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Design, synthesis, and biological evaluation of 4-(5-nitrofuran-2-yl)prop-2-en-1-one derivatives as potent antitubercular agents

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ABSTRACT

Based on stereoelectronic feature analysis using density functional theory (DFT) at B3LYP/3-21*G level, a series of 4-(5-nitrofuran-2-yl)prop-2-en-1-one derivatives with low LUMO energies (<−0.10 eV); concentrated over the nitro group, furan moiety and α,β-unsaturated carbonyl bridge were envisaged as potential antitubercular agents. The target compounds were prepared by condensation of 5-nitro-2-furaldehyde with various ketones under acidic condition. The compounds were evaluated for antitubercular activity against *Mycobacterium tuberculosis* H37Rv and their cytotoxicity in VERO cell line. Several synthesized compounds showed good antitubercular activity of <5 μM along with low cytotoxicity. In particular, compound ((*E*)-3-(5-nitrofuran-2-yl)-1-(4-(piperidin-1-yl)phenyl)prop-2-en-1-one) (**3v**) was found to be very potent (MIC: 0.19 μM) with good selectivity index (MIC_{90}/CC_{50}: >1800). Thus, this study shows the potential of stereoelectronic property analysis in developing improved nitroaromatics as antitubercular agents.

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb), a obligate pathogen remains one of the most fatal infectious disease.[1,2] World Health Organization (WHO) estimates that 1.3 million people died due to TB in 2008.[3] Despite elucidation of the genomic sequence of Mtb and emergence of multiple targets, TB continues to be a serious public-health problem at the global level.[4–7] Hence, there is a need to develop novel therapeutics to treat TB.

Currently, two molecules **PA-824** and **OPC-67683** belonging to the nitroimidazole class, having good in vitro and in vivo activity against Mtb in both its active and persistent forms, are being evaluated in phase II clinical trials to treat tuberculosis.[8] Recently, considerable research is focused on understanding the mechanistic details of this class of molecules.[9] It is proposed that the release of toxic intermediates, formed by the one electron and two electron bioreduction of the nitroimidazole moiety is the key component responsible for the high activity of these molecules.[10] Two series of nitrofurans have also been reported as antitubercular agents,[11] while other nitrofurans are reported to be antibacterial agents.[13]

Interactions between molecules are the consequence of their stereoelectronic properties that govern strength of bonds, strength of nonbonded interactions and molecular reactivity. Molecular electrostatic profile (MESP) is a very useful tool in understanding the chemical reactivity of molecules. Pharmacophore mapping and MESP analysis employing hybrid density functional theory with Becke's three-parameter exchange potential and the Lee–Yang–Parr correlation functional (B3LYP) using basis set 3-21G*, on the reported antitubercular nitrofurans[12] suggested that all the very active compounds possess characteristic localized negative potential regions near both the oxygen atoms of nitro group which extend laterally to the isoxazole ring system/amide bond.[14] Furthermore, higher negative values of LUMO energies located over the nitro group in the most active compounds were indicative of the electron acceptor capacity of the compounds, suggesting that these compounds are prodrugs and must be activated by TB-nitroreductase. Thus, this study indicated that compounds with good antitubercular activity could be designed by variation in LUMO energies that can be affected by changing the electronic features of the nitro heterocycle. It is worthy to note that, recently, while current work was under progress in our laboratory, Valdez et al. reported that molecules having olefins with a conjugated bridge connecting the nitro heterocycle and a substituted phenyl or heterocyclic ring showed a great increase in antigiardial activity and lower toxicity as compared to metronidazole in a murine giardiasis model.[15]

Considering the apparent importance of nitro reduction in the activation of nitroaromatic compounds, compounds were designed by coupling electronegative α,β-unsaturated carbonyl bridge to the nitrofuran ring and substituted phenyl or heterocyclic ring to minimize LUMO energies (Fig. 1). The designed compounds were studied by complete geometry optimization at DFT level of theory using the B3LYP functional and 3-21*G basis set (B3LYP/3-21*G). All calculations were carried in aqueous environment using the Poisson–Boltzmann solver as implemented in Jaguar.[14,16]

