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p-TSA Catalysted, One-Pot Synthesis and Antimicrobial Evaluation of some Novel Fused Dipyrazolo-1,4-Dihydropyridine Derivatives

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Abstract Some novel dipyrazolo-1,4-dihydropyridine derivatives have been synthesized *via* one-pot, multi-component condensation of N-phenylpyrazole, aldehyde and ammonium acetate using *p*-TSA as catalyst. The fused dipyrazolo-1,4-dihydropyridine molecules without using expensive starting materials and the molecules are obtained in excellent yield without using any special purification technique. The synthesized compounds were screened for their *in vitro* antibacterial and antifungal activity against six bacterial and four fungal species. Among these **4a**, **4e** and **4h** exhibited significant antibacterial activity against various bacterial and fungal strains.

Keywords *p*-TSA Catalysed, Multi-Component, 1,4-DHPs, MIC, Antibacterial Activity

1. Introduction

The chemistry of 1,4-dihydropyridines (1,4-DHP's) began in 1882 with Hantzsch condensation [1]. After Hantzsch, modifications of the original synthesis were developed [2]. 1,4-dihydropyridines ring system is of considerable interest because of its presence in the coenzyme, diphosphopyridine nucleotide (DPNH) [3] and found it as bio-active material. Now days many representatives have been commercialised such as nifedipine [4], felodipine [5], nicardipine [6], amlodipine [7] and many more have appeared in the market [8] in the treatment of angina and hypertension. It has been proved that their pharmaceutical action is related to binding to voltage dependent L-type of calcium channel and thus decreasing the passage of calcium ions to the cell. The result is relaxation of smooth muscle cells and lowering the blood pressure. DHPs have been explored to possess anti-tumor [8], anti-inflammatory [10], anticonvulsant activity [11], antitubercular activity [12] cerebral antischemic activity in the treatment of Alzheimer's disease, PAF-acether antagonists [13], etc. Apart from these many fused ring system such as Etazolate (SQ-20,009, EHT-0202), ICI-190,622, Tracazolate (ICI-136,753), *etc* are known to show many biologicial activities. By keeping above in mind and continuation of our on hetrocyclics [14], modification to the original Hantzsch pyridines have been carried out by one-pot condensation of 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one, aldehyde and ammonium acetate in acetonitrile using p-TSA as catalyst Scheme 1.

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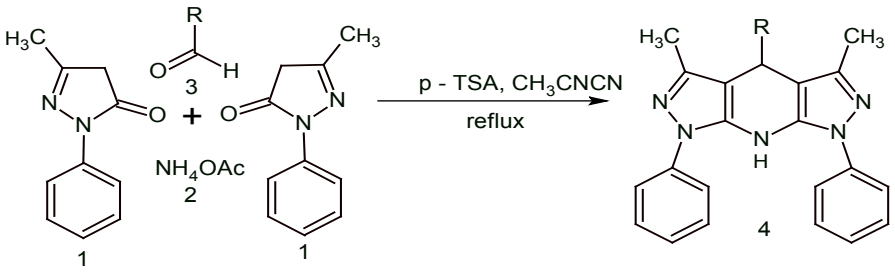
2. Experimental

Materials were obtained from commercial suppliers and were used without further purifications. Melting points were recorded in open end capillaries and are uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker Avance II 400 MHz spectrometer; chemical shifts (delta) are reported in ppm relative to TMS as internal standard. The mass spectrum and IR spectra were recorded at LC-MS Spectrometer Model Q-ToF Micro Waters and Perkin-Elmer Spectrum II infra-red spectrophotometer, respectively. Elemental analyses (C, H, and N) were performed using a Thermo Scientific elemental analyser.

3,5-dimethyl-1,4,7-triphenyl-1,4,7,8-tetrahydrodipyrazolo[*3,4-b:4',3'-e*] *pyridine* **4a**: In a conical flask, Benzaldehyde (1.06mL, 10mmol), N-phenylpyrazole (3.48g, 20mmol) and ammonium acetate (1.155g, 15mmol) in CH_3CN (10 mL) were heated at 90℃ for 3 hours. After the completion of reaction (*vide* TLC), the solvent was removed under reduced pressure. The residue obtained was washed with cold water and recrystalised from ethanol to afford 3,5-dimethyl-1, 4,7-triphenyl-1,4,7,8-tetrahydrodipyrazolo[3,4-*b*:4',3'-*e*]pyri dine 4a, , in 91% yield, colourless, M.p. 188-190℃ (Entry 1, Table 2). Similarly, other aldehydes 3b-h were reacted with N-phenylpyrazole and ammonium acetate to afford various dipyrazolo-1,4-dihydropyridine derivatives 4b-h.



