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In vitro Synthesis of 1,4-Dihydropyridines: An Anticancer Molecule

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ABSTRACT

As cancer is one of the leading causes of death across the globe. Various drugs are available in the market to treat such patients. Unfortunately all the drugs are very expensive due to their mode of synthesis. One such anticancer molecule is 1,4-Dihydropyridines. In the present study an attempt was made to synthesize 1,4-Dihydropyridines at fast and more economic and efficient way at *in vitro* level.

Key words *1,4-Dihydropyridines, In vitro synthesis, solvent free medium*

Cancer is the second most deadly disease worldwide. As per World Health Organization (Anonymous 2017) 8.8 million peoples died with cancer in 2015. The number of new cases is expected to rise by about 70% over the next 2 decades. Since ancient times various drugs are being utilized to treat this deadly disease. A number of drugs such as vinblastine, vincristine (Mukhtar et al. 2014), Podophyllotoxin (Ardalani et al. 2017), taxol (Rowinsky et al. 1992); (Malik 2017) protein phosphatase 2A (PP2A) etc. (Perrotti and Neviani 2013). are being used. As several cancers are characterized by the aberrant activity of oncogenic kinases, it was not surprising that a phosphatase like PP2A has progressively been considered as a potential tumor suppressor (Perrotti and Neviani 2008). Indeed, multiple solid tumors (e.g. melanomas, colorectal, carcinomas, lung and breast cancers) present with genetic and/or functional inactivation of different PP2A subunits and, therefore, loss of PP2A phosphatase activity towards certain substrates (Perrotti and Neviani 2008). Recently 1,4-dihydropyridines (a large group of structurally diverse compounds (Velena et al. 2016)) reported to have an anticancer potential (Viradiya et al. 2017). In the present study an attempt was made to synthesize 1,4-dihydropyridines in the laboratory with enhanced yield.

MATERIAL AND METHODS

All experiments were performed in oven dried glass apparatus. Melting points were measured in open capillaries on Perfit melting point apparatus and are uncorrected. The progress of the reaction was monitored by TLC (0.5-mm-thick plates using BDH silica gel G as adsorbent). Visualization of spots was effected by exposure to iodine vapours and Draggendroff reagent. Column chromatography was performed on silica gel (60-120 mesh) and compounds were eluted with graded solvent systems of petroleum ether and ethyl acetate. Recrystallization was achieved with ethyl acetate-petroleum ether (60-80) solvent system. IR spectra on KBr were recorded on Perkin-Elmer FTIR spectrophotometer. NMR (^{1}H and ^{13}C broadband decoupled) and ESIMS spectra were recorded on Brucker Ac-500 (500 & 125 MHz respectively) spectrometer and Micro Mass VG-7070H mass spectrometer respectively. Elemental analysis was performed on Leco CHNS 932 analyzer. ^{1}H chemical shifts are reported in parts per million (ppm) from tetramethylsilane (TMS) as internal standard. The abbreviations s, brs, d, dd, t, q, b, m in ^{1}H NMR spectra refer to singlet, broad singlet, doublet, double doublet, triplet, quartet, broad and multiplet respectively. All organic extracts were dried over anhydrous Na_2SO_4 and solvents were removed on rotary evaporator. Common abbreviations for solvents are used throughout, R.T. for room temperature, mix for mixture and aq. for aqueous. Commercial grade solvents were dried as per established procedure before use.

Preparation of catalyst ($SbCl_3$ / Al_2O_3):

To an antimony (III) chloride (2.28gm, 10 mmol) solution in100 ml of distilled ethanol was added 60 gm of neutral alumina. The mixture was stirred at room temperature for one hour followed by removal of solvent under reduced pressure on rotovap. The resultant free flowing powder was then activated at 110°C in an oven for two hours and was used throughout the experimentation (Kapoor et al. 2006).