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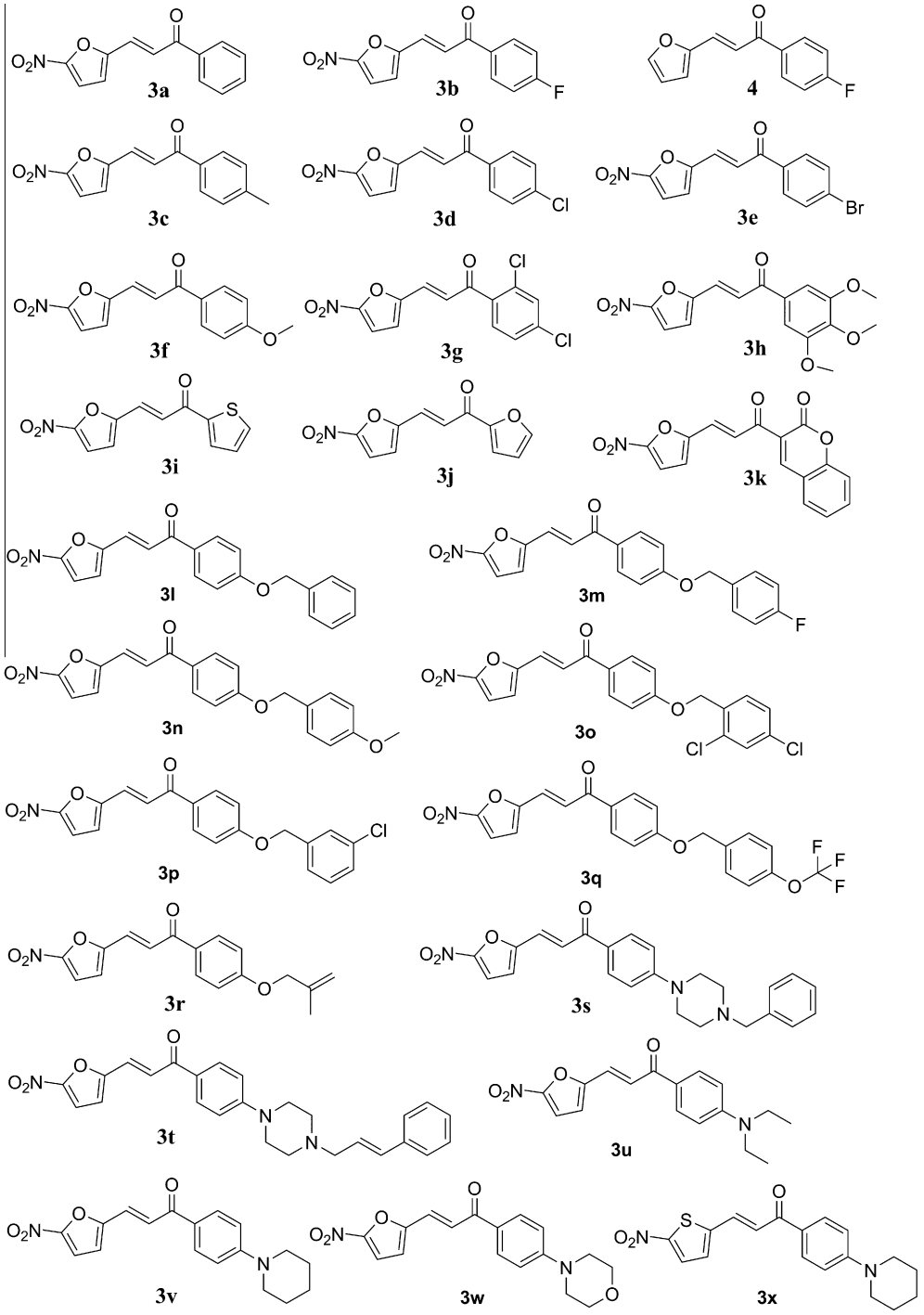


Figure 1. Chemical structures of the compounds synthesized.

