Bioorganic & Medicinal Chemistry Letters 22 (2012) 4629–4635

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Construction and functionalization of fused pyridine ring leading to novel compounds as potential antitubercular agents

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ARTICLE INFO

Article history:
Received 17 March 2012
Revised 15 May 2012
Accepted 28 May 2012
Available online 5 June 2012

Keywords:
Pyridine
Quinoline
X-ray
In vitro
Tuberculosis

ABSTRACT

A series of fused and functionalized pyridine derivatives were designed, synthesized and tested for their potential antitubercular properties. All these novel compounds were prepared by using multistep methods involving the construction of pyridine ring as a key synthetic step. Some of these compounds were found to be interesting when tested for their antitubercular properties in vitro and one of them appeared as an attractive and potential antitubercular agent.

Tuberculosis (TB), a major global health problem causes morbidity and mortality in developing countries.[1] It is estimated that one-third of the entire human population is infected with this disease.[2–4] Complicated by the human immunodeficiency virus (HIV), TB turns out to be most opportunistic co-infection posing twin trouble which is growing at an alarming rate with the increase of world human population.[5,6] Additionally, with the recent emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR)-TB, the disease poses a major threat to the human life and hence, requires an immediate attention for the identification and development of new and novel antitubercular drugs.[7]

The shikimate pathway constitutes the biosynthesis of aromatic rings from carbohydrate precursors in microorganisms and plants involving a range of chemical transformations. Through the seven enzymatic steps, phosphoenol pyruvate (PEP) and D-erythrose 4-phosphate (E4P) are condensed to a branching point compound called chorismate (the end product), which leads to several additional terminal pathways.[8] Shikimate dehydrogenase (EC 1.1.1.25) plays an important role in catalyzing the fourth step in the shikimate pathway (Fig. 1), involving the reversible conversion of 3-dehydro shikimate (DHS) and NADPH to yield shikimate, a 3,4,5-trihydroxycyclohexene-1-carboxylic acid and NADP+.[9–11] The absence of shikimate pathway in humans and its indispensible

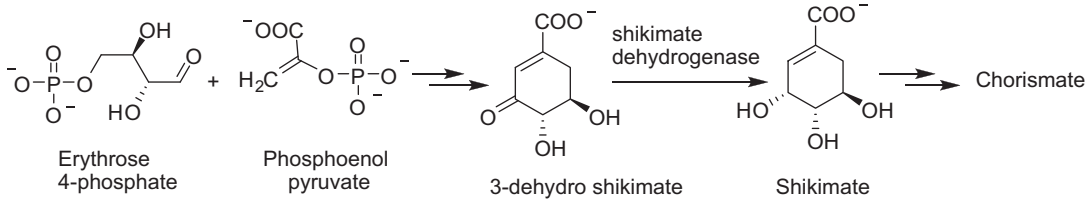

Figure 1. The role of shikimate dehydrogenase in the shikimate pathway.

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http://dx.doi.org/10.1016/j.bmcl.2012.05.096

