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**Research Article**

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# Synthesis, Charecterisation and biological Evaluation of Novel Hantzsch 1,4-dihydropyridines

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**ABSTRACT**

*We report a library consisting of some novel Hantzsch dihydropyridines of biological interest as well as their synthesis and analysis. The important steps in the synthetic part were found to be Hantzsch reactions. The synthesized compounds were screened for their in vitro antibacterial activity against two gram-positive bacteria: Staphylococcus aureus and Bacillus subtilis. The title compounds did not exhibit potential antibacterial activity. Furthermore, compounds were subjected to in vitro cytotoxicity against Vero cells. Compounds exhibited weak, moderate, or high cytotoxicity. Compounds 9b, 9c, 9d, 9e, 9f, 9g exhibited potential cytotoxicity.*

**Keywords:** Dihydropyridines Cytotoxicity, Antibacterial activity, Vero cells.

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## INTRODUCTION

Dihydropyridines (Eisner and Kuthan, 1972; Stout and Meyers, 1982; Kappe, 1993, 2003) represent important and extensively studied compounds belonging to the class of calcium channel blockers. Many researchers have attempted to determine the synthetic routes and various biological activities of these compounds. These developments led to the preparation and pharmacological evaluation of 1,4-dihydropyridines (DHP) of biological interest (Loev et al., 1972, 1974; Meyer et al., 1981; Mager et al., 1992; Kappe, 2000a, b; Dallinger et al., 2004). The discovery during the 1930s that a dihydropyridine (dihydronicotinamide derivative, NADH), ‘‘hydrogen transferring coenzyme’’ consequently became important in biological system, has generated numerous studies on the biochemical properties of dihydropyridines (Bruce and Bencovic, 1966). The dihydropyrimidine-5-carboxylate core has been found in several marine natural products (Heys et al., 2000; Aron and Overman, 2004).

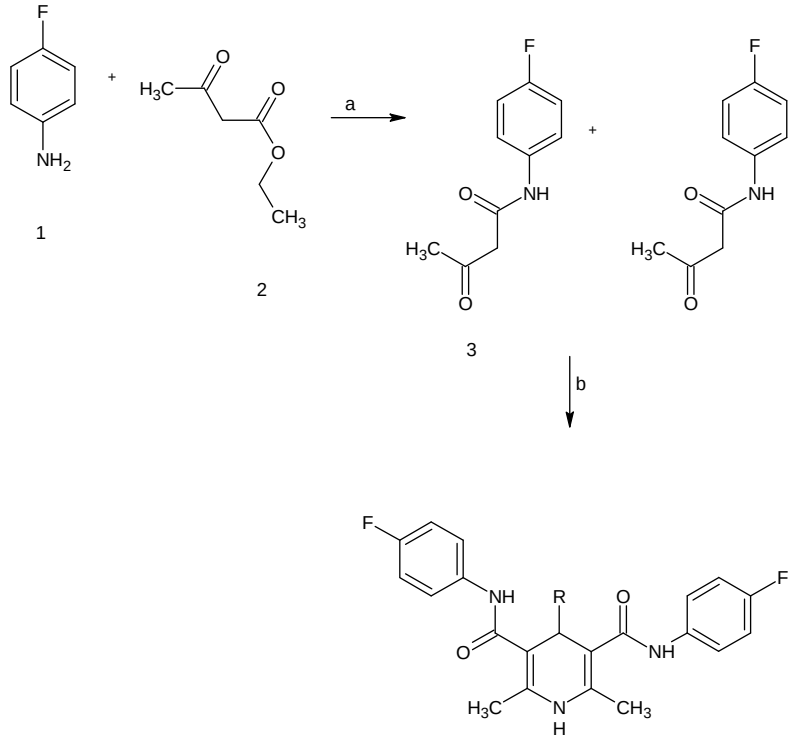
The credit for the first synthesis of dihydropyridines is attributed to Arthur Hantzsch for work performed a century ago (Hantzsch, 1882). The Hantzsch 1,4-dihydropyridines synthesis involves the reaction of a 1,3-dicarbonyl compound with aldehyde and ammonia (Jones, 1984). In 1893, The present interest for Hantzsch 1,4-dihydropyridines is mainly due to their close structural relationship to similar drugs and compounds reported in the literature for their antitubercular (Desai et al., 2001; Prashantha Kumar et al., 2008), antimicrobial (Hooper et al., 1982; Snider and Shi, 1993; Aron and Overman, 2004), and anticancer activities (Hattori et al.,2003; Tsuji et al., 2004).