Scheme 1. One-pot three component synthesis of Fused Dipyrazolo-1,4-dihydropyridine Derivatives

Characterization and spectral data for some selected compounds

*3,5-dimethyl-1,4,7-triphenyl-1,4,7,8-tetrahydrodipyrazolo[3,4-b:4',3'-e]pyridine (**4a**):* mp. 188–190°C; IR (KBr): υ = 3360, 3063, 3026, 2975, 1596, 1500, 1283, 1026 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): *δ(ppm)* = 13.84 (br s, 1H, NH), 7.12-7.92 (m, 15H, Ar-H), 5.20 (s, 1H, CH), 2.35 (s, 6H, 2xCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): *δ(ppm)* = 164.3, 159.4, 145.8, 144.9, 138.6, 133.8, 131.6, 129.9, 128.1, 125.2, 116.8, 52.6, 29.4 ppm. Anal. Calcd for $C_{27}H_{23}N_5$: C, 77.67; H, 5.55; N, 16.77. Found: C, 77.66; H, 5.51; N, 16.73. MS (EI) m/z 418 (M+1, 23%).

*3,5-dimethyl-1,7-diphenyl-4-(4-chlorophenyl)-1,4,7,8-tetrahydrodipyrazolo[3,4-b:4',3'-e] pyridine (**4b**)*: mp. 230-231°C; IR (KBr): υ = 3369, 3053, 2977, 2920, 1600, 1501, 1296, 1024 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): *δ(ppm)* = 13.89 (br s, 1H, NH), 7.16-7.91 (m, 14H, Ar-H), 5.24 (s, 1H, CH), 2.38 (s, 6H, 2xCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): *δ(ppm)* = 166.4, 160.1, 146.3, 145.8, 139.8, 136.2, 133.4, 130.1, 129.2, 127.4, 117.9, 52.8, 30.1 ppm. Anal. Calcd. for $C_{27}H_{22}N_5Cl$: C, 71.75; H, 4.91; N, 15.50. Found: C, 71.72; H, 4.91; N, 15.47; MS (EI) m/z 453 (M+1, 18%).

*3,5-dimethyl-1,7-diphenyl-4-(3-nitrophenyl)-1,4,7,8-tetrahydrodipyrazolo[3,4-b:4',3'-e] pyridine (**4c**):* mp. 246-248 °C; IR (KBr): υ = 3436, 3075, 2920, 1601, 1527, 1285, 1021 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): *δ(ppm)* = 13.92 (br s, 1H, NH), 7.26-8.10 (m, 14H, Ar-H), 5.31 (s, 1H, CH), 2.44 (s, 6H, 2xCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): *δ(ppm)* = 167.2, 161.2, 158.3, 155.5, 149.3, 146.4, 142.9, 140.2, 138.6, 137.5, 133.2, 129.2, 121.0, 55.3, 32.6 ppm. Anal. Calcd. for $C_{27}H_{22}N_6O_2$: C, 70.12; H, 4.79; N, 18.17. Found C, 70.10; H, 4.78; N, 18.17. MS (EI) m/z 463 (M+1, 20%).

*3,5-dimethyl-1,7-diphenyl-4-(4-methoxyphenyl)-1,4,7,8-tetrahydrodipyrazolo[3,4-b:4',3'-e] pyridine (**4e**)*: mp. 196-197 °C; IR (KBr): υ = 3412, 2919, 2839, 1600, 1507, 1180, 1032 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): *δ(ppm)* = 13.81 (br s, 1H, NH); 7.06-7.82 (m, 14H, Ar-H), 5.18 (s, 1H, CH), 3.87 (s, 3H, OCH_3), 2.31 (s, 6H, 2xCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): *δ(ppm)* = 163.4,158.2, 144.3, 143.7, 136.8, 132.9, 130.2, 128.6, 125.5, 124.3, 113.2, 59.6, 47.3, 28.2 ppm. Anal. Calcd. for $C_{28}H_{25}N_5O$: C, 75.15; H, 5.63; N, 15.65. Found C, 75.14; H, 5.61; N, 15.63. MS (EI) m/z 448 (M+1, 16%).

*3,5-dimethyl-1,7-diphenyl-4-(3,4-dimethoxyphenyl)-1,4,7,8-tetrahydrodipyrazolo[3,4-b:4',3'-e] pyridine (**4h**)*: mp. 215-217 °C; IR (KBr): υ = 3409, 2906, 2830, 1601, 1505, 1190, 1022 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): *δ(ppm)* = 13.71 (br s, 1H, NH), 6.92-7.70 (m, 13H, Ar-H), 5.09 (s, 1H, CH), 3.92 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 2.29 (s, 6H, 2xCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): *δ(ppm)* = 162.3, 155.8, 152.1, 149.3, 142.1, 140.2, 139.8, 138.9, 135.2, 133.4, 129.6, 126.2, 119.7, 62.5, 51.4, 28.2 ppm. Anal. Calcd. for $C_{29}H_{27}N_5O_2$: C, 72.94; H, 5.70; N, 14.66. Found: C, 72.90; H, 5.67; N, 14.65. MS (EI) m/z 478 (M+1, 13%).

