C, 89%, 4.2 hrs; IR (KBr): 3342, 2923, 2856, 1692, 1649, 1493, 831, 746, 680, 592 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.06 (t, J=7.5 Hz, 6H), 2.34 (s, 6H), 3.78 (s, 3H), 4.01 (q, J=8.32 Hz, 4H), 4.81 (s, 1H), 5.41 (br s, 1H), 6.71 (d, 2H), 7.12 (d, 2H); EI MS: m/z 359 (molecular ion), 252 (M-107) peak corresponds to the loss of 4-methoxy phenyl ring.

Compound 3b: Mp 139˚C, 86%, 4hrs; IR (KBr): 3337, 2980, 2930, 1682, 1504, 855, 753, 677 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): 1.21-1.23 (t, J=7.8 Hz, 6H), 2.41 (s, 6H), 4.01 (q, J=8.20 Hz, 4H), 4.89 (s, 1H), 5.54 (brs, 1H), 6.81-7.21 (m, 4H), ESI MS: m/z 347.

Compound 3c: Mp 144˚C, 92%, 3.4 hrs; IR (KBr): 3337, 2926, 2830, 1682, 1487, 855, 753, 677 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): 1.21 (t, J=7.45Hz, 6H), 2.32 (s, 6H), 4.12 (q, J=8.0 Hz, 4H), 4.89 (s, 1H), 5.58 (br s, 1H), 7.35 (d, 2H), 7.51 (d, J=9.06 Hz).

Compound 3d: Mp 115˚C, 91%, 3 hrs; IR (KBr): 3358, 2925, 1696, 1651, 1487, 849, 786, 610 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): 1.21 (t, J=8.3 Hz, 6H), 2.21 (s, 3H), 2.31 (s, 6H), 4.04 (q, J=8.4 Hz, 4H), 4.83 (s, 1H), 5.57 (br s, 1H), 6.95 (d, J=8.08 Hz, 2H), 7.16 (d, J=7.3 Hz, 2H); ESI MS: m/z 344.

Compound 3e: Mp 97˚C, 85%, 5.2 hrs; IR (KBr): 3355, 2926, 2856, 1702, 1682, 1484, 856, 681 cm^{-1} ; ^{1}H NMR (300 MHz, CDCl3): 1.11-1.32 (m, 12H), 2.60 (s, 6H), 2.89 (m, 1H), 4.12 (q, J=8.4 Hz, 4H), 4.91 (s, 1H), 5.45 (br s, 1H), 7.01 (d, J=8.36 Hz, 2H), 7.21 (d, J=8.3 Hz, 2H); ESI MS: m/z 291.

Compound 3f: Mp 95˚C, 90%, 5.1 hrs; IR (KBr): 3340, 2962, 2869, 1727, 1695, 1487, 680, 556 cm^{-1}; ^{1}H NMR (200MHz, $CDCl_3$): 1.01-1.19 (m, 15H), 2. 81 (s, 6H), 4.05 (q, J=8.4 Hz, 4H), 4.86 (s, 1H), 5.42 (br s, 1H), 7.01–7.20 (m, 3H), 7.41–7.50 (m, 1H); ESI MS: m/z 385.

Compound 3g: Mp 128–129˚C, 81%, 3.3 hrs; IR (KBr): 3322, 2927, 2856, 1702, 1647, 1486, 859, 753, 601 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.25 (t, J=8.2 Hz, 6H), 2.34 (s, 6H), 4.09 (q, J=8.4 Hz, 4H), 5.02 (s, 1H), 5.76 (br s, 1H), 7.41 (d, J=8.56 Hz, 2H), 8.08 (d, J=8.56 Hz, 2H); ESI MS: m/z 374.

Compound 3h: Mp 178˚C, 83%, 4.3 hrs; IR (KBr): 3358, 2925, 2854, 1646, 1484, 808, 663 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): 1.24 (t, J=8.3 Hz, 6H), 2.31 (s, 6H), 4.09 (q, J=8.4 Hz, 4H), 5.02 (s, 1H), 5.71 (br s, 1H), 7.01–7.16 (m, 4H); ESI MS: m/z 374.

Compound 3i: 165˚C, 87%, 4.5 hrs; ^{1}H NMR (200 MHz, $CDCl_3$): 1.30 (t, J=8.3 Hz, 6H), 1.71 (s, 6H), 4.19 (q, J=8.4 Hz, 4H), 4.42 (s, 1H), 4.71 (br s, 1H), 6.16 (d, 2H), 6.83 (d, 2H); ESI MS: m/z 374.