## EXPERIMENTAL SECTION

### Materials and Methods

The entire chemicals were supplied by E.Merck (Germany) and S.D fine chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system ethanol, chloroform, ethylacetate (7:2:1), the spots were located under iodine vapors or UV light. IR spectrums were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets).1H–NMR spectra were recorded or a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO/ CDCl3. The chemical shifts were reported in d ppm. Mass spectra were obtained using Shimadzu LCMS 2010A under ESI ionization technique.

**Scheme: I**




*Scheme: Reagents and conditions for the synthesis of DHPs: (a) neat, reflux, 6 h and (b) RCHO, ethanol, 25–30% aqueous ammonia solution, reflux for 13–17 h Procedure for the preparation of N-(4-fluorophenyl)-3-oxobutanamide (1)*

1 (0.01 M) and 2 (0.01 M) were mixed and refluxed for approximately 6 h. The Colourless liquid formed was then heated on a water bath to remove the alcohol formed during the reaction. After allowing the reaction mixture to cool, crude crystals were obtained. Purification was performed by stirring crude crystals with cold diethyl ether for approximately 20 min using a mechanical stirrer. After allowing it to stand for 30 min, followed by filtration, gave 3 in a pure form.

*N*-(4-fluorophenyl)-3-oxobutanamide (1)
White crystalline solid, mp 168 $^{o}$C, Yield 30%, IR (KBr, cm-1): 3246 (N–H), 3072 (ArC–H), 2980 (AliC–H), 1716(C=O, ketone), 1658 (C=O, amide), 1554 (C=C), 1344 (C–N), 1H-NMR (DMSO-d6) d: 2.21 (s, 3H, CH3), 3.58 (s,2H, CH2), 7.36 (d, 2H, ArH), 7.64 (d, 2H, ArH), 10.28 (s, 1H, NH).

General procedure for the preparation of 1,4-dihydropyridines by one pot-multicomponent, Hantzsch method of synthesis (9a–k):
Preparation of 9a–k by one pot multicomponent reaction was performed according to Scheme-I . The mixture of intermediate product I (0.01 M), appropriate aldehyde (0.005 M), and 3 ml of 25–30% aqueous ammonia solution were transferred to a round bottom flask containing 12 ml of ethanol. The reaction mixture was refluxed for 10–16 h. One milliliter of 25% aqueous ammonia solution was added for every 2 h during the reflux. The reactions were monitored through TLC using 20% ethyl acetate in pet ether as solvent system. Soon after the reaction was completed, the reaction mixture was allowed to cool. The solid product formed was filtered and washed with cold methanol to get Hantzsch compounds. Hantzsch condensation reactions can be performed in a parallel synthetic way with ease.

N,N-Bis(4-flurophenyl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxamide (9a)
Colorless amorphous solid, IR (KBr, cm-1): 3290 (N–H), 3074 (ArC–H), 3000 (AliC–H), 1654 (C=O, amide), 1492 (ArC=C), 1334 (C–N), 706 (C– $NO_2$), 1H-NMR (DMSO-d6) d: 2.09 (s, 6H, (CH3)2), 5.04 (s, 1H, CH), 6.18 (bs, 1H, NH), 7.24 (d, 4H, ArH), 7.32 (d, 4H, ArH), 7.48 (m, 1H, CH), 7.62 (m,4H, ArH), 9.31 (s, 2H, (NH)2).

N,N-Bis(4-flurophenyl)-4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9b)
White amorphous solid, IR (KBr, cm-1): 3338 (N–H), 3001 (ArC–H), 2920 (AliC–H), 1666 (C=O, amide), 1512 (ArC=C), 1316 (C–N), 1242 (C–O), 678 (C– $NO_2$), 1H-NMR (DMSO-d6) d: 2.02 (s, 6H, (CH3)2), 3.67 (s, 3H, OCH3), 5.10 (s, 1H, CH), 5.82 (bs, 1H, NH), 6.78 (d, 2H, ArH), 7.18 (d, 2H, ArH), 7.34 (d, 4H, ArH), 7.64 (d, 4H, ArH), 9.46 (s, 2 H, (NH)2).

