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Facile synthesis and stereochemical investigation of Mannich base derivatives: Evaluation of antioxidant property and antituberculostic potency

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ABSTRACT

A mini-library of diversely substituted 2,4-diaryl-3-azabicyco[3.3.1]nonan-9-one *O*-methyloximes and their *N*-methyl analogs were synthesized by a non-laborious, modified and an optimized Mannich condensation in good yields. Both the ring N-methylation and oxime O-methylation were employed by various methods; of them, the usage of ^{t}BuOK was found to be the superior in terms of good yield in short time. Stereochemistry of all the synthesized compounds was unambiguously established by their NMR spectral (^{1}H, ^{13}C, ^{1}H–^{1}H COSY, ^{1}H–^{13}C one and multiple bond COSY and NOESY) as well as single-crystal XRD studies. Irrespective of the nature and position of the substituents, all the synthesized oxime ethers of the bicyclic Mannich bases as well as their *N*-methyl analogs adopted the twin-chair conformation with equatorial orientations of all the substituents. All the synthesized oxime ethers were evaluated for their antioxidant property by DPPH radical scavenging method. According to the structure–activity correlations, compound **4y** was found to be a lead molecule with the IC_{50} of 0.187 mg/mL. Thus, the present study exploits the scope of finding more active analogs by further optimization with the incorporation of more electron enriched alkoxy/amino and/or phenolic groups on the heterocycle as well as oxime ether pharmacophore. Most of the synthesized molecules were screened for their antituberculostic potency against *Mycobacterium tuberculosis* $H_{37}Rv$ by zone of inhibition method. Of them, **4w/5d** and **4x** showed very promising inhibition zones of 21 and 23 mm, respectively, which leads to the optimization of **4x** by introducing various substituents on the *O*-benzyl moiety to enhance the antituberculostic potency.

Heterocyclic systems with 3-azabicyclononane nucleus are present in the molecular structure of various diterpenoid/norditerpenoid alkaloids such as kobusine, hetisine, delcorine, deltaline, condelphine, elatine, methyllycaconitine, karacoline, delsoline, nudicauline, lycoctonine, aconitine, lappaconitine, inuline, etc., and it has been isolated from a range of plants including aconitum, delphinium, consolida, thalictrum and spiraea species. They are displaying interesting chemical reactions and important biological actions such as antibacterial, antimycobacterial, antiinflammatory, antiarrhythmic, antifungal, antiallergic, antiprotozoan, anticholinergic, antitumor, anticonvulsant, antiviral, antimalarial, local anesthetic, antineoplastic, hypotensive, cytotoxic, muscle relaxant, analgesic, herbicidal, tyrosinase inhibitor, tranquilizer and nicotinic acetylcholine receptor activity.[1] Similarly, the biological actions of oxime ether pharmacophore, C=N–O–R is also well documented.[2]

In light of the above all, we designed to synthesize a mini-library of *O*-methylated oximes of 2,4-diaryl-3-azabicyco[3.3.1]nonan-9-ones and their *N*-methyl analogs, by combine the bio-active azabicycle and oxime pharmacophores, together. An essential component of the search for new leads in drug designing program is the synthesis of molecules, which are novel still resembling known biologically active molecules by virtue of the presence of some critical structural features.[3] Moreover, the nature and position of the substituents are important factors toward significantly effect the biological actions.[4]

Phenolic and poly-phenolic as well as the EDGs such as OCH_3 and $N(CH_3)_2$ are the key factors to expose the antioxidant property of a molecule.[5] Hence, we synthesized the title compounds with a range of alkoxy substituents such as OCH_3, OCH_2CH_3, $OCH_2CH_2CH_3$, $OCH_2CH_2CH_2CH_3$, $OCH(CH_3)_2$, $OCH_2CH{=}CH_2$, $OCH_2C_6H_5$ and $OCOCH_3$ at various positions of the phenyl groups on both sides