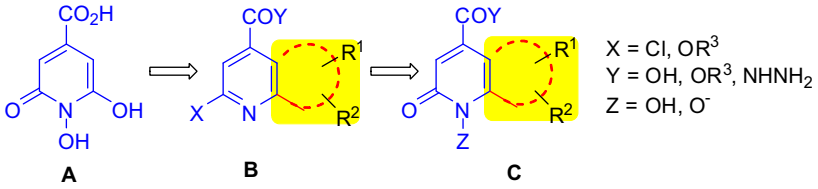
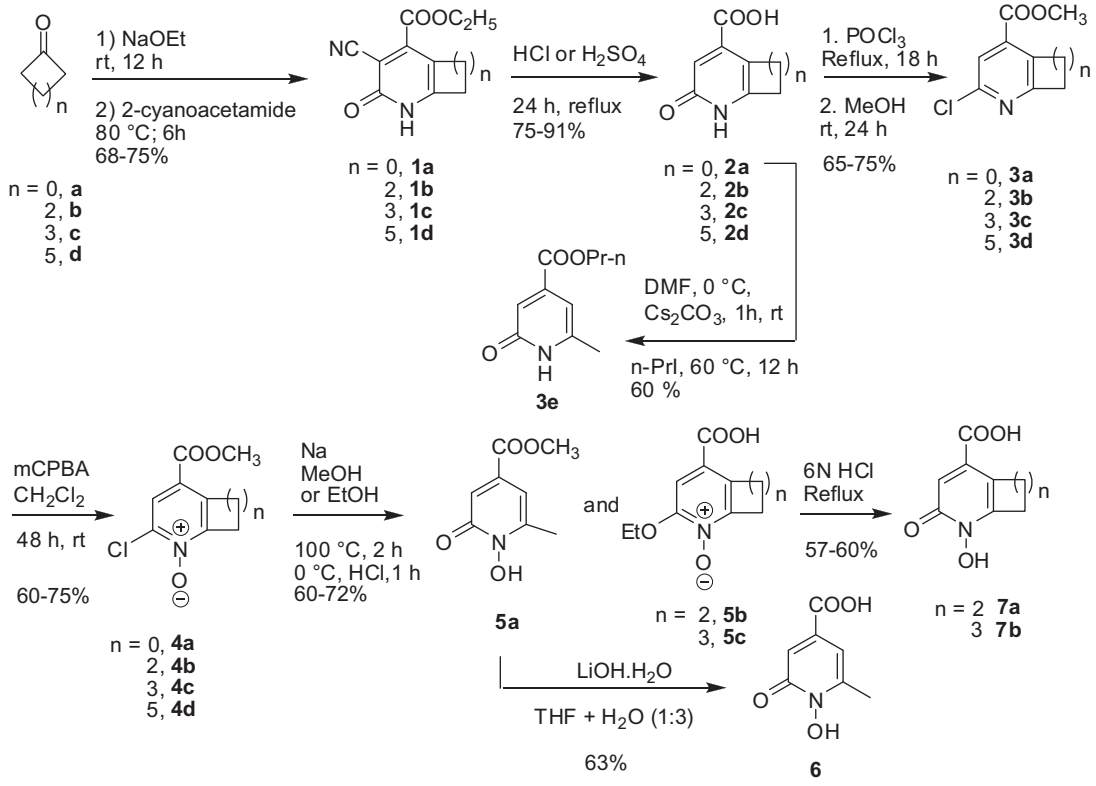
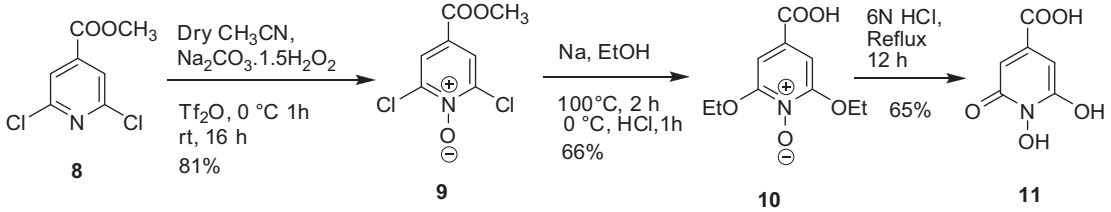


Figure 2. Design of new and potential antitubercular agents from a known DHS analog A.



Scheme 1. Synthesis of 2-oxo-1,2-dihydropyridine-4-carboxylic acid derivatives (**B**).

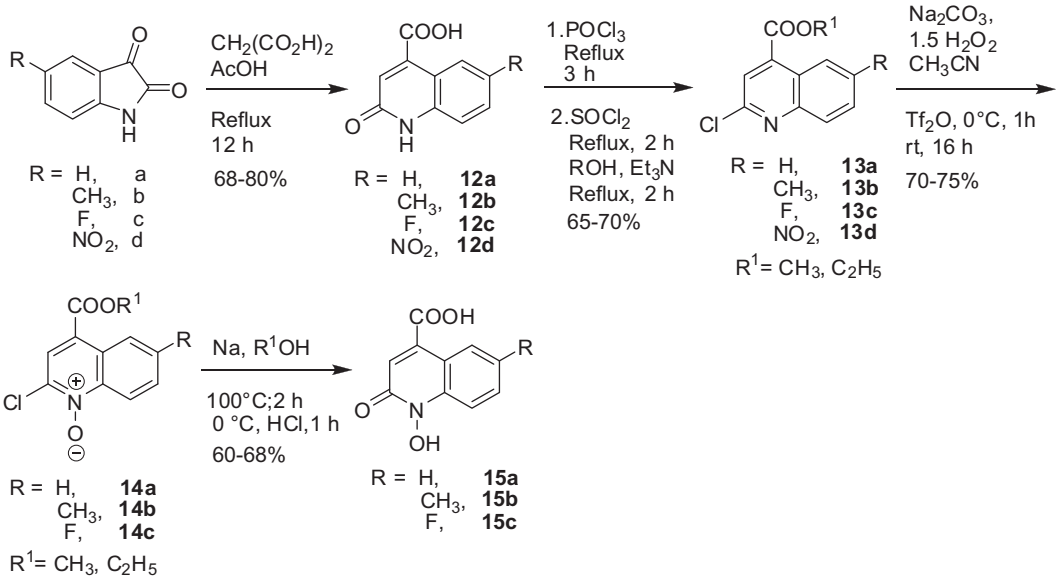


Scheme 2. Synthesis of 1,6-dihydroxy-2-oxoisonicotinic acid **A** (**11**).[13]