Compound 3j: Mp 199˚C, 89%, 5.1 hrs; IR (KBr): 3342, 2978, 2929, 1725, 1692, 1488, 860, 756 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.16 (t, J=8.2 Hz, 6H), 2.16 (s, 6H), 4.01 (q, J=8.4 Hz, 4H), 5.08 (s, 1H), 5.67 (br s, 1H), 7.33-8.01(m, 7H); ESI MS: m/z 380.

Antimicrobial Activity Evaluation: In Vitro studies

Antibacterial activity, Cup plate method: The antibacterial activity study of synthesized compounds (**3a-3j**) was conducted against two gram positive bacteria viz., *Bacillus subtilis, Bacillus pumilis*, and two

gram negative bacteria viz., *Escherichia coli* and *Proteus vulgaris* by using cup plate method. Ampicillin sodium was employed as standard to compare the results. Nutrient broth was used for the preparation of inoculum of the bacteria and nutrient agar was used for the screening method.

The test organisms were sub-cultured and inoculated onto the nutrient agar medium. After incubation at 37°C ± 1°C for 24 h, they were stored in a refrigerator. Bacteria inoculum was prepared by transferring a lapful of stock culture to the nutrient broth (100 mL) in conical flasks (250 mL). The flasks were incubated at 37 °C ± 1 °C for 48 h before the experimentation. Solutions of the test compounds were prepared by dissolving 10 mg each in dimethylformamide (10 mL Anal R. Grade). A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of Ampicillin sodium in sterile distilled water, separately. The nutrient agar medium was sterilized by autoclaving at 121 °C (151 lb sq.inch^{-1}) for 15 min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot-air oven at 160°C for an hour and into each sterilized petriplate (10 cm diameter) about 27 mL of molten nutrient agar medium was poured and inoculated with the respective strain of bacteria (6 mL of inoculum to 300 mL of nutrient agar medium) aseptically. The plates were left at room temperature to allow the solidification. On each plate, three cups of 6 mm diameter were made with sterile borer. Then 0.1 mL of the test solution was added to the respective cups aseptically and labeled accordingly. The plates were kept undisturbed for at least 2 h in refrigerator to allow diffusion of the solution properly into the nutrient agar medium. After incubation of the plates at 37 °C ± 1 °C for 24 h, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1mL of dimethylformamide to observe the solvent effects. The results are presented in table 2.

Antifungal activity: All those compounds (**3a-3j**) screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were *Aspergillus niger, Rhizopus oryzae* and *Aspergillus flavus*. The test compounds were sub-cultured using potato-dextrose-agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25°C for 48 h, they were stored at 4 °C in refrigerator. The inoculum was prepared by taking a loopful of stock culture to about 100mL of nutrient broth in 250mL conical flasks. The flasks were incubated at 25°C for 24 h before use. The solutions of test compounds were prepared by a similar procedure described under antibacterial activity. A reference standard (1 mg mL^{-1} conc) was prepared by dissolving 10 mg of clotrimazole in 10mL of dimethylformamide (Anal R grade). Further, the dilution was made with dimethylformamide itself to obtain a solution of 100 µg mL^{-1} concentration.

The potato-dextrose-agar medium was sterilized by autoclaving at 121°C (15 lb sq.inch^{-1}) for 15 min. The petri plates, tubes and flasks with cotton plugs were sterilized in hot-air oven at 150°C, for an hour. In each sterilized petri plate, about 27 mL of molten potato-dextrose-agar medium inoculated with the respective fungus (6 mL of inoculum in 300 mL of potato-dextrose-agar medium) was added, aseptically. After solidification of the medium at room temperature, three cups of 6 mm diameter were made in each plate with a sterile borer and 0.1 mL (100µg cup^{-1}) of test solution was transferred accurately to the cups aseptically and labeled accordingly. The reference standard, 0.1 mL (10 µg disc) was also added to the discs in each plate. The plates were kept undisturbed at room temperature at least for 2 h to allow the solution to diffuse properly into the potato-dextrose-agar medium. Then the plates were incubated at 25°C for 48 h. The diameter of the zone of inhibition was read with the help of an antibiotic zone reader. The experiments were performed in triplicate in order to minimize the errors.

Molecular Modeling Studies

Methodology: The series of compounds **3a-j** was docked to Catalase (PDB ID: 4QOL) in Bacillus pumilus and ferulic acid esterase from Aspergillus Niger (PDB ID: 1UZA, R1UZASF). After the unnecessary chains and hetero atoms were removed using SPDBV software, hydrogens were added to the protein and used for active site identification.

Active site Identification: Active sites of Catalase and ferulic acid esterase were identified using CASTp server, for automatically locating and measuring protein pockets and cavities, based on precise computational geometry methods, including alpha shape and discrete flow theory. It identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining the pockets, pocket openings, buried cavities; the volume and area of pockets, cavities and the area, circumference of mouth openings [22].