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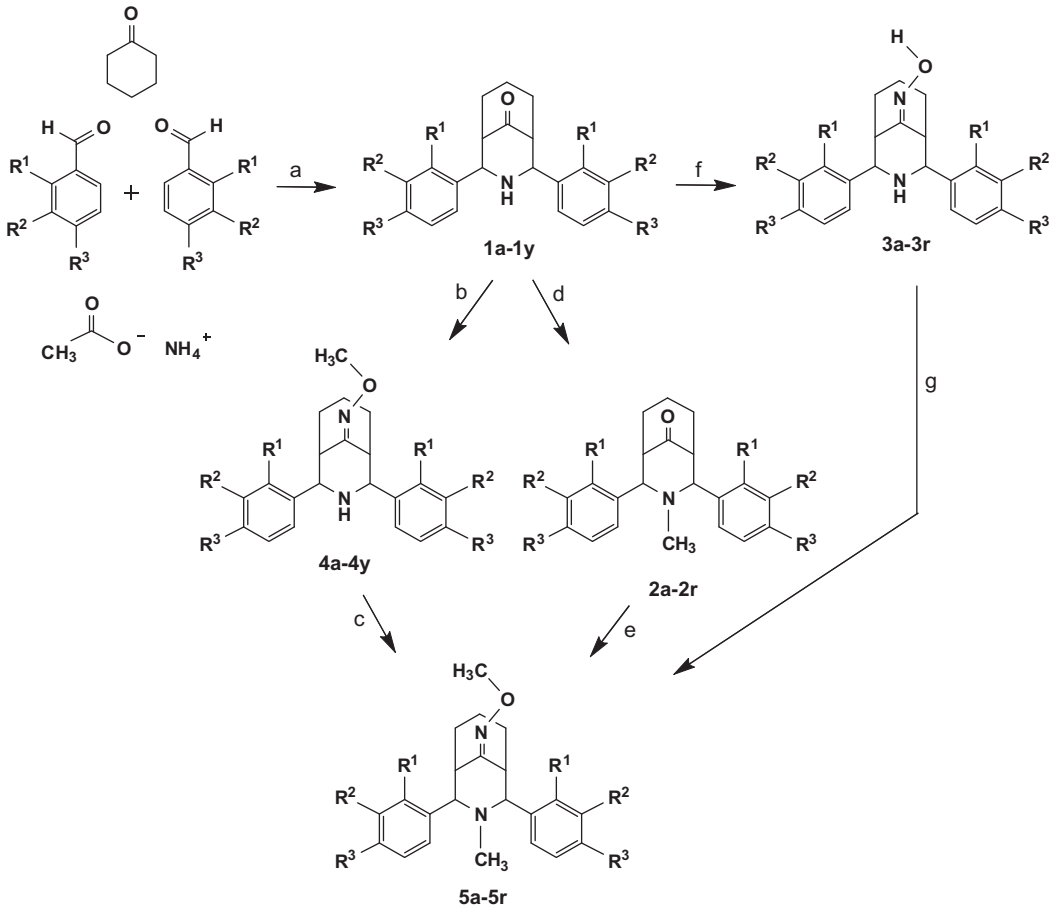
of the secondary amino group; besides, an electron-donating CH_3 group was also introduced on the secondary amino group to improve the activity. Finally, all the Mannich bases were converted as *O*-methyloximes (=N–O–CH_3). Thus, the target molecules were synthesized with a large number of EDGs, but, we could not achieve the Mannich base with OH or $N(CH_3)_2$ substituents on the phenyl.

The 2,4,-diaryl-3-azabicyclo[3.3.1]nonan-9-ones were conveniently synthesized by a modified[1b] and an optimized successive double Mannich condensation of cyclohexanone, substituted benzaldehydes and ammonium acetate in 1:2:1.5 ratio in ethanol (Scheme 1). A typical Mannich condensation[6] involves the use of amine, aldehyde and ketone in acidic medium with poor to moderate yield, whereas, the modified Mannich condensation affords an improved yield by the usage of NH_4OAc and EtOH, instead of amine and acetic acid, respectively. Further, to increase the yield and decrease the tedious work-up procedure/reaction, we have optimized the reaction conditions by the following aspects, eventually achieved good result. They are, (i) we used 1.5 mol of NH_4OAc for 1 mol of cyclohexanone, which completely prevented the by-product chalcone formation, (ii) the addition of ether to the reaction mixture was avoided, which avoided the loss of yield, and (iii)



Com	R^1	R^2	R^3	Com	R^1	R^2	R^3
1a-5a	H	H	H	**1n-4n**	H	H	$CH(CH_3)_2$
1b-4b	F	H	H	**1o-4o**	H	H	SCH_3
1c-4c	H	F	H	**1p-5p**	OCH_3	H	H
1d-5d	H	H	F	**1q-5q**	H	OCH_3	H
1e-5e	Cl	H	H	**1r-5r**	H	H	OCH_3
1f-4f	H	Cl	H	**1s-4s**	OCH_2CH_3	H	H
1g-5g	H	H	Cl	**1t-4t**	H	H	OCH_2CH_3
1h-4h	Br	H	H	**1u-4u**	H	H	$OCH_2CH_2CH_3$
1i-4i	H	Br	H	**1v-4v**	H	H	$OCH_2CH_2CH_2CH_3$
1j-5j	H	H	Br	**1w-4w**	H	H	$OCH_2CH{=}CH_2$
1k-5k	CH_3	H	H	**1x-4x**	H	H	OCH_2-C_6H_5
1l-5l	H	H	CH_3	**1y-4y**	H	OCH_3	$OCOCH_3$
1m-4m	H	H	CH_2CH_3				

Scheme 1. Reagents and conditions: (a) ethanol, warm; (b), (e) CH_3–O–NH_2·HCl, $CH_3COONa·3H_2O$, ethanol, reflux; (c), (d) methyl iodide, dry acetone, anhydrous K_2CO_3, reflux; (f) HO–NH_2·HCl, $CH_3COONa·3H_2O$, ethanol, reflux; (g) NaH, DMF (or) ^{t}BuOK, DMF by stirring at 0 °C–rt. Yields of **5a**–**5r**: 84–89% by "c"; 84–92% by "d"; NaH 75–88% and ^{t}BuOK 83–95% by "g".

