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An atom efficient, solvent-free, green synthesis and antimycobacterial evaluation of 2-amino-6-methyl-4-aryl-8-[(*E*)-arylmethylidene]-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitriles

Raju Ranjith Kumar,[a] Subbu Perumal,[a,*] Palaniappan Senthilkumar,[b] Perumal Yogeeswari[b] and Dharmarajan Sriram[b]

[a]*Department of Organic Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai 625021, India*
[b]*Medicinal Chemistry Research Laboratory, Pharmacy Group, Birla Institute of Technology and Science, Pilani 333031, India*

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Abstract—An atom efficient, green protocol for the synthesis of fifteen 2-amino-6-methyl-4-aryl-8-[(*E*)-arylmethylidene]-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitriles in quantitative yields from the reaction of 1-methyl-3,5-bis[(*E*)-arylmethylidene]-tetrahydro-4(1*H*)-pyridinones with malononitrile in presence of solid sodium ethoxide under solvent-free condition is described. The compounds were tested for their in vitro activity against *Mycobacterium tuberculosis* H37Rv (MTB), multi-drug resistant tuberculosis (MDR-TB), and *Mycobacterium smegmatis* using agar dilution method. 2-Amino-4-[4-(dimethylamino)phenyl]-8-(*E*)-[4-(dimethylamino)phenyl]methylidene-6-methyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]-pyridine-3-carbonitrile was found to be the most potent compound (MIC: 0.43 μM) against MTB and MDR-TB, being 100 times more active than standard, isoniazid against MDR-TB.

Tuberculosis (TB) is a chronic bacterial infection and most people infected with *Mycobacterium tuberculosis* (MTB) harbor the bacterium without symptoms (latent TB), but some develop into active TB disease. The World Health Organization (WHO) estimates disclose that 2 billion people are infected with MTB, among which 8 million develop active TB and nearly 2 million die each year.[1] Currently, TB is treated with agents that target mycolic acid biosynthesis including isoniazid, inhibitors of nucleic acid biosynthesis such as rifampicin which binds and inhibits mycobacterial DNA-dependent RNA polymerase, and the aminoglycoside antibiotic streptomycin which targets protein synthesis.[2] With the increasing incidence of TB[3] and the emergence of multi-drug resistant strains (MDR-TB),[4] the development of new TB therapeutics is imperative and of paramount importance.

Random screening of compounds from one of our laboratories identified various antitubercular leads.[5,6] In the course of screening to discover new compounds that could be useful for the chemotherapy of tuberculosis, we identified tetrahydro-4*H*-pyrano[3,2-*c*]pyridine derivatives, which inhibited in vitro *M. tuberculosis* H37Rv (MTB) and multi-drug resistant *M. tuberculosis* (MDR-TB). We present the preliminary results on the synthesis and the in vitro antimycobacterial activities of the first representative series of this family.

The tetrahydro-4*H*-pyrano[3,2-*c*]pyridine derivatives (**3a**–**o**) were obtained in quantitative yields, except for the slight loss during workup, from the reaction of a series of 1-methyl-3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones (**1a**–**o**) and malononitrile (**2**) in presence of solid sodium ethoxide at ambient temperature under solvent-free conditions (Scheme 1). This reaction affords solely tetrahydro-4*H*-pyrano[3,2-*c*]pyridines **3** without any side product and hence neither crystallization nor column chromatographic purification is necessary. In a typical experiment, a mixture of 1 mmol of each: **1**, malononitrile, and solid sodium ethoxide was ground thoroughly at ambient temperature. The reac-

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* Corresponding author. Tel./fax: +91 452 2459845; e-mail: subbu.perum@gmail.com

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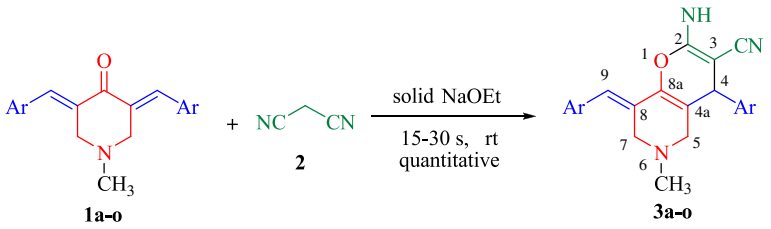


Scheme 1. Synthesis of 4*H*-pyrans **3**.

tion progress is indicated by the diminishing color of the reaction mixture, which became colorless at the end of the reaction. Then water (50–70 mL) was added to the reaction mixture and the precipitate filtered to obtain pure tetrahydro-4*H*-pyrano[3,2-*c*]pyridines (**3a–o**) in quantitative yield (Table 1). This reaction does not require monitoring as it is completed within 30 s. The only solvent employed is the green solvent, water for washing the product. Scaling up of the reaction does not envisage any reduction in either the yield or purity of the product, as the reaction requires only a thorough mixing of the reactants at ambient temperature, which can be readily ensured by appropriate mills.