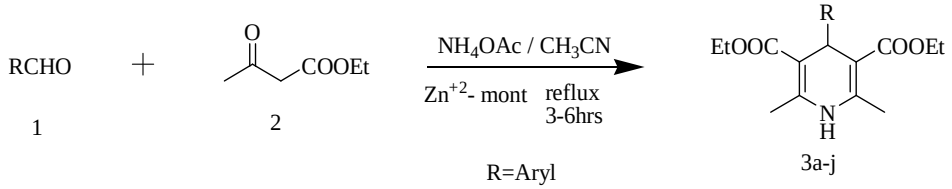
Docking method: Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows partial flexibility of protein and full flexibility of ligand. The compounds were docked to the active site of the protein in Bacillus pumilus and ferulic acid esterase from Aspergillus Niger. The interaction of these compounds with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0A° (dH-X) for hydrogen bonds and 6.0A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the ligand binding site in the Ferulic Acid Esterase from Aspergillus Niger was defined within a 10A° radius with the centroid as CE atom of ARG154. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5A° RMSD (root mean square deviation). After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each ligand was selected [23].

Gold Score fitness function: Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3. Ligand internal vanderWaals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)
Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

RESULTS AND DISCUSSION

Chemistry: In this present work, using Zn^{+2}- montmorillonite, a cost effective procedure for the synthesis of 1,4-DHPs is depicted. It used aromatic aldehydes, ethylacetoacetate and ammonium acetate in 1:2:1.5 ratio in ethanol-dimethyl formamide (3:1) with little excess over 0.1equivalents of catalyst under reflux for 3-6 hrs resulted in products- 1,4-DHPs- in 80-91% overall yield (Scheme-1).



R= a) 4-OCH_3-Ph-, b) 4-F-Ph, c) 4-Cl-Ph, d) 4-Me-Ph, e) 4-^{i}Pr-Ph, f) 4-^{t}Bu-Ph, g) 2-NO_2-Ph, h) 3-NO_2-Ph, i) 4-OH-Ph, j) 2-Naphthyl

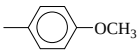
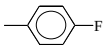
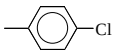
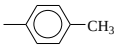
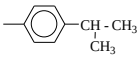
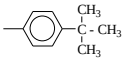
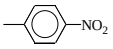
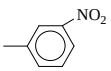
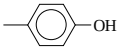
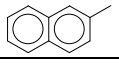
Scheme-1: Synthesis of 1,4-dihydropyridines

Encouraged by this, a series of ten compounds was synthesized that differed in the nature of the substituent on the aromatic aldehyde. Both the electron donating and electron withdrawing sunbstituents like alkyl, alkoxy, nitro and hydroxy groups on the ring (mono or bicyclic systems) gave very good yields of 1,4-DHP esters. The synthesized compounds were identified by comparing their ^{1}H-NMR chemical shifts and melting point values with the already reported literature [24].

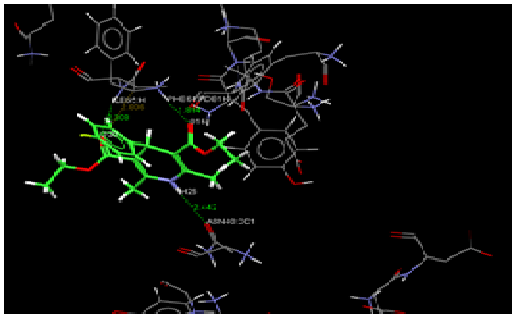
APPLICATIONS

Antimicrobial activity (In vitro studies): The synthesized compounds were tested for their antibacterial activity against two gram positive bacteria (*Bacillus subtilis, Bacillus pumilis*), two gram negative bacteria (*Escherichia coli, Proteus vulgaris*) by using cup plate method and antifungal activity against *Aspergillus niger*, *Rhizopus oryzae* and *Aspergillus flavus strains.* Out of the ten compounds (**3a-3j**) synthesized, compound **3a** with 4-methoxy phenyl substitution was found to be the most effective against the bacterial strains used, with a zone of inhibition matching the standard Ampicillin (18-24 mm) except against E. Coli where the test compound (**3a**) exhibited the better inhibition property over the Control. Even against the fungal strains, **3a** exhibited equivalent potency as that of clotrimazole standard. This is followed by **3d** and **3e** in the order in exhibiting the antimicrobial activity (Table 1). This suggested that aromatic aldehydes with substituents rendering purely either +M.effect or +I.effect exhibited substantial activity whereas other substituents with both +M.effect , -I.effect and –M.effect such as halogens in **3b**, **3c** and nitro, hydroxyl group in **3g**, **3h, 3i** respectively resulted in poor activity. But **3f** with a bulky tertiary butyl group due to steric factor showed the poor activity. This gave some insights regarding the size and electronic effect of the substituents towards the studied properties. It is further reverberated in **3j** where a bicyclic ring - (following the strategy-*fusion of rings*) - as aromatic system- nowhere promoted the activity.