Corroborating with our design postulate, DFT results showed that all compounds posses localized negative potential regions near the oxygen atom of carbonyl group and both the oxygen atoms of nitro group that extend laterally to the furan ring oxygen (Fig. 2a and b). Furthermore, the compounds have smaller values of LUMO energies (<−0.1 eV) located over the nitro group, furan ring and α,β-unsaturated carbonyl bridge attached to it (Table 1, Fig. 2c).

Thereafter, these compounds were considered for synthesis and biological activity evaluation against Mtb. Initially, compounds, **3a–k** (Fig. 1) were synthesized as exploratory set, with various aromatic substitutions and changing the aromatic moiety with heteroaromatic moiety; using acid catalyzed reaction (Scheme 1),[17] as the starting material, 5-nitro-2-furaldehyde is alkali sensitive and standard Claisen–Schmidt condensation under basic condition could not be used. The purity of the compounds was checked by HPLC and the structures were confirmed by spectral data (FT-IR, ^{1}H NMR, ^{13}C NMR, and MS). The synthesized compounds (**3a–k**) were screened against Mtb H37Rv using twofold dilution, in order to determine the actual minimum inhibitory concentration (MIC) with Resazurin microtiter assay (REMA).[18,19] MIC is the lowest concentration at which complete inhibition was observed and was