N,N-Bis(4-flurophenyl)-4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9c)
Colorless amorphous solid, IR (KBr, cm-1): 3299 (N–H), 3126 (ArC–H), 3068 (AliC–H), 1668 (C=O, amide), 1539 (ArC=C), 1253 (C–N), 1166 (C–O), 690 (C– $NO_2$). 1H-NMR (DMSO-d6) d: 2.12 (s, 6H, (CH3)2), 5.16 (s,1H, CH), 6.16 (bs, 1H, NH),7.27 (d, 4H, ArH), 7.48 (d, 4H, ArH), 7.52 (t, 1H, CH), 7.88 (d, 1H, ArH), 7.97 (d, 1H, ArH), 9.18 (s, 2H, (NH)2).

N,N-Bis(4-flurophenyl)-4-(4-hydroxy-3-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9d)
Bluish amorphous solid, IR (KBr, cm-1): 3380 (O–H), 3366 (N–H), 3126 (ArC–H), 3022 (AliC–H), 1664 (C=O, amide), 1518 (ArC=C),1307 (C–N), 1252(C–O), 688 (C– $NO_2$). 1H-NMR (DMSO-d6) d : 2.54 (s, 6H, (CH3)2), 3.58 (s, 3H, OCH3), 5.24 (s, 1H, CH), 5.84 (bs, 1H, NH), 6.84–6.98 (m, 3H, ArH), 7.08(s, 1H, ArH), 7.38 (d, 4H, ArH), 7.49 (d, 4H, ArH), 2H, (NH)2).

N,N-Bis(4-flurophenyl)-4-(4-pyridyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9e)
Colorless amorphous solid, IR (KBr, cm-1): 3282 (N–H), 3114 (ArC–H), 2992 (AliC–H), 1676 (C=O, amide), 1635 (C=C), 1597(C=N), 1326 (C–N), 1246 (C–O), 690 (C– $NO_2$), 1H-NMR (DMSO-d6) d: 2.12 (s, 6H, (CH3)2), 5.08 (s, 1H, CH), 6.22 (bs, 1H, NH), 7.10 (d, 2H, ArH), 7.34 (d, 4H, ArH), 7.64 (d,4H, ArH), 8.10 (d, 2H, ArH), 9.67(d, 2H, (NH)2).

N,N-Bis(4-flurophenyl)-4-(3-nitrophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9f)
Yellowish amorphous solid, 1H-NMR (DMSO-d6) d:2.14 (s, 6H, (CH3)2), 5.36 (s, 1H, CH), 6.38 (bs, 1H, NH), 7.22 (d, 4H, ArH), 7.58 (t,1H, CH), 7.59 (d, 4H, ArH), 7.68 (d, 1H, CH), 7.99 (d, 1H, ArH), 8.08 (s, 1H, ArH), 9.66 (s, 2H, (NH)2).

4-(3-flurophenyl)-N,N-bis(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9g)
Brownish semisolid, 1H-NMR (DMSO-d6) d: 2.02 (s, 6H,(CH3)2), 5.71 (s, 1H, CH), 6.26 (bs, 1H, NH), 7.18 (d, 1H, CH), 7.28 (t, 1H, CH), 5.38 (d, 4H, ArH), 7.46 (d, 2H, ArH), 7.26 (s, 1H, ArH), 7.67 (d, 4H, ArH), 9.58 (s,2H, (NH)2).

(E)-N,N-Bis(4-flurophenyl)-2,6-dimethyl-4-styryl-1,4-dihydropyridine-3,5-dicarboxamide (9h)
Colorless amorphous solid, 1H-NMR (DMSO-d6) d:2.27 (s, 6H, (CH3)2), 5.32 (s, 1H, CH), 6.08 (s, 1H, NH), 6.26 (d, 1H, CH), 6.68 (d,1H, CH), 7.146 (m, 5H, ArH), 7.498 (d, 4H, ArH), 7.62 (d, 4H, ArH), 9.44 (s, 2H,(NH)2).

N,N-Bis(4-flurophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9i)
Yellowish amorphous solid, 1H-NMR (DMSO-d6) d:2.08 (s, 6H, (CH3)2), 3.37 (s, 2H, CH2), 7.38 (d, 4H, ArH), 7.69(d, 4H, ArH), 7.82(s, 1H, NH), 9.81 (s, 2H, (NH)2).