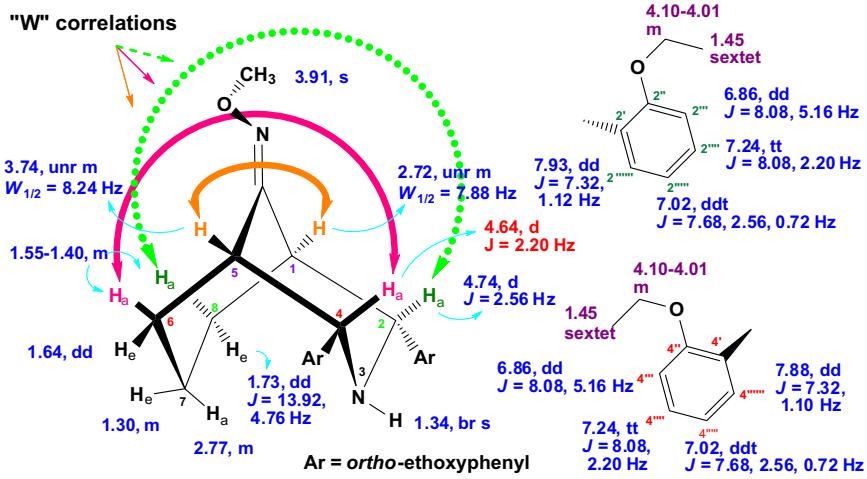


Figure 1. The ^{1}H NMR chemical shifts of compound **4s** are assigned by H,H-COSY and NOESY correlations. The long-range couplings between the protons that are in "W" arrangement are identified by H,H-COSY; of them, between H-4a and H-6a is weaker.

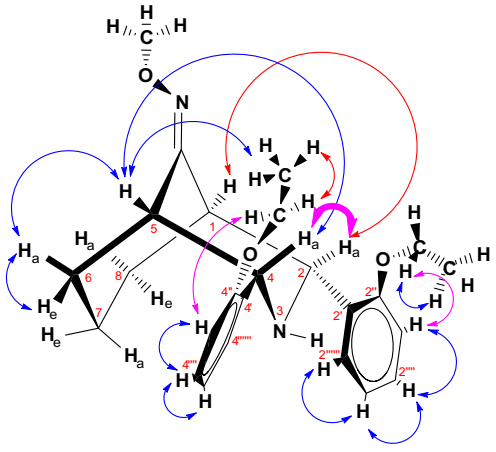


Figure 2. The NOE correlations are represented by the NOESY spectrum of compound **4s**. Accordingly, the 3-azabicycle exists in a twin-chair conformation with equatorial orientations of the *ortho*-ethoxyphenyl groups at C-2 and C-4.

instead of making the Mannich base via the hydrochloride salt, the reaction mixture was moderately stirred between 30 and 35 °C to obtain the base directly as well as non-laboriously.

The 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones were N-methylated by use of methyl iodide in acetone to obtain a good yield 84–92% of *N*-methyl azabicycles. Then, the oxime ethers were obtained by direct condensation of the corresponding azabicycle/*N*-methyl azabicycle with *O*-methylhydroxylamine hydrochloride in ethanol using sodium acetate trihydrate as base. Since the azabicylic oximes **3a**–**3r** are easily preparable in higher yields, we performed the methylation simultaneously on the ring nitrogen and oxime functionality of the azabicyclic oximes **3a**–**3r** by using NaH as well as ^{t}BuOK, eventually achieved good yields both bases. However, the yield of *N*-methylated azabicyclic *O*-methyloximes **5a**–**5r** were improved to 83–95% by condensation of the **3a**–**3r** with methyl iodide and ^{t}BuOK in THF rather than use of the NaH in DMF 75–88% or direct condensation of the ketones with *O*-methylhydroxylamine hydrochloride as discussed for the non-N-methylated compounds.

The stereochemistries of 2,4-bis(2-ethoxyphenyl)-3-azabicyclo[3.3.1]nonan-9-one *O*-methyloxime **4s** and 2,4-bis(4-ethoxyphenyl)-3-azabicyclo[3.3.1]nonan-9-one *O*-methyloxime **4t** are shown in Figures 1–4. According to NMR studies, it is established that all the synthesized bicyclic oxime ethers adopted the twin-chair conformation.

The ^{1}H as well as ^{13}C chemical shifts of the *ortho* isomers are varying (Figs. 1 and 5a) from the *para* isomers (Figs. 3 and 5b).