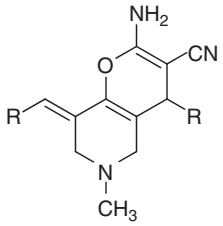
The present investigation assumes importance from the viewpoints of (i) ~100% atom efficiency,[7] (ii) solvent-free and mild (ambient temperature) reaction conditions further amplifying the greenness of the transformation, and (iii) biological importance of tetrahydro-4*H*-pyrano[3,2-*c*]pyridines (**3**) described in this work. It is pertinent to note that a previous study by El-Subbagh et al.[8] reported the formation of three pyrano[3,2-*c*]pyridines (**3c**, **3d**, and **3k**), respectively, in 72%, 97%, and 65% yields from the reaction of 1-methyl-3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones (**1**) and malononitrile (**2**) in butanol under reflux for 5 h. This literature procedure suffers from long reaction time, use of solvent, and inconsistent yields for the compounds reported.

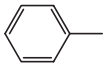
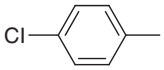
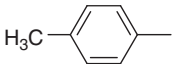
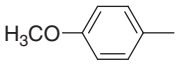
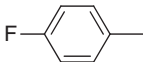
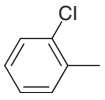
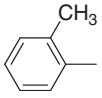
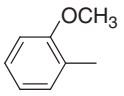
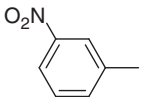
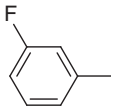
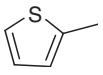
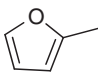
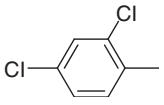
The structure of **3** was thoroughly elucidated using NMR spectroscopy, as discussed in detail for one example,[9] **3f**. The ^{1}H NMR spectrum of **3f** has singlets for the benzylidene proton (H-9), NH_2, and N–CH_3, respectively, at 6.96, 4.81, and 2.24 ppm and a multiplet for the aromatic protons in the range of 7.13–7.43 ppm. The C,H–COSY correlation of H-9 assigns C-9 to 120.3 ppm. The HMBC correlations of H-9 (Fig. 1) with the carbon at 54.4 ppm and C-8a at 140.1 ppm assign the former to C-7. The C,H–COSY spectrum assigns the 7-CH_2 protons to the doublets at 3.39 and 3.27 ppm (J = 13.8 Hz). The 7-CH_2 protons show a HMBC correlation with a carbon at 127.9 ppm assigning it to C-8. The 5-CH_2 protons appear as doublets at 3.01 and 2.71 ppm (J = 16.2 Hz) and show: (i) a C,H–COSY correlation with the carbon at 54.8 ppm due to C-5 and (ii) a HMBC correlation with C-8a and the carbon at 37.4 ppm due to C-4. From C,H–COSY spectrum the singlet at 4.74 ppm is readily assigned to H-4. From the HMBC correlations of H-4, the carbon signals at 58.9, 113.0, 119.4, and 159.5 ppm were assigned to C-3, C-4a, the nitrile carbon, and C-2, respectively. The structures of all the new compounds are in accord with their analytical and NMR spectroscopic data, while the physical and spectroscopic data of the known compounds agree well with those available in the literature.[8]

The compounds were screened for their in vitro antimycobacterial activity against MTB, MDR-TB, and *Mycobacterium smegmatis* ATCC 14468 (MC^2) by agar dilution method for the determination of MIC in duplicate.[10] The MDR-TB clinical isolate was resistant to isoniazid, rifampicin, ethambutol, and ofloxacin. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to completely inhibit bacterial growth and MICs of the synthesized compounds along with the standard drug for comparison are reported in Table 1. In the first phase of screening against MTB, all the compounds showed excellent in vitro activity against MTB with MIC ranging from 0.43 to 37.31 μM. Six compounds (**3b**, **3e**, **3f**, **3i**, **3j**, and **3n**) inhibited MTB with MIC less than 2 μM and were more potent than standard fluoroquinolone gatifloxacin (MIC: 2.08 μM). When compared to isoniazid (MIC: 0.36 μM), one compound **3n** was found to be almost equipotent against MTB. 2-Amino-4-[4-(dimethylamino)phenyl]-8-(*E*)-[4-(dimethylamino)phenyl]methylidene-6-methyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]-pyridine-3-carbonitrile (**3n**) was found to be the most active compound in vitro with MIC of 0.43 μM against MTB and was 4.8 times more potent than gatifloxacin. Subsequently, some of the compounds were evaluated against MDR-TB, and the compounds inhibited MDR-TB with MIC ranging from 0.43 to 3.68 μM and all the six compounds screened were found to be more active than isoniazid (MIC: 45.57 μM) and gatifloxacin (MIC: 8.34 μM). Two compounds (**3e** and **3n**) inhibited MDR-TB with MIC less than 1 μM. Compound **3n** was found to be the most active in vitro with MIC of 0.43 μM against MDR-TB, being 19 and 105 times more potent than gatifloxacin and isoniazid, respectively. All the compounds inhibited MC^2 with MIC ranging from 3.68 to 70.42 μM and nine of them were found to be more active than isoniazid (MIC: 45.57 μM) (see Table 1).