3. Result and Discussion

The reactions of benzaldehyde, N-phenylpyrazole and ammonium acetate were refluxed in the varying amount of *p*-TSA as catalyst in acetonitrile. The reaction conditions were optimised and it was found that 3 mol % (50 mg) *p*-TSA is sufficient to catalyse the reaction. It was found that decrease in the amount of catalyst will decrease the reaction yield and increase the reaction time but on increasing the amount of catalyst will not much affect both reaction time and yield.

Table 1. Effect of catalyst on the synthesis of 3,5-dimethyl -1,4,7-triphenyl -1,4,7,8-tetrahydrodipyrazolo[3,4-*b*:4',3'-*e*]pyridine **4a**

S. No	Amount of catalyst (mol %)	Time (hr.)	Yield[a] (%)
1	1	3.8	73
2	2	3.4	77
3	3	3	91
4	4	3	91
5	5	3	92

[a]yield refer to combined yield of different crops.

Structure of compound 4a is confirmed by advanced spectroscopic techniques. In the IR spectra, peak at 1596 cm^{-1} indicated the presence of C=N group. Another peak at 3360 cm^{-1} shows the presence of NH group. In the NMR spectra, peak at δ 7.12-7.92 (m, 15H) indicated the presence of aromatic protons and peak at δ 13.84 (br s, 1H) represents NH group. Peak for CH proton is observed at δ 5.20 (s, 1H). Also peak at δ 2.35 (s, 6H, CH_3) is observed for two methyl groups (Figure 1). To check the versatility of this process, we have reacted N-phenylpyrazole, ammonium acetate with different aldehydes and results are summarized in Table 2. Reactions proceed smoothly with aldehydes bearing electron withdrawing as well as electron donating

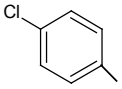
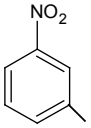
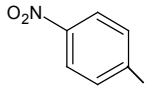
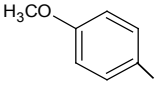
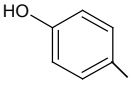
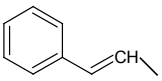
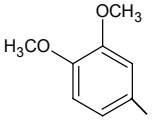
substituents (Table 2).

In the proposed mechanism (Scheme 2), for the synthesis of dihydropyridines follow the Knoevenagal condensation between two N-phenylpyrazoles **1** and aldehyde **2** to give **5**, which on tautomerisation exists as **6**. Then addition of ammonium acetate **3** to **6** yields **7** which further change to **8** followed by cyclization to produced the dipyrazolo-1, 4-dihydropyridine (**4 a-h)** (Table 2). This method tolerates various functionalities like nitro, ether, halogen etc. on the aldehydes. Efficacy of this method is fairly general and afforded the resultant products in excellent yield and products are obtained by simple work up.