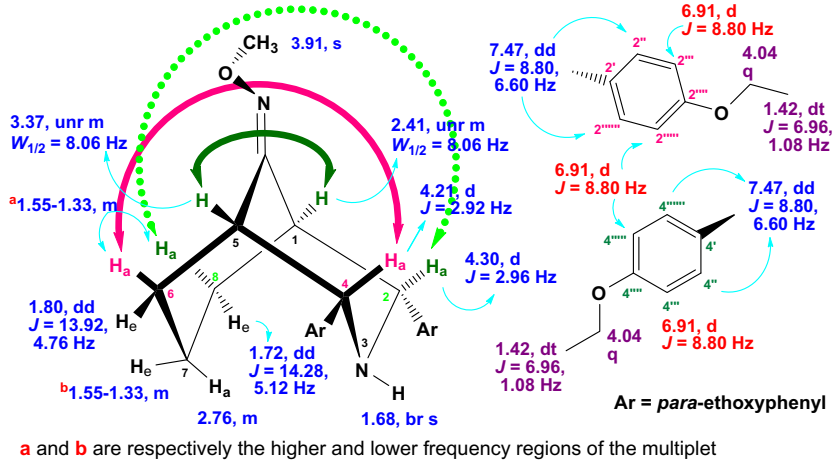


Figure 3. The proton NMR chemical shifts of compound **4t** are assigned by H,H-COSY and NOESY correlations. The long-range couplings between the protons that are in "W" arrangement are identified by the correlations from H,H-COSY spectrum.

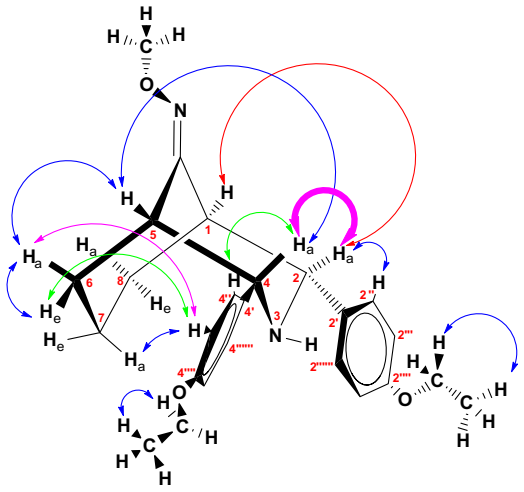


Figure 4. The important NOE correlations obtained from the NOESY spectrum of compound **4t** is represented. Accordingly, **4t** also adopts the same conformation as compound **4s** with equatorial orientations of the *para*-ethoxyphenyl groups on both sides of the secondary amino group.

The proton and carbon chemical shifts are deshielded and shielded, respectively, compared to the unsubstituted phenyl, according to the varying impact on the chemical shifts with varying magnitude of electronegativity of the substituents. The deshielding of protons is reasonably attributed by the interaction between the halogens and benzylic/bridge-head protons, indeed, which is more with the bridge-head protons by their spatial proximity, than benzylic protons.

The established stereochemistry by NMR in solution state is further confirmed by the single-crystal XRD of **4e** (Fig. 6). The detailed XRD analysis shows that the piperidine ring C1–C2–C8–C6–C7–N1 adopts a near ideal chair conformation with the deviation of ring atoms N1 and C8 from the best plane C1–C2–C6–C7 by −0.618 and 0.707 Å, respectively. Similarly, the analysis of cyclohexane C2–C3–C4–C5–C6–C–8 indicates that which also adopts the chair, however, deviated from the ideal chair as follows. The ring atoms C4 and C8 deviated from the best plane C2–C3–C5–C6 by 0.449 Å and 0.652 Å, respectively. The torsion angles of C8–C6–C7–C15 and C8–C2–C1–C9 of the *ortho*-chlorophenyl rings are 179.9(10) and 179.6(11)°, respectively, and they are orientated at an angle of 21.01(3)° with respect to one another.[7] Thus, the bicycle exists in a twin-chair conformation.

In N-methylated bicycles, the benzylic carbons C-2/C-4 and their protons H-2a/H-4a are deshielded and shielded about 9–10 and <1 ppm, respectively, due to the effect of N-methylation. In addition, the vicinal coupling constants $J_{2a,1}$ and $J_{4a,5}$ are higher than corresponding non-N-methylated bicycles by means of