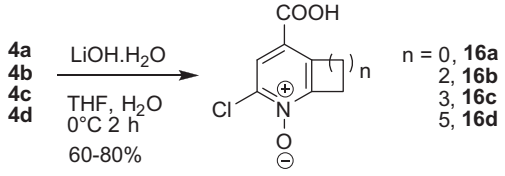
importance in algae, higher plants, bacteria, and fungi makes the pathway a potential target for the development of novel antitubercular agents.[12]

Accordingly, a novel DHS analog that is, 1,6-dihydroxy-2-oxoisonicotinic acid (**A**, Fig. 2) was reported as a potential herbicide based on the results obtained in the enzyme assay of shikimate dehydrogenase.[13] Indeed, the compound **A** has been reported as a powerful inhibitor of shikimate dehydrogenase from pea (K_i = 0.12 mM) but was found to be less effective against couch enzyme (K_i = 0.32 mM).[13] Prompted by these observations and due to our continuing interest in the identification of novel small molecules as potential antitubercular agents we became interested in evaluating a series of pyridine-4-carboxylic acid derivatives (**B** and **C**, Fig. 2) for their potential antitubercular properties. The structures of all target compounds were attained by introducing a fused ring with or without substituents into the 2-pyridone moiety of **A** and/or converting the carboxylate group into an ester or hydrazide moiety (Fig. 2). The newly introduced fused ring was expected to provide additional hydrophobicity into the molecule which was thought to be beneficial for the potential and enhanced pharmacological properties of the resulting molecules.

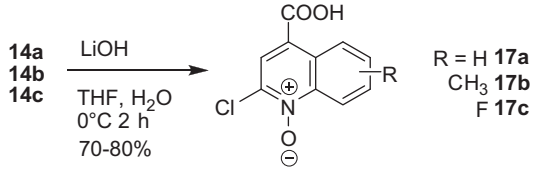
The synthesis[14] of our target molecules based on **B** and **C** are shown in the following Schemes. The 3-cyano-2-pyridone derivatives (**1a–d**) prepared by treating appropriate ketones with cyano-



Scheme 3. Synthesis of 2-oxo-1,2-dihydropyridine-4-carboxylic acid derivatives (**B**).

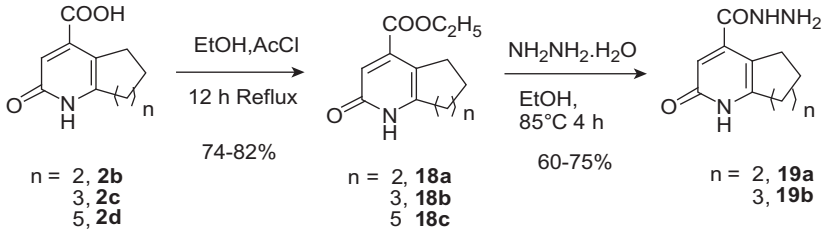


Scheme 4. Synthesis of 2-chloro acid derivatives (**16**).

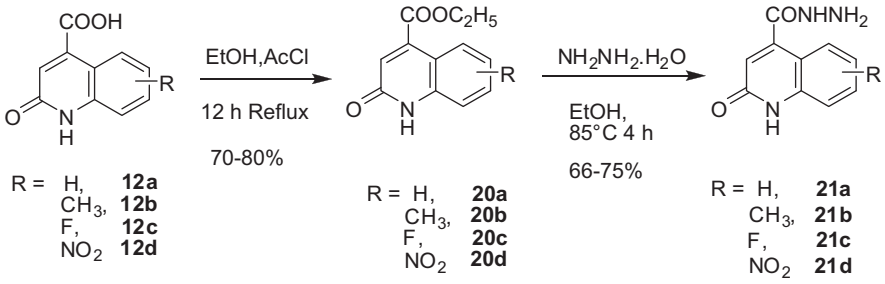


Scheme 5. Synthesis of 2-chloro acid derivatives (**17a–c**).