N,N-Bis(4-flurophenyl)-4-(2-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxamide (9j)
Brownish amorphous solid, IR (KBr, cm-1): 3396 (O–H), 3351 (N–H), 3062 (ArC–H), 2984 (AliC–H), 1662 (C=O, amide), 1525(ArC=C), 1316 (C–N), 1234 (C–O), 668 (C– $NO_2$), 1H-NMR (DMSO-d6) d: 2.16 (s,6H, (CH3)2), 5.29 (s, 1H, CH), 5.86 (bs, 1H, NH), 6.63 (d, 1H, ArH), 6.74 (t, 1H,ArH), 6.93 (t, 1H, CH), 7.20 (d, 1H, CH), 7.31 (d, 4H, ArH), 7.61 (d, 4H, ArH), 9.33 (s, 2H, (NH)2), 10.16 (s, 1H, OH).

N,N-Bis(4-flurophenyl)-4-(4-(dimethylamino)phenyl)-2,6-dimethyl-1,4-dihydropyridine -3,5-dicarboxamide (9k)
Yellowish amorphous solid, IR (KBr, cm-1): 3292 (N–H), 3046 (ArC–H), 2938 (AliC–H), 1636 (C=O, amide), 1564 (ArC=C), 1358 (C–N), 1232 (C–O), 633 (C– $NO_2$). 1H-NMR (DMSO-d6) d: 2.14 (s, 3H, (CH3)2), 3.06 (s,6H, 2 9 NCH3), 3.74 (s, 3H, OCH3), 5.72 (s, 1H, CH), 6.08 (bs, 1H, NH), 6.96 (d, 4H, ArH), 7.14 (m, 4H, ArH), 7.38 (d, 4H, ArH), 9.28 (s, 2H, NH). MS (m/z): M?calculated 535, found 535.45.

**Antibacterial activity**

Dihydropyridines are known to posses' antitubercular activity. All the synthesized compounds were screened for their potential antibacterial activity by cup plate method against two gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus subtilis*, because gram-positive bacteria share some common structural features with the Mycobacterium tuberculosis. However, none of the titled compounds exhibited significant antibacterial activity when tested in triplicate at 50 lg concentration, as preliminary antibacterial study. Possibly, it seems that the p-fluroanilide portion of structures would not have contributed toward their antibacterial activity to come out as candidate compounds to investigate further for the same.

**In vitro cytotoxicity assay**

The synthesized compounds were subjected to in vitro cytotoxicity assay against *Vero cells*. The motive for us to check the cytotoxicity for the synthesized compounds was that some reports in the past have claimed significant anticancer activity for similar kind of substructures (Kawase et al., 2002). The assay was performed by the sulforhodamine B (SRB) method (Philip et al., 1990). Almost all of the titled compounds exhibited weak, moderate, or high cytotoxicity. Compounds, 9c, 9d, 9e, 9f, 9g, 9j, 9k exhibited significant cytotoxic activity with lesser CTC50 values (Table 1). system indicate the substitution favoring the activity. Evidence for this is that all the dihydropyridines and dihydropyrimidines possessed the different substitution at that position. The small yellow contour on the nitrogen atoms of the basic bioisosteric scaffolds indicates that there should not be any substitution or steric extension over them or in that region. The small green contour map at the one side of anilide portion indicates that 1,4-dihydropyridines are relatively more potent having anilide portions on either side of the scaffold (9a and 9g). In contrast to this, dihydropyrimidines had anilide portion on one side only and still exhibited the cytotoxic activity (9c exhibited least activity amongst all, whereas 9f and 9g showed significant activity). The yellow and blue contours at the para position of phenyl ring indicated that substitution with bulky (methyl groups) and electropositive substitutions will not favor toward cytotoxic activity .