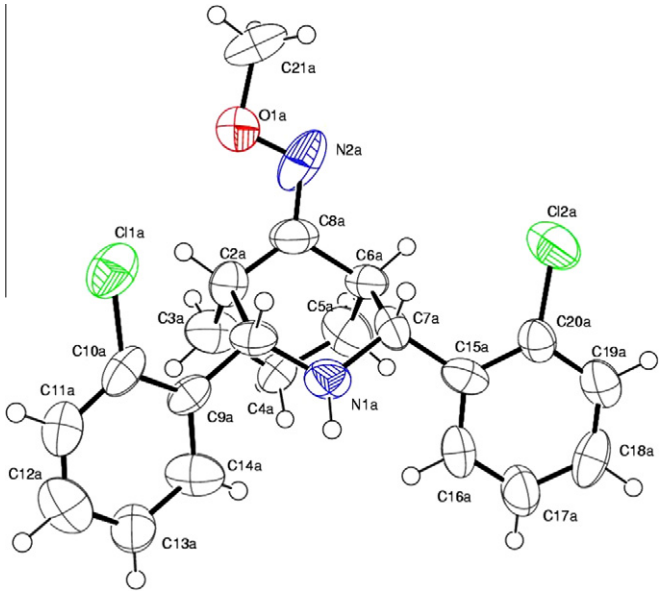


Figure 6. Anisotropic displacement representation of the molecule **4e** with atoms represented with 30% probability ellipsoids (for clarity of the picture, one part of the asymmetric unit only shown in Figure). The 3-azabicycle exists in a chair conformation with equatorial orientations of the *ortho*-chlorophenyl rings on both sides of the secondary amino group. The asymmetric unit of this molecule, $C_{42}H_{44}Cl_4N_4O_2$, crystallized in a monoclinic system under the space group $P2_1/n$ with cell parameters, $a = 16.3022(18)$ Å, $b = 15.2021(14)$ Å, $c = 16.3172(18)$ Å, $\beta = 107.352(4)$ and $Z = 4$.

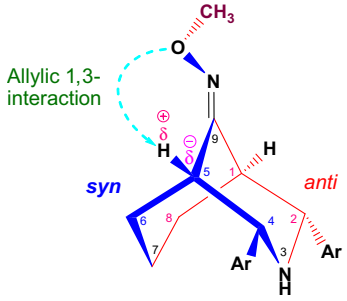


Figure 7. Non-bonded interaction between the N-O and C(5)-H bonds.

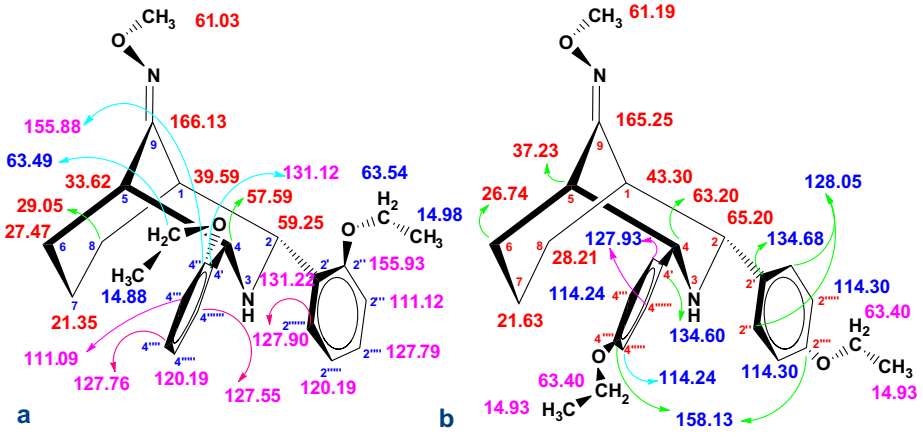
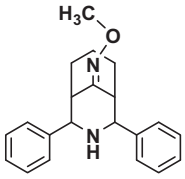
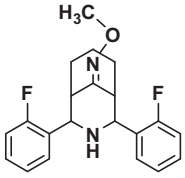
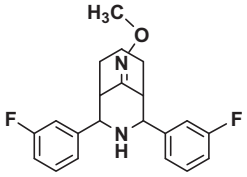
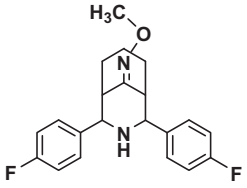
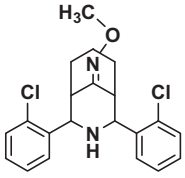
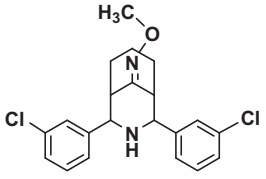
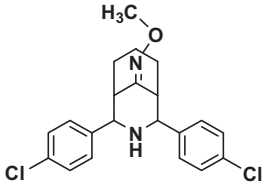
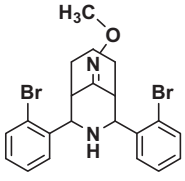


Figure 5. The ^{13}C NMR chemical shifts of compounds **4s** and **4t** are assigned by their one and multiple bond ^{1}H–^{13}C-COSY correlations.