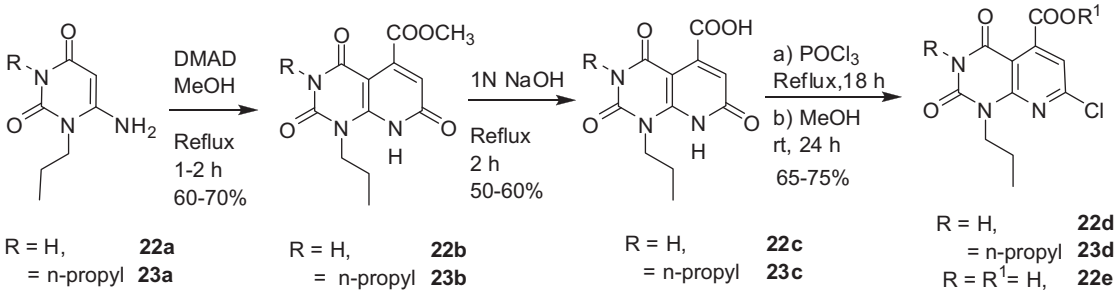
acetamide in the presence of NaOEt were converted to 2-pyridone carboxylic acids (**2a–d**) and subsequently to 2-chloro ester derivatives (**3a–d**) (Scheme 1). The acid **2a** was also converted to the corresponding ester **3e** as shown in Scheme 1. Treating the compounds **3a–d** with *m*-CPBA provided the corresponding N-oxides (**4a–d**) that were converted to 1-hydroxy 2-pyridone derivative (**5a**) or 2-ethoxy pyridine N-oxides (**5b–c**) depending on the reaction conditions employed. Finally, the ester **5a** was hydrolyzed to the corresponding acid **6** or the acids **5b–c** were transformed into the corresponding 1-hydroxy 2-pyridone derivatives (**7a–b**). The DHS analog **A** (or **11**) was prepared according to a known method as shown in Scheme 2.[13] The synthesis of our other target molecules based on **B** is shown in Scheme 3. The 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives (**12a–d**) prepared from appropriate indoline-2,3-diones were converted to the corresponding 2-chloro ester derivatives (**13a–d**). Treating these compounds with H_2O_2 provided the corresponding N-oxides (**14a–c**) that were



Scheme 6. Synthesis of 2-oxo-1,2-dihydropyridine-4-carbohydrazide derivatives I.



Scheme 7. Synthesis of 2-oxo-1,2-dihydropyridine-4-carbohydrazide derivatives I.



Scheme 8. Synthesis of 7-chloro-2,4-dioxo-1-propyl-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine derivatives.

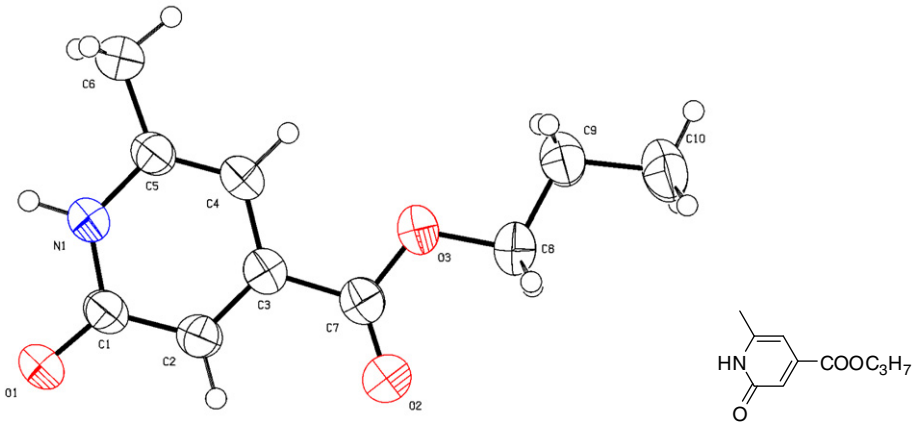


Figure 3. ORTEP representation of the **3e** ($C_{10}H_{13}NO_3$). (Thermal ellipsoids are drawn at 50% probability level).

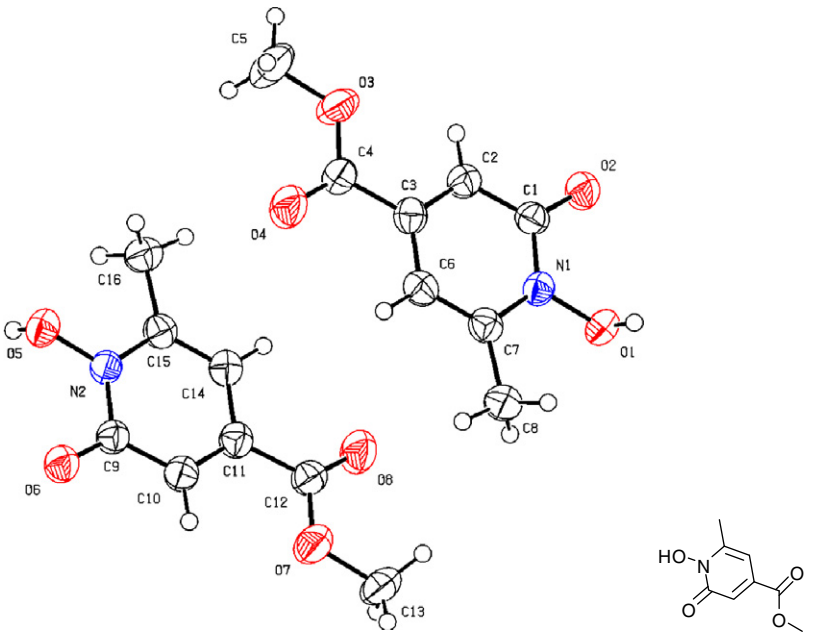


Figure 4. ORTEP representation of the **5a** ($C_8H_9NO_4$). (Thermal ellipsoids are drawn at 50% probability level).