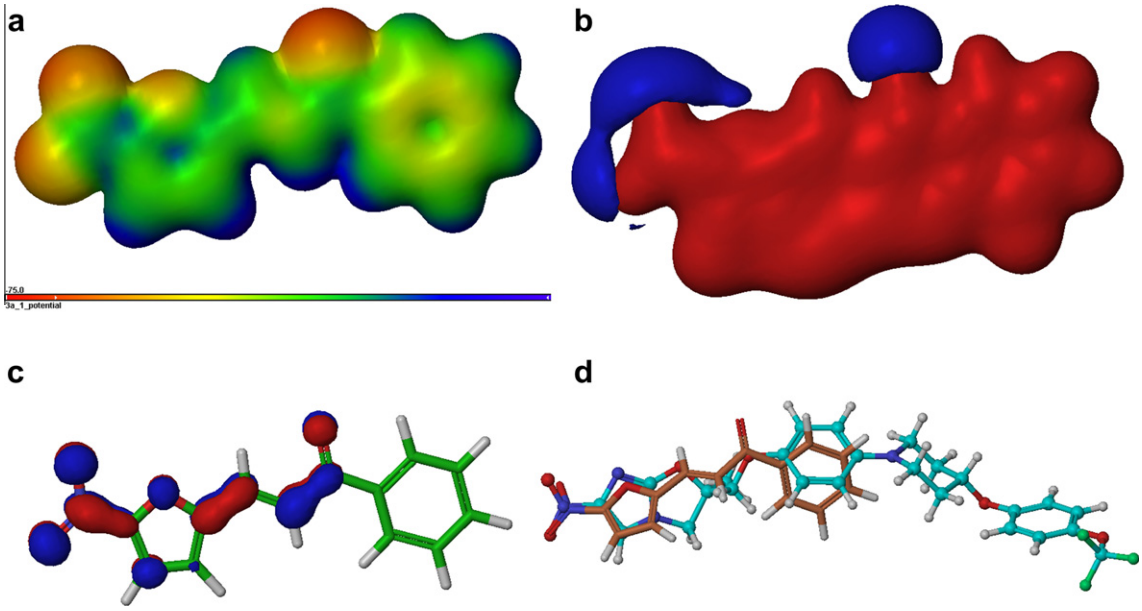


Figure 2. (a) MESP (−75 to 110 kcal/mol) superimposed onto a surface of constant electron density (0.01 e/au^3) for compound **3a** showing the most positive potential region (deepest blue color) by the protons of C_3 and C_4 of the furan ring and protons of α,β-unsaturated system and the most negative potential region (deepest red color) by the carbonyl oxygen and oxygen atoms of the nitro group extending laterally over the oxygen atom of furan ring. (b) Three-dimensional isopotential contours of MESP at −30 kcal/mol for compound **3a** showing negative potential regions around the carbonyl oxygen and oxygen atoms of the nitro group extending laterally over the oxygen atom of furan ring. (c) LUMO energy distribution for compound **3a**. (d) Superimposition of compound **3a** (violet color) and **OPC67683** (cyan color).

Table 1
Results of antitubercular activity and cytotoxicity values for the synthesized compounds, along with their calculated electronic properties

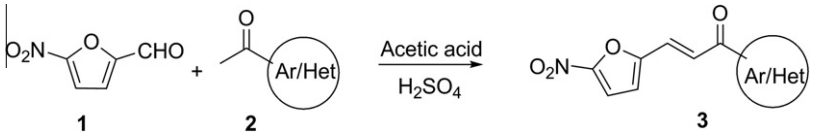
Sr. no.	Code	MW	MIC[a] (μM)	CC_{50}[a] (μM)	HOMO (eV)	LUMO (eV)
1	**3a**	243.21	5.51	444.06	−0.25	−0.12
2	**3b**	261.21	4.17	463.23	−0.25	−0.12
3	**4**	216.21	>93.89	661.39	−0.23	−0.09
4	**3c**	257.24	4.35	439.28	−0.25	−0.12
5	**3d**	277.66	4.50	558.24	−0.25	−0.12
6	**3e**	322.11	3.88	353.92	−0.25	−0.12
7	**3f**	273.24	3.84	570.93	−0.24	−0.12
8	**3g**	312.10	14.13	416.53	−0.25	−0.12
9	**3h**	333.29	6.06	384.05	−0.22	−0.12
10	**3i**	249.24	5.86	742.26	−0.25	−0.12
11	**3j**	233.18	8.28	776.22	−0.25	−0.12
12	**3k**	311.25	29.94	359.84	−0.25	−0.12
13	**3l**	349.34	3.29	515.26	−0.24	−0.12
14	**3m**	367.33	19.79	430.13	−0.24	−0.12
15	**3n**	379.36	1.85	311.05	−0.22	−0.12
16	**3o**	418.23	>46.63	339.53	−0.24	−0.12
17	**3p**	383.78	6.10	307.47	−0.24	−0.12
18	**3q**	433.33	1.66	334.62	−0.24	−0.12
19	**3r**	313.30	5.59	392.59	−0.24	−0.12
20	**3s**	417.46	>31.74	299.43	−0.20	−0.12
21	**3t**	443.49	>32.70	342.74	−0.20	−0.12
22	**3u**	314.34	0.48	334.03	−0.20	−0.12
23	**3v**	326.35	0.19	349.32	−0.20	−0.11
24	**3w**	328.32	0.38	386.82	−0.20	−0.12
25	**3x**	344.38	15.62	403.62	−0.20	−0.12
26	INH	137.10	1.80	>500.0	ND	ND

ND—not determined.
[a] Results of three independent experiments performed in duplicate.

determined by visual inspection (color change from blue to pink) (Table 1). Isoniazid (INH) was used as the reference drug. The compounds were also evaluated for toxicity in a mammalian VERO cell line (C1008) in 96-well microtitre plates and the CC_{50} values were determined.[19]

Among the synthesized compounds, **3a–k**; five compounds showed good antitubercular activity of <5 μM. The *p*-methoxy substituted compound, **3f**, was found to be the most active compound. It was observed that there was little difference in activity between unsubstituted and *p*-monosubstituted aromatic ring in compounds, **3a–f**. Di- and tri-substitution led to lower activity as seen in compounds, **3g,h**. Replacing the aromatic ring by heteroaromatic moiety, **3i–k**, led to decrease in activity. To evaluate the effect of nitro group on the activity; des-nitro derivative, **4**, of compound, **3b**, was synthesized and evaluated. The des-nitro compound was found to be inactive at high concentration (~90 μM), thus, indicating the importance of nitro group for the activity of these compounds. This observation is in accordance with the recently published results on **PA-824** and **OPC-67683**, where the corresponding des-nitro derivatives were devoid of antitubercular activity.[20,21]