With respect to structure–MTB activity relationship, the results demonstrated that the antimycobacterial activity is enhanced by the presence of weakly electron-withdrawing groups like chloro and fluoro in

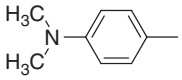
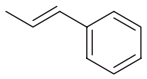
Table 1. Yields, physical constants, and antimycobacterial activities of **3**



Compound	R	Yield (%)[a]	Mp (°C)	MIC		
				MTB	MDRTB	MC[2]
3a		99	166	35.21	NT	70.42
3b		98	181	0.92	1.84	3.68
3c		99 (72)[b]	212	16.32	NT	65.27
3d		99 (97)[b]	200	30.12	NT	60.24
3e		99	177	0.97	0.97	31.97
3f		99	171	1.84	3.68	58.96
3g		98	99	16.32	NT	32.64
3h		99	159	15.06	NT	30.12
3i		98	164	1.75	1.75	56.18
3j		98	153	0.99	1.99	31.97
3k		99 (65)[b]	193	68.12	NT	34.06
3l		99	162	37.31	NT	37.31
3m		98	199	25.36	NT	50.71

(*continued on next page*)

Table 1 (*continued*)

Compound	R	Yield (%)[a]	Mp (°C)	MIC		
				MTB	MDRTB	MC[2]
3n		99	190	0.43	0.43	28.34
3o		99	152	30.71	NT	30.71
Isoniazid	—	—	—	0.36	45.57	45.57
Gatifloxacin	—	—	—	2.08	8.34	2.08

[a] Yields are quantitative, except the loss during workup.
[b] Yields in parentheses from Ref. 8.

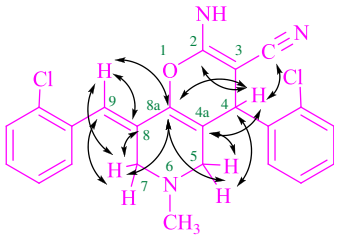


Figure 1. HMBC correlations of **3f**.

the aromatic rings (**3b**, **3e**, **3f**, and **3j**), whilst the presence of two chlorines at 2- and 4-positions (**3m**) diminished the activity. Strongly electron-withdrawing group, nitro at the 3-position of the aromatic ring, is uninfluential leading to the retention of good activity (**3i**). Electron-donating groups at the aryl rings reduce the activity greatly, except in **3n**. Replacement of phenyl by other heterocyclic rings also reduces the activity markedly (**3k**–**l**).

The present investigation reports an atom efficient, green protocol for the synthesis of several tetrahydro-4*H*-pyrano[3,2-*c*]pyridines, which is significantly more advantageous than the literature method. The antimycobacterial potency of these compounds renders them valid leads for synthesizing new compounds endowed with enhanced activity. Further, studies on the synthesis of a wide range of heterocyclic compounds with similar and different structures, and examination of structure–activity relationships, with a view to disclosing new leads and unearthing the factors lying behind the antimycobacterial activity, are in progress in our research groups.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.095.

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9. 2-Amino-4-(2-chlorophenyl)-8-[(*E*)-(2-chlorophenyl)methylidene]-6-methyl-5,6,7,8-tetrahydro-4*H*-pyrano[3,2-*c*]pyridine-3-carbonitrile **3f**: white solid, 99%, mp 170–172 °C, Anal. Calcd for $C_{23}H_{19}Cl_2N_3O$: C, 65.10; H, 4.51; N, 9.90. Found: C, 65.13; H, 4.48; N, 9.94; IR (KBr): υ3380, 2942, 2778, 2190, 1679, 1639, 1602, 1390, 1103 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 2.24 (3H, s, N–CH_3), 2.71 (1H, d, J = 16.2 Hz, 5-CH_2), 3.01 (1H, d, J = 16.2 Hz, 5-CH_2), 3.27 (1H, d, J = 13.8 Hz, 7-CH_2), 3.39 (1H, d, J = 13.8 Hz, 7-CH_2), 4.74 (1H, s, 4-CH), 4.81 (2H, s, NH_2), 6.96 (1H, s, C–CH), 7.13–7.43 (8H, m, ArH); δ_C (75 MHz, $CDCl_3$) 37.5, 44.6, 54.4, 54.8, 58.9, 113.0, 119.4, 120.3, 126.4, 127.8, 127.9, 128.7, 128.8, 129.6, 129.7, 130.4, 133.3, 134.0, 134.3, 139.3, 140.1, 159.5.
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