**Table:1 Synthesized dihydropyridines physical parameter and its cytotoxicity**

| Compound. No; | R | Reaction time | Yield **(%)** | mp $^{o}$C | $CTC_{50}$ (µg/ml) | $pCTC_{50}$ (µMl) |
|---|---|---|---|---|---|---|
| 9a | phenyl | 23 | 51 | 196 | 215 | 0.146 |
| 9b | 4-pyridyl | 21 | 48 | 228 | 138 | 0.222 |
| 9c | 4-pyridyl | 26 | 44 | 199 | 160 | 0.443 |
| 9d | 2-hydroxy phenyl | 20 | 38 | 282 | 160 | 0.554 |
| 9e | 2-hydroxy phenyl | 19 | 42 | 278 | 143 | 0.434 |
| 9f | 3-nitrophenyl | 23 | 38 | 201 | 64 | 0.676 |
| 9g | 3-nitrophenyl | 21 | 41 | 69 | 72 | 0.772 |
| 9h | 3-chlorophenyl | 24 | 39 | 182 | 234 | 0.122 |
| 9i | 3-chlorophenyl | 21 | 42 | 120 | 226 | 0.138 |
| 9j | 2-furyl | 22 | 36 | 173 | 189 | 0.298 |
| 9k | 2-furyl | 20 | 39 | 153 | 152 | 0.352 |

## RESULTS AND DISCUSSION

**Chemistry**

Synthesis of Hantzsch 1,4-dihydropyridines derivatives was achieved by adopting steps as outlined in the Scheme. Refluxing ethyl acetoacetate 1 and 4-fluro aniline 2 under neat conditions for 6h gave *N*-(4-fluorophenyl)-3-oxobutanamide 3 at 30% yield. The *N*-(4-fluorophenyl)-3-oxobutanamide 3 was subjected to Hantzsch method of 1,4-dihydropyridines synthesis. Hantzsch one pot multicomponent reaction involved 0.01 mole of *N*-(4-fluorophenyl)-3-oxobutanamide 3, 0.005 moles of aryl or heteroaryl aldehydes, and excess of 25% aqueous $NH_3$ using ethanol as a solvent under reflux condition for 13–17 h gave a corresponding 1,4-dihydropyridines 9a–k. After recrystallization, yields were found to be between 38 and 51% (Table 1). Most of the reported Hantzsch dihydropyridines were found to be novel.

**Antibacterial activity**

All of the synthesized compounds were screened for their antibacterial activity against two gram-positive bacteria*: B. subtilis* and *S. aureus* (Frankel et al., 1970). The primary screening was performed by using the agar disc-diffusion method using Muller-Hinton agar medium. The compounds were tested at the concentration of 50 lg per well. Sterile nutrient agar plates were prepared in petri dishes under aseptic conditions; 0.1 ml of each standardized test organism was spread onto agar plates and cavity was done by using a sterile borer of diameter 6 mm. Then, 50 lg per well of compounds or standard drug solution of streptomycin (10 lg) and DMSO solvent were placed in each cavity, separately. The plates were maintained at ?4 $^{o}$C for 1 h to allow diffusion of solution into the medium; then the plates were incubated at 37 $^{o}$C for 24 h and observed for zone of inhibition.

**In vitro cytotoxicity**

Short-term in vitro cytotoxicity assay was performed using *Vero cells* according to the standard procedure (Moldeus et al., 1978). SRB is a bright-pink aminoxanthene dye with two sulfonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in trichloroacetic acid fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. After incubation, the solutions in the wells were flicked off and 100 ll of different concentrations (2–500 lg) of compounds were added to the cells and incubated at 37 $^{o}$C for 3 days in 5% CO2 atmosphere. The microscopic examinations were performed and observations were recorded every 24 h. After, 72 h, 50% trichloroacetic acid (25 ll) was added to each well and the plates were incubated for 1 h at 4 $^{o}$C. The supernatant was then removed, and the cells were washed with water, air-dried, and stained, each well with SRB for 30 min. The unbound dye was removed by washing with 1% acetic acid and the plates were airdried. Tris base (10 mM, 100 ll) was added to wells to solubilize the dye. The plates were vigorously shaken for 5 min, and the absorbance was measured using microtiter plate reader at 540 nm. The mean absorbance of triplicate was recorded. Mean absorbance taken from cells grown in the absence of the test compound was taken as 100% cell survival (control). The percentage growth inhibition was calculated using the following formula:

Growth inhibition %= 100 – (sample absorbance/control absorbance) X 100
The percentage growth inhibition was plotted against concentration and the CTC50 (concentration required to reduce viability by 50%) value was calculated.

## CONCLUSION

A series of novel Hantzsch dihydropyridines of biological interest were synthesized and analyzed for their structures. Compounds, such as 9h, 9f, and 9g, exhibited potential cytotoxicity and are considered the candidates to investigate further for the same.

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