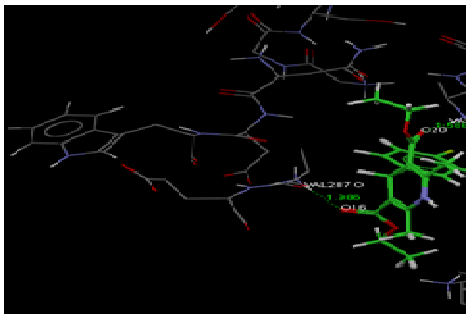
Table 1. Antimicribial activity of 1,4-dihydropyridines (3a-3j): Antibacterial activity and Antifungal activity

Compd	R	Zone of inhibition (in mm)						
		B. pumilis	***B. subtilis***	***E. coli***	***P. vulgaris***	***A. niger***	***R. oryzae***	***A. flavus***
3a		20	22	17	16	17	16	14
3b		3	4	6	-	3	2	-
3c		4	6	7	4	7	5	-
3d		10	9	11	10	11	9	8
3e		10	12	12	11	9	8	7
3f		8	7	8	6	8	7	-
3g		6	7	6	2	7	6	-
3h		3	6	7	-	6	3	-
3i		6	9	9	6	8	7	-
3j		7	9	8	7	8	6	5
Ampicillin (10g/cup)		20	24	13	18	-	-	-
Clotrimazole (10 µg/cup)		-	-	-	-	20	18	16

Antimicrobial activity (Insilico studies): Invitro results were further verified in molecular modeling studies. In the active site of Catalase protein of Bacillus pumilus, among the synthesized derivatives (**3a-j**), according to the fitness scores (Table 2), **3a** was found to be the better binding ligand. It actually binds with amino acid residues ASN40:OD1 through the hydrogen bonding (d= 2.442 ˚A) using its secondary amine (H26) and amino groups of ILE65:H, PHE 64 coordinated with ester carbonyls O20, O16 of DHP via the hydrogen bondings (d= 2.308 ˚A, 1.894 ˚A) respectively. Similarly all the other compounds interacted with the same amino acid residues in the active site but with poor coordination resulting in the feeble fitness score. In case of the antifungal studies the synthesized compounds were docked in the active site of the Ferulic Acid Esterase from Aspergillus Niger and the aminoacid residues which held the ligands were VAL 287 and VAL 325 (Table 3). Out of all the compounds **3a** was held firmly by the intermolecular hydrogen bonding (d= 1.986 ˚A, 1.588 ˚A) between the ester carbonyls O16, O20 with the amino groups of VAL 287, VAL 325 respectively. In the rest of the analogues **3d, 3e** and **3g** were moderately active.



3a in the active site of
Catalase protein of Bacillus pumilus



3a in the active site of the
Ferulic Acid Esterase from Aspergillus Ni

Table 2: Docking fitness score for Antibacterial activity

Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	Ligand name
20.38	12.00	12.21	0.00	-8.41	3a
9.62	8.90	7.92	0.00	-10.18	3b
9.49	9.04	9.78	0.00	-12.99	3c
18.66	8.53	14.63	0.00	-9.98	3d
17.82	6.00	15.79	0.00	-9.90	3e
0.47	8.82	9.17	0.00	-20.97	3f
17.61	6.07	15.31	0.00	-9.51	3g
10.59	9.05	8.28	0.00	-9.84	3h
11.03	8.91	9.37	0.00	-10.76	3i
12.48	8.82	9.81	0.00	-9.83	3j

Table 3: Docking fitness score for Antifungal activity

Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	Ligand name
31.53	12.44	24.13	0.00	-14.10	3a
25.90	12.00	19.08	0.00	-12.34	3b
26.45	11.89	18.26	0.00	-10.54	3c
31.08	12.03	22.51	0.00	-11.90	3d
28.90	12.76	19.09	0.00	-10.11	3e
19.23	12.00	20.34	0.00	-20.73	3f
28.04	16.81	15.67	0.00	-10.31	3g
25.91	12.00	18.56	0.00	-11.61	3h
26.09	12.00	17.50	0.00	-9.97	3i
27.51	12.00	20.02	0.00	-12.01	3j

CONCLUSIONS

For three component Hantsch 1,4-dihydropyridine synthesis, Zn^{+2} – montimorillonite was found to be an effective catalyst with an overall yield of 80-91% under less than 6 h of reluxing. At the end of the reaction, product can recovere in high purity simply by filtering off the catalyst followed by the removal of solvent under vaccum.

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