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Research paper

Highly functionalized tetrahydropyridines are cytotoxic and selective inhibitors of human puromycin sensitive aminopeptidase

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ABSTRACT

Efficient one-pot five-component synthetic protocols for highly functionalized tetrahydropyridines (THPs) and their biological evaluation have been illustrated. Synthesis of novel functionalized tetrahydropyridines containing differential substitutions at 2,6-positions has been achieved *via* a modified MCR. Cytotoxic studies of 23 synthesized compounds have been carried out against three different cell lines, namely A-549, HeLa and HepG2, wherein some compounds have displayed appreciable cytotoxicity. Further, investigation of enzyme inhibition by the synthesized THPs has been carried out against four members of M1 family aminopeptidases. Several compounds have selectively inhibited only one member of this enzyme family i.e., human puromycin sensitive aminopeptidase (hPSA). Among the compounds; **4b**, **9b**, **9e** and **10a** demonstrated best inhibition against hPSA.

1. Introduction

Heterocyclic nitrogenous compounds and their analogues represent an important class of bioactive compounds. Many of these compounds are employed in the treatment of human diseases due to their specific activity [1]. Among a wide range of heterocyclic compounds that have been explored for the development of pharmaceutically important molecules, tetrahydropyridines constitute an interesting class of pharma actives due to their versatility and broad-spectrum activities such as anticancer [2], anti-hypertensive [3], antibacterial [4], anti-malarial [5], anticonvulsant [6] and anti-inflammatory [7] activities. Moreover, they are reported as inhibitors of farnesyl transferase [8], dihydroorotate dehydrogenase [9] and also involved in the MAO-based mechanism in Parkinson's disease [10]. In addition, the 2,6-disubstituted tetrahydropyridine framework has been regarded as a valuable basic unit since the possibility of modification and functionalization of the double bond enables the preparation of polysubstituted piperidines [11]. They exist in numerous natural products such as (+)-cannabisativine, (−)-palustrine, (−)-sedacrine. 1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) is a neurotoxin which induces Parkinson's disease [12]. In addition, this skeleton is an integral part of many drugs such as xanomeline, sabcomeline, tazomeline, alvameline, milameline etc., involved in treatment of cognitive diseases like Alzheimer's and Schizophrenia [13]. Diverse activity profile has inspired development of many new strategies to construct the 2,6-disubstituted 1,2,5,6-tetrahydropyridine skeleton.

Our interest in developing new chemical entities with antiproliferative activity led us to pay much attention towards the synthesis of polysubstituted tetrahydropyridines. Multicomponent reactions (MCRs) have become important tools for the rapid generation of molecular complexity and diversity with predefined functionality in chemical biology and drug discovery. MCRs have been extensively used in the construction of target compounds by the introduction of several diversity elements in a single operation, resulting minimization of waste, labor, time, and cost. Therefore, new protocols with operational simplicity, efficiency, high selectivity and minimal environmental hazards are still in demand and offer a challenging task to chemists. Recently, we have published a MCR approach [14] based on modified Hantzsch synthesis for construction of polysubstituted tetrahydropyridines. An improved

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version of the reported protocol has been currently extended for construction of a library of 2,6-differentially substituted THPs for the first time. Further the THPs have been used as synthons for construction of piperidine-4-one and piperidine-4-ol frameworks, to generate an exhaustive library of six membered *N*-heterocycles. The library has been evaluated for cytotoxicity and enzyme inhibition.

2. Results and discussion

2.1. Chemistry

Requisite compounds were synthesized in two phases. In the first phase, highly substituted tetrahydropyridines containing same substitutions at 2 and 6 positions (Fig. 1) were synthesized adopting an earlier reported procedure. In this protocol we have demonstrated a highly efficient one-pot five component MCR protocol using benign Lewis acid namely, zirconium tetrachloride and a green solvent, ethanol for construction of THPs. This protocol has advantage of higher yields in lesser reaction times and occurs at ambient temperatures. A series of tetrahydropyridines were synthesized employing a variety of aromatic aldehydes, aromatic amines and methyl/ethyl acetoacetate as shown in Scheme 1.

In the second phase, a series of tetrahydropyridines containing different substitutions at 2 and 6-positions have been synthesized. A brief introspection of the mechanism has led to the conclusion that the reaction proceeds *via* inter and intramolecular Mannich reactions to result in the formation of THP. With this understanding it was envisaged that a one-pot sequential addition protocol would yield desired molecules. Therefore, a one-pot reaction was carried out by sequential addition of different aromatic aldehydes (one equivalent each) for synthesis of THPs with different 2,6-substitutions. At the outset, a model reaction was carried out by taking two equivalents of *p*-toluidine and one equivalent of ethyl acetoacetate in ethanol at room temperature. After formation of enamine (confirmed by TLC), one equivalent of pyridine-3-carbaldehyde was added followed by catalyst ($ZrCl_4$). An *in situ* attack of enamine on imine formed between pyridine-3-carbaldehyde and *p*-toluidine is expected to take place through intermolecular Mannich reaction. Later one equivalent of benzaldehyde was added to the reaction mixture, which yields iminium species. An intramolecular Mannich reaction completes the protocol. After completion of the reaction, solid precipitate was filtered off and purified by column chromatography to obtain the desired product i.e., ethyl 6-phenyl-2-(pyridin-3-yl)-1-*p*-tolyl-4-(*p*-tolylamino)-1,2,5,6-tetrahydropyridine-3-carboxylate (**9a**) in 60% yield (Scheme 2).