converted to the desired 1-hydroxy-2-oxo-1, 2-dihydroquinoline-4-carboxylic acid derivatives (**15a–c**).

Synthesis of 2-chloro acid derivatives (**16a–d**) from **4** is shown in Scheme 4 whereas preparation of other 2-chloro acid derivatives (**17a–c**) from **14a–c** is shown in Scheme 5. The reactions were carried out carefully in the presence of LiOH in these cases.

The synthesis of target molecules based on **C** is shown in Schemes 6 and 7. The ethyl-2-oxo-1,2-dihydropyridine-4-carboxylate derivatives (**18a–c** and **20a–d**) were prepared from the appropriate 2-oxo-1,2-dihydroquinoline-4-carboxylic acid derivatives (**2b–d** and **12a–d**). The resulting ethyl esters were treated with hydrazine hydrate to afford 2-oxo-1,2-dihydropyridine-4-carbohydrazide derivatives (**19a–b** and **21a–d**).

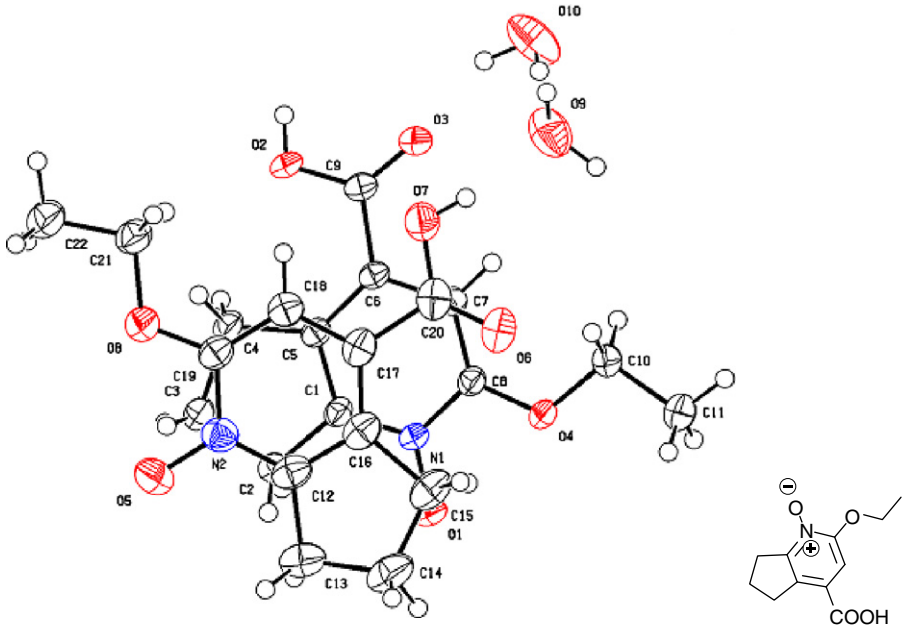
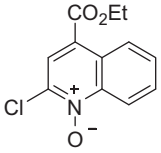
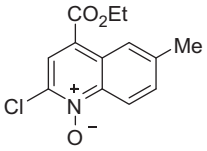
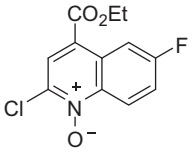
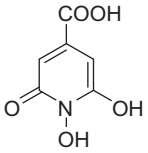
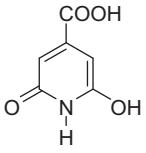


Figure 5. ORTEP representation of the **5b** ($C_{11}H_{13}NO_4.H_2O$). (Thermal ellipsoids are drawn at 50% probability level).

Table 1
In vitro assay results of selected compounds

Compound	Structure	MIC (μg/mL)		IC_{50} (μg/mL)
		MABA	LORA	Vero cell
14a		9.2	7.3	n.d.
14b		4.33	3.65	<8.0 (96%)
14c		10.9	13.1	n.d.
11		>100	n.d.	n.d.
Citrazinic acid		>50	n.d.	n.d.
Rifampicin		0.05	1.47	n.d.
Isoniazide		0.33	>128 (84%)	n.d.
Moxifloxacin		0.39	15.93	n.d.

n.d. = not determined.