General procedure for the synthesis of 3-methoxycarbonyl-2-methyl-1,4-dihydropyridine and 3-aceto-2-methyl-1,4-dihydropyridine:

An equimolar mixture of 1,3-dicarbonyl compound, cinnamaldehyde and aromatic amines was taken in mortar and grinded with 2 mol% of $SbCl_3/Al_2O_3$ using pestle at room temperature till the completion of reaction (TLC). The reaction mixture was diluted with ethyl acetate (30 ml for a 5 mmol scale) and washed with water (2 x 10 ml) and brine (1 x 10 ml). The organic layer was dried over anhydrous Na_2SO_4, followed by the removal of ethyl acetate under reduced pressure and the residue was column chromatographed to give corresponding 1,4-dihydropyridines (85-90% yield).

RESULTS AND DISCUSSION

A reaction of methyl-acetoacetate, (5 mmol, 0.58g), cinnamaldehyde, (5mmol, 0.66g), aniline, (5 mmol, 0.46g) and 2 mol% of $SbCl_3/Al_2O_3$ (0.25 mmol, 0.62g) was carried out by grinding using pestle and mortar under solvent-free conditions (1stScheme, Fig. 1 (A)).

After one hour, the formation of product was noticed (TLC) which showed a distinct spot at Rf-value 0.75 when