Table 1
Antioxidant and antituberculostic activities of the 3-azabicyclic oxime ethers **4a**–**5r**

Compound	Compound structure	Antioxidant potency		Anti-MTB activity
		[a]Concentration	% of inhibition	
4a		100 200 400	10.50 18.67 24.42	–
4b		100	2.33	++
4c		100	0.97	+
4d		100	3.60	+++
4e		100	6.47	+
4f		100	4.77	–
4g		100	6.58	++
4h		100	5.28	NT

(*continued on next page*)

Table 1 (*continued*)

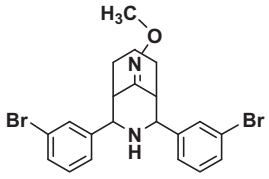
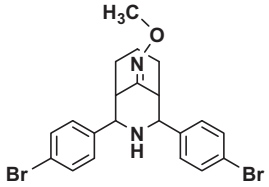
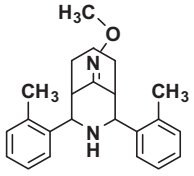
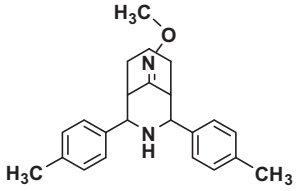
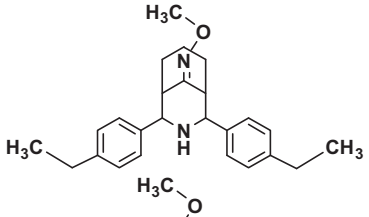
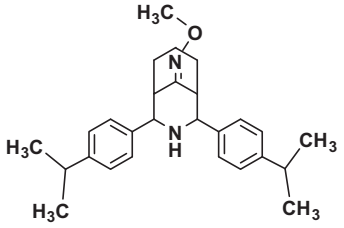
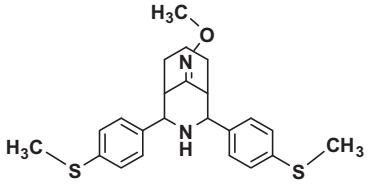
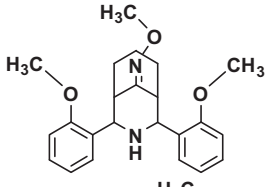
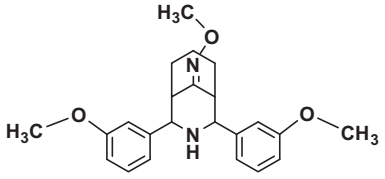
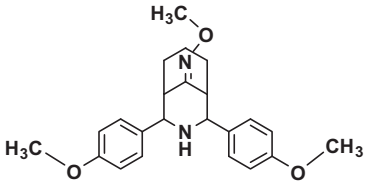
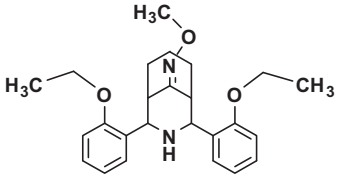
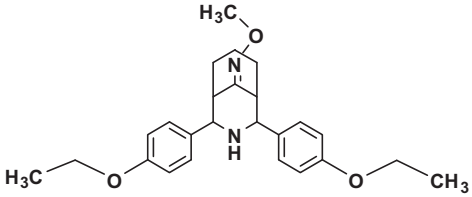
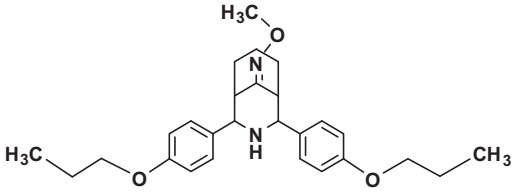
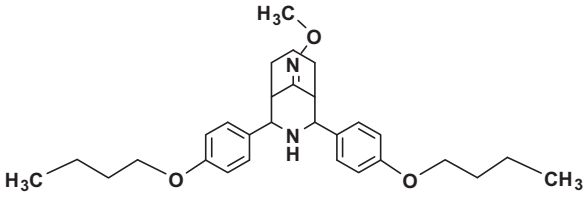
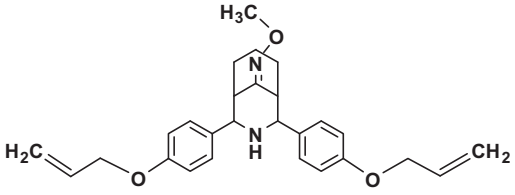
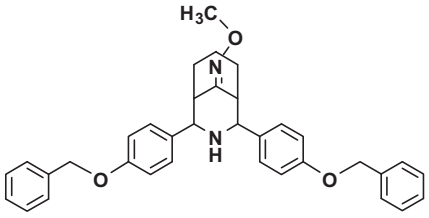
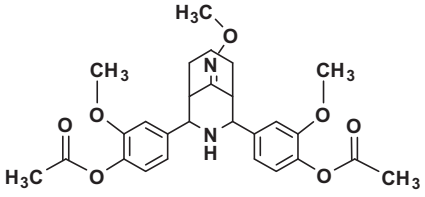
Compound	Compound structure	Antioxidant potency		Anti-MTB activity
		[a]Concentration	% of inhibition	
4i		100	4.35	NT
4j		100	5.72	–
4k		100	9.50	–
4l		100	14.46	–
4m		100	11.91	–
4n		100	15.29	+
4o		100 200	17.64 22.53	++
4p		100 200	16.04 20.71	+
4q		100	13.50	NT