Table 2
Dock scores obtained after docking the molecules into the SDHmt protein

Molecules	MOE Dock score (K cal/mol)
14a	−10.03
14b	−12.84
14c	−10.93
11	−8.87

The 4-methylcarboxylate-2-pyridone derivatives (**22b, 23b**) were prepared by treating appropriate uracil derivatives (**22a**, **23a**) with dimethyl acetylenedicarboxylate in MeOH. These compounds were converted to 2-pyridone carboxylic acids (**22c, 23c**) and subsequently to 2-chloro ester derivatives (**22d, 23d**) (Scheme 8). Treating the compounds **22d, 23d** with several per acids did not provide the corresponding N-oxides. Saponification of **22d** with $LiOH{\cdot}H_2O$ provided the compound **22e**.

While all the compounds synthesized were characterized by spectral (NMR, IR and MS) data the molecular structure of few representative compounds for example, **3e**, **5a**, and **5b** were confirmed unambiguously by single crystal X-ray diffraction (Figs. 3–5).[15]

All the compounds synthesized initially were tested for their activity against *Mycobacterium tuberculosis* $H_{37}Rv$ that was determined by using fluorescence readout in the Microplate Alamar Blue Assay (MABA).[16] The MIC value obtained through this assay was defined as the minimum concentration inhibiting fluorescence by 90% relative to bacteria-only controls. The compounds found promising in this assay were then tested for their activity against non-replicating *M. tuberculosis* that was determined by using the Low-Oxygen Recovery Assay (LORA).[17] Like earlier case, MIC value obtained was defined as the lowest concentration inhibiting recovery of luciferase signal, greater or equal to 90% relative to bacteria-only controls. The well-known agents such as rifampicin (a semisynthetic bactericidal antibiotic drug), isoniazide (or isonicotinylhydrazine, an antitubercular agent) and moxifloxacin (a fluoroquinolone antibacterial agent) were used as reference compounds in these assays. Finally, the cytotoxicity assay[18] of the best compound was carried out using Vero cells. Among all

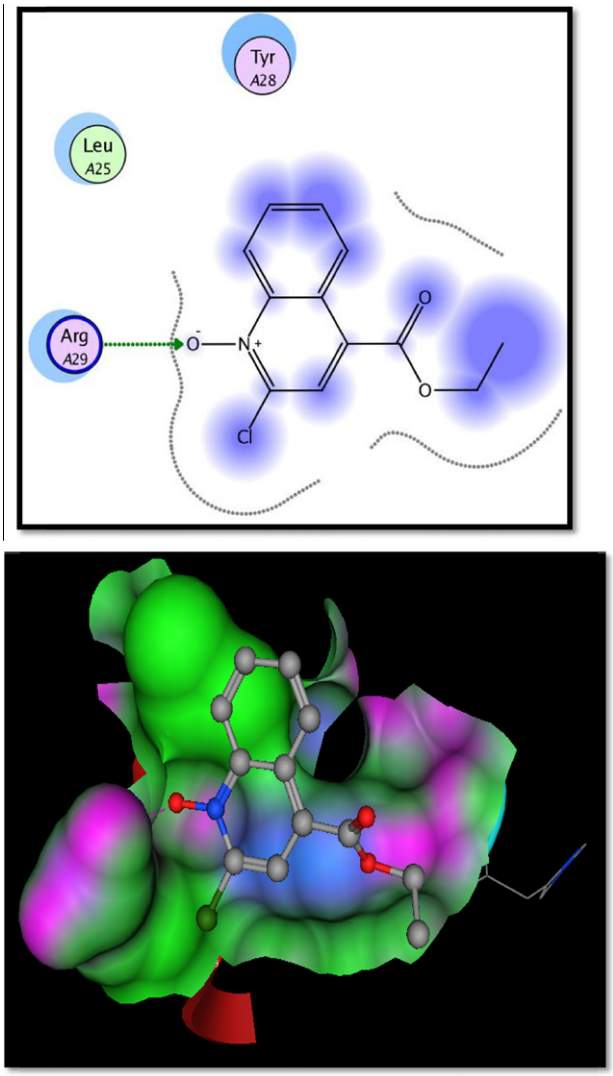


Figure 6. The ligand interaction and binding pose of compound **14a** with SDHmt protein.