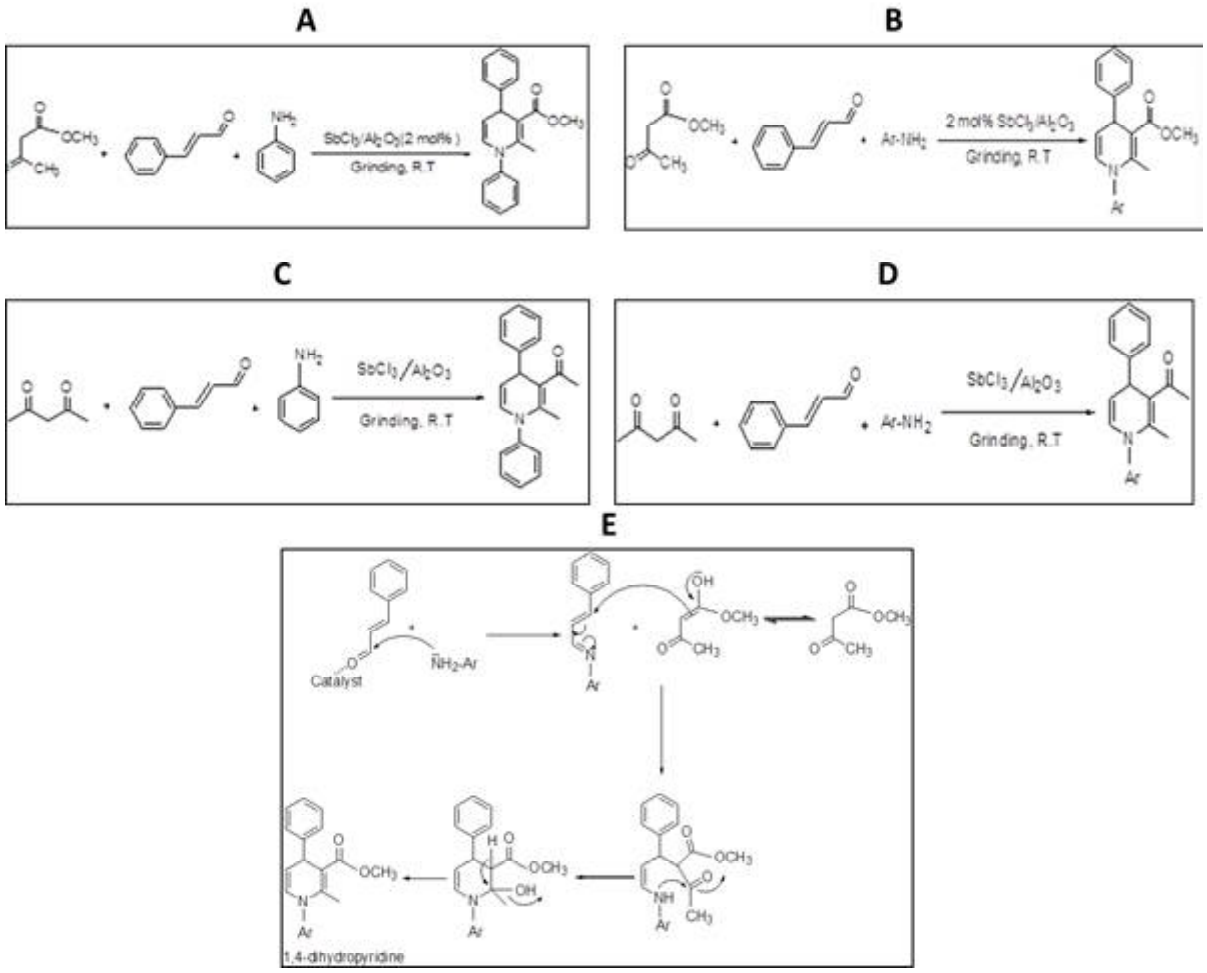

Fig. 1. Different schemes of 1,4-dihydropyridine synthesis: A: 1st Scheme, B: 2nd Scheme, C: 3rd Scheme, D: 4th Scheme, E: 5th Scheme portraying the mechanism of 1,4-dihydropyridine synthesis.

eluted with 20% ethylacetate-petroleum ether. After completion of the reaction, the product was isolated and purified by column chromatography. The structure of the isolated product was arrived at by spectral means such as ^{1}H NMR, ^{13}C NMR, IR and MS. It's ^{1}H NMR showed three characteristic resonances i.e., a doublet at ä 4.75 (J = 5.5 Hz), doublet of doublet at ä 5.07 (J = 5.5 Hz, J = 7.6 Hz) and another doublet at ä 6.23 (J = 7.6 Hz) arising out of C^4-H, C^3-H and C^2-H of the heterocyclic ring respectively. The presence of singlets at ä 3.58 and 2.15 can be attributed to methoxy and methyl protons. A multiplet at ä 7.01-7.36 for ten protons speaks the presence of two phenyl rings in the product. IR spectrum showed characteristic bands [(õcm^{-1}) at 1690, 1568, 1221] indicative of the formation of product 1,4-diphenyl-3-methoxycarbonyl-2-methyl-1,4-dihydropyridine. Further corroboration comes from the MS data (306(M+H)$^{+}$) and CHN analysis and comparison with the spectral data of known compound (Sridharan et al. 2007). Embracing the same protocol, the reaction was also carried out with anilines (2nd Scheme, Fig. 1(B)) and results are shown in Table 1.

In another experiment, acetylacetone, was used in place of methyl acetoacetate, and reaction was conducted with equimolar amounts of cinnamaldehyde, and aniline in presence of 2 mol% of $SbCl_3/Al_2O_3$ to realize the formation of expected product as 1,4-diphenyl-3-aceto-2-methyl-1,4-dihydropyridine (3rd Scheme, Fig. 1(C)). The structure integrity of was established by comparison of our spectral and physical data with the one known in literature(Sridharan et al. 2007).The same methodology was extended to a number of substituted anilines (4th Scheme, Fig. 1 (D)) and reaction was found successful and results are shown in Table 2. The reaction seems to be following the mechanism as shown in 5th Scheme (Fig. 1 (E)).

CONCLUSION

Based on the study carried out a rapid and efficient method for the synthesis of 1,4-dihydropyridines (1,4-DHPs) through the one pot condensation of 1,3-dicarbonyl compounds, various aromatic amines and cinnamaldehyde using 2 mol % $SbCl_3/Al_2O_3$ as the catalyst was reported.