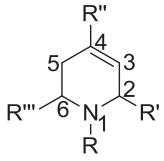


Fig. 1. Pictorial representation of 1,2,5,6-tetrahydropyridine.

Later, the scope of the reaction was extended by varying both aromatic amines and substituted aromatic aldehydes as shown in Scheme 3. The isolated yields of products obtained by this route are in the range of 50–65%. To the best of our knowledge this is the first report on synthesis of 2,6-unsymmetrical THPs using a one pot protocol.

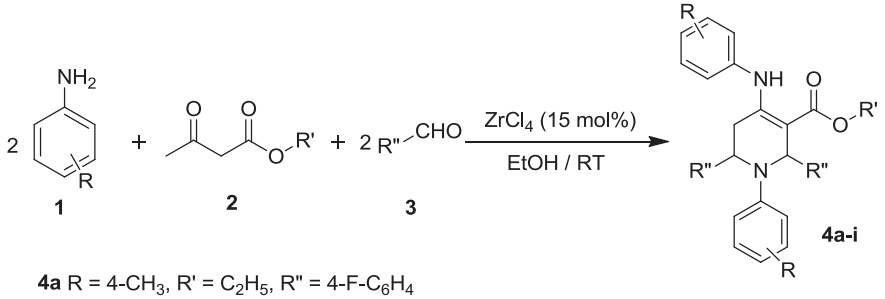
Additionally, piperidine-4-one-3-carboxylates and piperidine-4-ol derivatives, required for the comparative study, were obtained from corresponding polysubstituted 1,2,5,6-tetrahydropyridines. Piperidine-4-one-3-carboxylates (**10a** & **10b**) were synthesized by acidic hydrolysis of polysubstituted 1,2,5,6-tetrahydropyridines. Due to enamine nature of –NH on C-4 carbon of tetrahydropyridine ring, it could be easily hydrolyzed in acetone at ambient temperatures to obtain piperidine-4-one-3-carboxylates as shown in Scheme 4. The isolated yields of products were about 60%.

Piperidine-4-ol derivatives (**11a** & **11b**) could be obtained by simple basic hydrolysis of corresponding 1,2,5,6-tetrahydropyridine in presence of lithium hydroxide (LiOH) in methanol/tetrahydrofuran/water (1:1:1) under reflux conditions as shown in Scheme 5. The isolated yields were 60 and 58% respectively.

2.2. Biological assays

2.2.1. Cytotoxic assay

All synthesized compounds were evaluated for their *in vitro* cytotoxicity against three different cancer cell lines. Table 1 depicts the active compounds among those tested. Surprisingly, many compounds showed high selectivity towards HepG2 cell lines. None of the compounds were active against the A-549 cell lines. Compounds **9d** and **10a** were effective against HeLa and HepG2 cell lines in low micromolar concentrations while **4b** had feeble effect on both these cell lines. Compounds **4a** (symmetric substitution of 4-F-C_6H_4 at C-2 and C-6 positions) and **9e** (unsymmetric substitutions with 3-pyridinyl and 4-CN-C_6H_4 at C-2 and C-6 positions, respectively) selectively inhibit the growth of HepG2 cell lines in low micromolar concentration while **9b** and **9f** had marginal effect on HeLa cells. Although aromatic groups at C-2 and C-6 positions



4a R = 4-CH_3, R' = C_2H_5, R'' = 4-F-C_6H_4
4b R = H, R' = C_2H_5, R'' = 3-pyridinyl
4c R = 3-Br, R' = C_2H_5, R'' = 4-Br-C_6H_4
4d R = H, R' = CH_3, R'' = 4-NO_2-C_6H_4
4e R = 4-OCH_3, R' = C_2H_5, R'' = 4-CH_3-C_6H_4
4f R = 4-F, R' = C_2H_5, R'' = 4-Cl-C_6H_4
4g R = H, R' = C_2H_5, R'' = C_6H_5
4h R = H, R' = C_2H_5, R'' = 2-thiophenyl
4i R = H, R' = C_2H_5, R'' = 2-pyridinyl.

Scheme 1. One-pot MCR approach for synthesis of tetrahydropyridines.