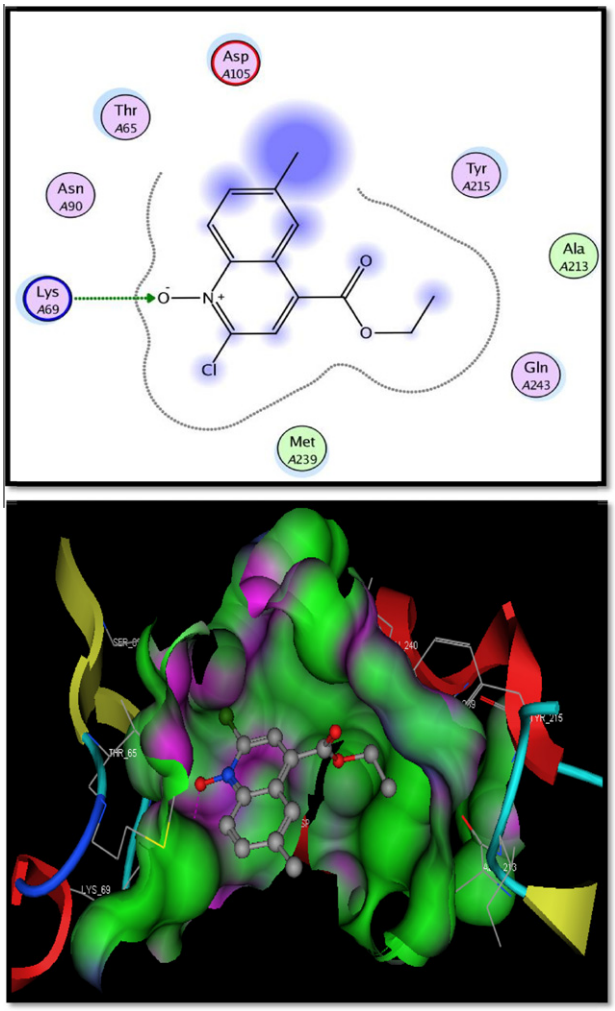


Figure 7. The ligand interaction and binding pose of compound **14b** with SDHmt protein.

the compounds tested quinoline-1-oxides that is, **14a**, **14b**, and **14c** were found to be promising in MABA (Table 1). Indeed, compound **14b** showed the lowest MIC that is, 4.33 μg/mL among them. This compound also showed the lowest MIC that is, 3.65 μg/mL in LORA (Table 1). Finally, this compound showed IC_{50} value <8.0 μg/mL in the cytotoxicity assay in Vero cells. Among the other compounds **2a–d, 4d**, **6**, **12a–d** and **16b** showed MIC >50 μg/mL whereas the rest of the compounds showed MIC >100 μg/mL when tested in MABA (see ESI). Notably, MIC of the known inhibitor of shikimate dehydrogenase[13] **11** and citrazinic acid were found to be >50 and 100 μg/mL, respectively, in the same assay (Table 1). Nevertheless, the compound **14b** though found to be slightly less effective than the reference compounds employed in MABA but was comparable or better than rifampicin, isoniazide and moxifloxacin in LORA. Overall, the compound **14b** appeared as attractive new chemical entity due to its interesting and novel structural features.

To understand the nature of interactions of these molecules with the shikimate dehydrogenase protein of Mycobacterium tuberculosis (SDHmt) in silico docking studies were performed using compounds **14a–c** along with the known inhibitor of shikimate dehydrogenase[13] **11**. An SDHmt protein homology model was developed and used for this purpose (see ESI). The dock scores obtained after docking these molecules into the SDHmt protein are summarized in Table 2. It is evident from Table 2 that in compared to compound **11** these molecules (**14a–c**) bind better with SDHmt the compound **14b** being the best among them. The ligand interaction and binding pose of compound **14a–c** with SDHmt protein is shown in Figs. 6–8. All these molecules were found to interact with the SDHmt protein through H-bonding. For example, compound **14a** with Arg 29 or **14b** with Lys69 or **14c** with Arg29 residue of the protein used. Additionally, the fused ring (introduced by drug design approach, see Fig. 2) that is, the benzene ring fused with the pyridine moiety participated well in hydrophobic interactions in all these cases. The compound **11** showed H-bonding interactions with the Pro57 and Glu58 residues of SDHmt protein (see ESI). Overall, the docking studies indicated that the present N-oxides[19] **14a–c** could be potential inhibitors of shikimate dehydrogenase and are of further interest.

In summary, based on the reported assay results of a novel DHS analog that is, 1,6-dihydroxy-2-oxoisonicotinic acid against shikimate dehydrogenase a series of fused and functionalized pyridine derivatives were designed, synthesized and tested for their potential antitubercular properties. All these pyridine based compounds were prepared by using multistep methods involving the construction of pyridine ring as a key synthetic step followed by further functionalization reactions. Few of these compounds were found to be interesting when tested in vitro. Based on overall results the compound **14b** appeared as an attractive and potential antitubarcular agent.