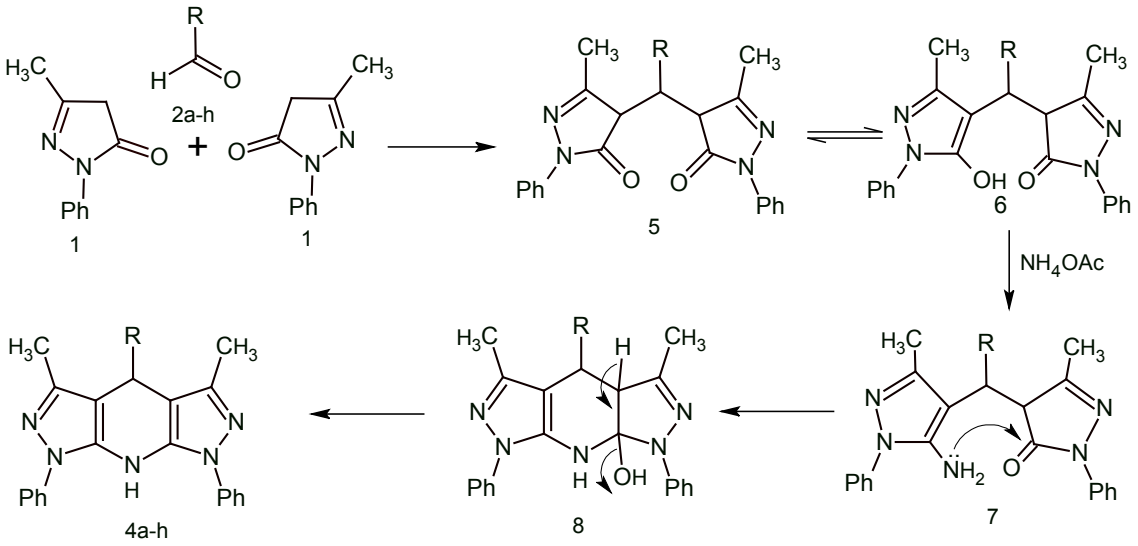
4. Conclusions

The present work provides an excellent route for the synthesis of novel fused dipyrazolo-1,4-dihydropyridine using readily available starting material. In addition, this protocol is easy to reproduce and do not require any tedious workup conditions. The targeted molecules are obtained in excellent yield without any side product and all the compounds evaluated against various bacterial and fungal strains. Compounds **4a**, **4e** and **4h** exhibited significant antibacterial activity against various bacterial and fungal strains comparable to standard drug. The importance of such work lies in the possibility that the new compounds might be more efficacious drugs against bacteria for which a thorough investigation regarding the structure–activity relationship, toxicity and their biological effects could be helpful in designing more potent antibacterial agents for therapeutic use.

Table 2. Synthesis of 3,5-dimethyl-1,7-diphenyl-4-aryl-1,4,7,8-tetrahydrodipyrazolo[3,4-b:4',3'-e] pyridine

Entry	R	Yield[a]	Melting Point
4a		91	188-190
4b		90	230-231
4c		92	246-248
4d		89	245-246
4e		92	196-197
4f		88	234-235
4g		81	159-160
4h		91	215-217

[a]Yield refer to combined yield of different crops



Scheme 2. Mechanism for the synthesis of Fused Dipyrazolo-1,4-dihydropyridine Derivative

Table 3. Minimal inhibitory concentration (MIC, μg/mL) of the newly synthesized dipyrazolo-1,4-DHPs

Entry	Gram (+ve) bacteria			Gram (+ve) bacteria			Fungi			
	E. coli	***K. Pneumonia***	***P. Aeruginosa***	***S. aureus***	***B. subtilis***	***S. pyrogens***	***A. Janus***	***P. glabrum***	***A. niger***	**A. Sclerotiorum**
4a	16	**8**	**8**	16	32	64	16	16	**8**	32
4b	16	32	64	16	32	32	32	32	32	32
4c	32	64	64	16	32	32	16	64	16	32
4d	64	32	64	16	16	64	32	16	16	16
4e	**8**	16	32	**8**	64	16	**8**	32	16	32
4f	16	32	64	16	16	32	16	**8**	32	16
4g	32	16	64	16	16	32	16	32	16	16
4h	**8**	16	**8**	32	16	16	32	64	**8**	**8**
Amoxicillin	**4**	**4**	**4**	**2**	**2**	**4**	-	-	-	-
Fluconazole	-	-	-	-	-	-	**2**	**2**	**2**	**2**

5. Antibacterial Activity

Synthesized compounds **4a-h** were screened for their *in vitro* antibacterial and antifungal activity against six bacterial and four fungal species namely *Klubsellia pneumonia* (MTCC 3384)*, Pseudomonas aeruginosa* (MTCC 424)*, Escherichia coli* (MTCC 443), *Straphylococcus aureus* (MTCC 96), *Bacillius subtilis* (MTCC 441), *Streptoccus pyrogens (MTCC 442)* and *Aspergillius janus* (MTCC 2751), *Pencillium glabrum* (MTCC 4951), *Aspergillus sclerotiorum (MTCC 1008)*, *Aspergillus niger (MTCC 281)* respectively. Amoxicillin and fluconazole were used as the standard drug as positive control while the DMSO was used as negative control.

The minimum inhibitory concentrations of the newly prepared compounds (4a–4h) were determined by using Serial tube dilution method at the concentration of 128, 64, 32, 16, 8, 4, 2 and 1 lg/ml against above said microorganisms. The bacterial and fungi strains susceptibility to the studied compounds was determined by the appearance of turbidity after 24 h of incubation at 37℃ and 72 h of incubation at 28℃, respectively. The observed MIC values (μg/ml) for the compounds **4a-4h** are represented in table 3.

It is evident from Table 3 that the compounds **4a**, **4e** and **4h** show significant activities against the most of bacterial and fungal strains at (MIC-8 μg/ml). The compound 4a showed significant activity (MIC-8 μg/ml) against the strains, K. pneumonia, P. aeruginosa, S. aureus, B. subtilis (bacterial strains) and A. niger (fungi strains) while **4e** displayed activity of same order against E. coli, S. aureus and A. janu. Further **4h** showed significant activity (MIC-8 μg/ml) against E. coli, P. aeruginosa (bacterial strains) and A. niger, A. sclerotiorum (fungi strains).

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