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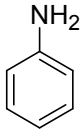
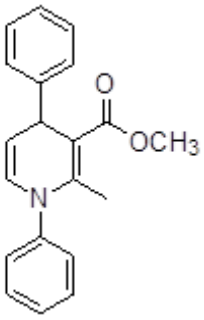
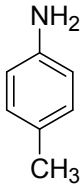
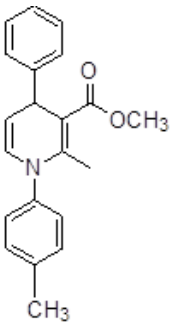
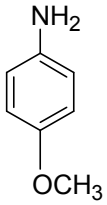
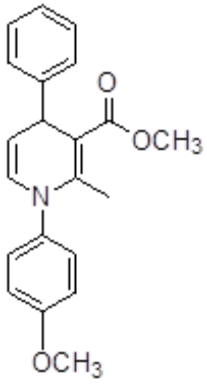
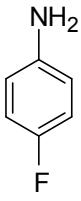
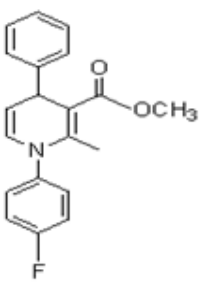
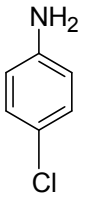
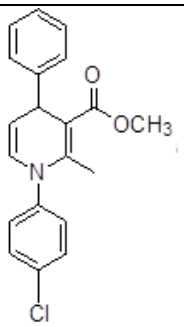
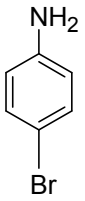
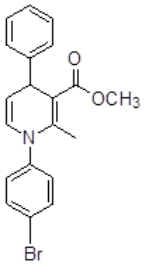
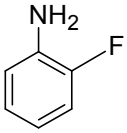
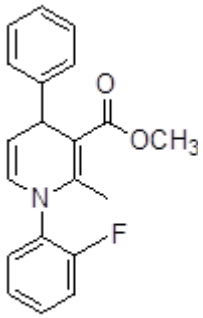
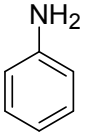
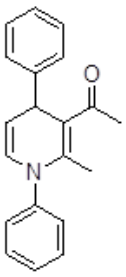
Table 1. Synthesis of 1,4-dihydropyridines

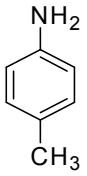
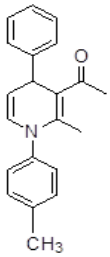
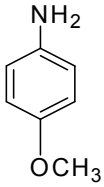
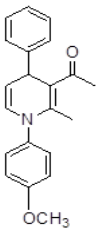
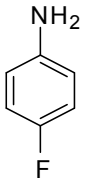
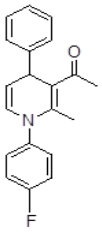
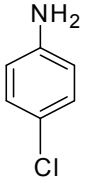
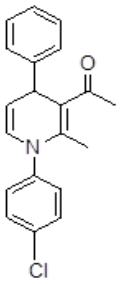
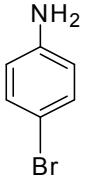
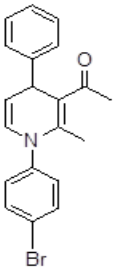
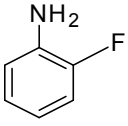
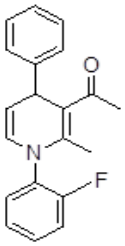
Entry	Ar-NH_2	1,4-Dihydropyridines 40	Time (min.)	Yield (%)
1			80	88
2			76	89
3			70	90
4			90	88

Entry	Ar-NH_2	1,4-Dihydropyridines 40	Time (min.)	Yield (%)
5			86	87
6			83	86
7			96	85

40g

Table 2. Synthesis of 1,4-dihydropyridines

Entry	Ar-NH_2	1,4-dihydropyridines 44	Time (min.)	Yield(%)
1			80	89

Entry	Ar-NH_2	1,4-dihydropyridines 44	Time (min.)	Yield(%)
2			75	90
3			70	91
4			90	88
5			85	87
6			83	86
7			98	85

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