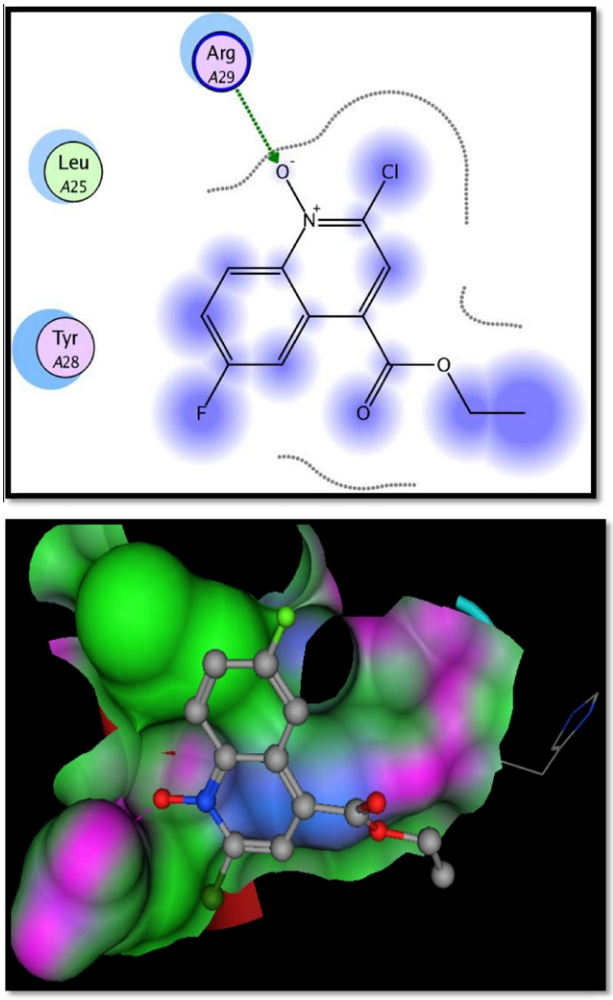


Figure 8. Ligand interaction and binding pose of compound **14c** with SDHmt protein.

Acknowledgements

The authors thank the management of ILS for encouragement and support, Dr. M. Zabel and S. Stempfhuber for single crystal X-ray structure analyses and Dr. G. Gopikrishna for literature help. B.D. thanks A.K. Reiser, University of Regensburg, Germany and DAAD (Indigo) for providing a fellowship. B.D. also thanks Dr. T. D. Neelima for support and DST for providing a fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.096.

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15. (a) *Crystal data of* ***3e***: Molecular formula = $C_{10}H_{13}NO_3$, formula weight = 195.21, crystal system = monoclinic, space group = P2(1)/n, a = 8.918 (11) Å, b = 7.969 (7) Å, c = 14.721 (18) Å, V = 1009.73 (2) Å^3, T = 296 K, Z = 4, D_c = 1.284 Mg m^{-3}, μ(Mo Kα) = 0.79 mm^{-1}, 5081 reflections measured, 1808 independent reflections, 1612 observed reflections [I >2.0 σ (*I*)], R_1_obs = 0.009, goodness of fit = 1.081. Crystallographic data (excluding structure factors) for **3e** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 871206. (b) *Crystal data of* ***5a***: Molecular formula = $C_8H_9NO_4$, formula weight = 183.16, crystal system = orthorhombic, space group = Pna2$_1$, a = 23.033 (6) Å, b = 4.038 (10) Å, c = 18.018 (5) Å, V = 1676.2 (8) Å^3, T = 296 K, Z = 8, D_c = 1.456 Mg m^{-3}, μ(Mo Kα) = 1.01 mm^{-1}, 5768 reflections measured, 2506 independent reflections, 2371 observed reflections [I >2.0 σ (*I*)], R_1_obs = 0.026, goodness of fit = 1.05. Crystallographic data (excluding structure factors) for **5a** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 871207. (c) *Crystal data of* ***5b***: Molecular formula = $C_{11}H_{13}NO_4$,H_2O, formula weight = 241.24, crystal system = monoclinic, space group = P2(1)/n, a = 10.413 (19) Å, b = 19.895 (3) Å, c = 11.164 (2) Å, V = 2256.54 (7) Å^3, T = 296 K, Z = 8, D_c = 1.387 Mg m^{-3}, μ(Mo Kα) = 0.95 mm^{-1}, 17064 reflections measured, 4439 independent reflections, 3792 observed reflections [I >2.0 σ (*I*)], R1_obs = 0.031, goodness of fit = 1.05. Crystallographic data (excluding structure factors) for **5b** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 871209.
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19. Pyridine N-oxides are well known in the literature and found to be beneficial in terms of their pharmacological properties. For example, pyridine N-oxide showed protective effects against 3-chloropyridine-induced cytotoxicity and clastogenicity (see: Anuszewska, E. L.; Koziorowska, J. H. *Toxicol. In Vitro* **1995**, *9*, 91).