Table 1 (*continued*)

Compound	Compound structure	Antioxidant potency		Anti-MTB activity
		[a]Concentration	% of inhibition	
4r		100	13.95	+
4s		100	10.17	+
4t		100 200	23.22 30.82	+
4u		100 200	23.93 38.52	+
4v		100 200	27.80 43.87	+
4w		100 200 400	21.70 25.82 31.77	++++
4x		100 200	27.85 47.76	++++
4y		100 200 400 800	32.39 52.91 78.30 88.85	NT

(*continued on next page*)

Table 1 (*continued*)

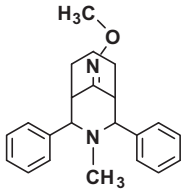
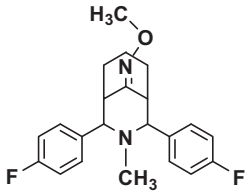
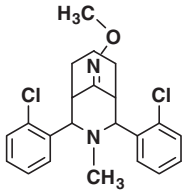
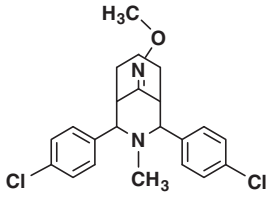
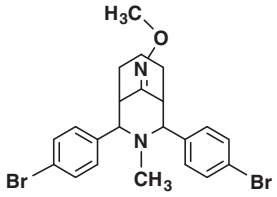
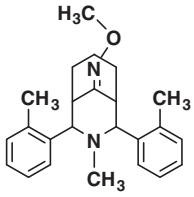
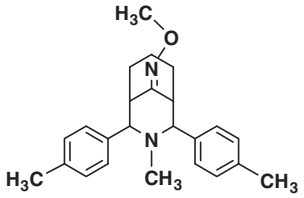
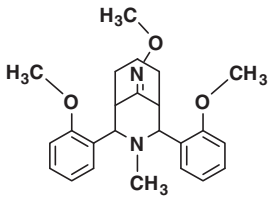
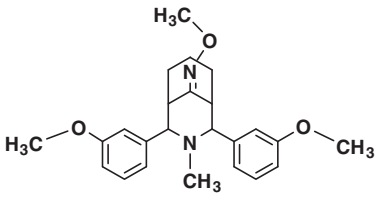
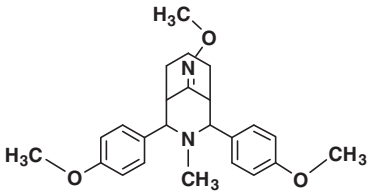
Compound	Compound structure	Antioxidant potency		Anti-MTB activity
		[a]Concentration	% of inhibition	
5a		100 200	10.73 13.80	NT
5d		100	2.40	++++
5e		100	11.37	++
5g		100	11.90	++
5j		100	12.13	–
5k		100	15.29	+
5l		100 200	19.75 22.02	+
5p		100 200 400	15.39 24.90 40.20	NT

Table 1 (*continued*)

Compound	Compound structure	Antioxidant potency		Anti-MTB activity
		[a]Concentration	% of inhibition	
5q		100 200 400	10.72 13.80 26.76	NT
5r		100 200 400	20.88 41.88 58.82	+
L-Ascorbic acid		10	98.46	
Isoniazid				++++

Activity Index: "–": 0–5 mm; "+": 6–10 mm; "++": 11–15 mm; "+++": 16–20 mm; "++++": ⩽21 mm.
NT: Not tested; NA: Not applicable.
Anti-MTB: Zone of inhibition against *Mycobacterium tuberculosis* $H_{37}Rv$.
[a] Concentration is represented in μg/mL.

lowering the electronegativity of NH by the introduction of methyl group. However, all the synthesized N-methylated bicyclic oxime ethers **5a**–**5r** adopted the twin-chair conformation with equatorial orientations of the substituents as their non-*N*-methyl analogs.

The oximination effect on $^{1}H/^{13}C$ chemical shifts are significant by the allylic 1,3-interaction between the N–O and C(5)–H bonds besides the decrease in electronegativity at C-9 by the reduction of C=O as C=N. In fact, $A^{1,3}$ interaction is noteworthy (Fig. 7) as H-5 (*syn* α-proton) deshielded >1 ppm and C-5 (*syn* α-carbon) shielded about 7 ppm besides the electronegativity (oximation) effect on that proton/carbon. Thus, $A^{1,3}$ interaction dominated the electronegativity effect on consecutive *syn* β position and reduced the impact of electronegativity to some extent on *syn* γ-carbon.

Since the antioxidants are gaining a lot of importance as panacea for a large number of life-style diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases, it is of immense significance to establish some new antioxidants by a convenient synthetic methodology. Accordingly, we synthesized a library of Mannich derivatives and were evaluated for their in vitro antioxidant activity by DPPH radical scavenging method of Blois[8] with slight modifications.[9] Although a number of methods such as ORAC, ABTS, DMPD, FRAP, TRAP, TBA, superoxide radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, xanthine oxidase, cytochrome C, reducing power method, etc. available, the DPPH method is very common and proved as the best.[10]

Although we designed the target molecules in such a way with a large number of EDGs on the phenyl groups and methyl group on the ring nitrogen and oxime functionality, most of the compounds did not express a good antioxidant activity according to our expectation. Generally, halo substituents do not hold a good antioxidant profile due to their electron-withdrawing nature, but we expect that the OCH_3 group on the oxime functionality will exhibit antioxidant activity with an added influence of CH_3 on the ring nitrogen. In fact, a careful analysis of the data from Table 1 reveals that most of the alkyl/alkoxy compounds did not exhibit a good antioxidant property, except a few compounds **4t**–**4y**; very particularly, **4y** exhibited its best activity at the IC_{50} of 0.187 mg/mL.

The Mannich derivatives were screened for their in vitro antituberculostic activity against *M. tuberculosis* $H_{37}Rv$ by zone of inhibition method and their inhibition levels are reproduced in Table 1. The inhibition by **4a** (compound with no substitution on the phenyl) is negligible, whereas, the inhibition efficiency increased by incorporating halo substituents on the phenyl in this order F > Cl > Br. Of the F substituted compounds **4b**–**4d**, **4d** (*para* substituted compound) registered a good inhibition of 16 mm and its *N*-Me analog **5d** registered an improved inhibition of 21 mm. As fluoro, the *N*-methyl analogs of Cl and Br compounds also showed an improvement in their inhibition zone; however, they did not cross the moderate level. Moreover, the replacement of halo by alkyl/alkoxy substituents also did not exhibit a remarkable enhanced activity. Surprisingly, the allyloxy **4w** and benzyloxy **4x** compounds registered an excellent inhibition of 21 and 23 mm, respectively.

In conclusion, a mini-library of 35 Mannich derivatives was synthesized very conveniently in high yields. All the molecules were designed in such a manner to possess electron-donating alkoxy/hydroxy/amino substituents on the phenyl. Although amino/hydroxyl substituents failed to yield the Mannich base, a variety of alkoxy/alkyl substituents afforded the desired product as a single isomer. Further, to increase the electron-donating tendency, methyl group was introduced on the secondary amino group and oxime functionality. However, most of the halo/alkyl/alkoxy substituents exhibited a poor activity, compound **4y** (methoxy and acetyloxy substituents at *meta* and *para* positions of the phenyl groups, respectively) exhibited its best DPPH radical scavenging activity at the IC_{50} 0.187 mg/mL. Thus, the compound **4y** is identified as a lead molecule. Further optimization of the lead molecule **4y** to improve its antioxidant property by incorporating more alkoxy and/or polyhydric phenolic groups on the phenyl as well as on the oxime functionality is under progress. Since the synthesis of the above molecules are very convenient and high yielding as well as stereospecific, optimization of this molecule would provide a better class of compounds with a good antioxidant profile, comparable to flavonoids.

Of the tested compounds against *M. tuberculosis* $H_{37}Rv$, **4x** registered a promising activity, which suggest us to optimize this molecule for further investigation toward its antituberculostic potency, and hence, incorporation of various groups on OBn moiety is planned to carry out with toxicity and mechanistic studies.

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Supplementary data

Supplementary data (complete experimental details, ^{1}H and ^{13}C NMR data of all compounds, 2D NMR data of the representative compounds, and single crystal XRD data of **4e**. Supplementary crystallographic data for **4e** (CCDC No. 812239) can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.103.

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9. Briefly, 160 μL of a methonal solution with various concentrations (0.625–320 μg/mL) were added to a 40 μL DPPH methonal solution (1.5×10^{-4} M). After mixing gently and standing at rt for 30 min, the optical density was measured at 530 nm using a microplate reader spectrometer VERSA max (Molecular Devices). The antioxidant activity IC_{50} of the sample required to inhibit DPPH radical formation by 50% was calculated from the log-dose inhibition curve. L-Ascorbic acid was used as the positive control. In its radical form, DPPH absorbs at 520 nm, but upon reduction by an antioxidant or a radical species, the absorption disappears. The reduction of DPPH as indicated above is followed by monitoring the decrease in its absorbance at a characteristic wavelength during the reaction.
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