To further modify and enhance the potency of these compounds, the unsubstituted compound, **3a**, was aligned on the structure of **OPC-67683** using PHASE (Fig. 2d).[22] Alignment studies revealed the scope for introduction of a substituted benzyloxy moiety at the *para*-position. In this light, compounds, **3l–q**, were synthesized (Scheme 1). Commercially unavailable substituted acetophenone derivatives required for the synthesis of **3l–q** were prepared by etherification of *p*-hydroxyacetophenone with corresponding



Scheme 1. Synthesis of 4-(5-nitrofuran-2-yl)prop-2-en-1-one derivatives. Reagents and conditions: acetic acid, catalytic H_2SO_4, 60–100 °C, 3–6 h.

benzyl halide in presence of K_2CO_3 using CH_3CN as solvent.[23] Within these molecules, compounds with *p*-methoxy, **3n** (MIC: 1.85 μM) and *p*-trifluromethoxy, **3q** (MIC: 1.66 μM) substitution showed good activity. It is interesting to note that both **PA-824** and **OPC-67683** posses *p*-trifluromethoxy substitution in the distal part of their structures. The compound, **3r**, with a flexible methyallyloxy substitution at the distal part was found to be less active than the compound with a distal aromatic ring, **3l**.

Results of Tangallapally et al.[24] study and **OPC-67683** SAR revealed the importance of various tertiary amines as important features responsible for potent antitubercular activity of nitroaromatics. In this light, compounds, **3s,t**, were synthesized. The required ketones for the synthesis of **3s**,**t** were synthesized by substituting the fluorine of 4-fluoroacetophenone by conventional aromatic nucleophilic displacement with secondary amines 1-benzylpiperazine and 1-cinnamylpiperazine.[25] However, the biological activity evaluation revealed that both compounds were less active, this could be due to increase in bulk at the *para*-position. Thereafter, compounds, **3u–w**, were synthesized by introducing small tertiary amine substitutions at the *para*-position. All of these compounds exhibited potent antitubercular activity at <0.5 μM. Of particular Letter, compound **3v** ((*E*)-3-(5-nitrofuran-2-yl)-1-(4-(piperidin-1-yl)phenyl)prop-2-en-1-one) was found to be most potent (MIC: 0.19 μM). The compound, **3v**, was ~10 times potent than the first line antitubercular agent INH.

Dramatic decrease in activity was found for compound, **3x**, which was synthesized by replacing the furan moiety of **3w**, with thiophene moiety from corresponding 5-nitrothiophene-2-carbaldehyde, pointing to the importance of the electronegative furan moiety in the vicinity of the nitro group. Thus, compounds with small tertiary amine feature as structural features (**3u**, **3v**, and **3w**) were more active than other compounds. Fitting of ligand in the receptor site plays an important role for activity. This has been achieved by actual experiments with systematic modification of substitutions. This coupled with lower LUMO energies is responsible for overall activity picture and therefore no direct correlation between the LUMO, HOMO energies and biological activity was observed.

The compounds were also evaluated for toxicity in a mammalian VERO cell line (C1008) in 96-well microtitre plates and the CC_{50} values were determined (Table 1). All compounds showed good safety profile, particularly, the most potent compound, **3v**, had a selectivity index >1800.

In summary, several 4-(5-nitrofuran-2-yl)prop-2-en-1-one derivatives were designed and synthesized using stereoelectronic feature analysis. Screening of the antimycobacterial activity of these compounds identified ((*E*)-3-(5-nitrofuran-2-yl)-1-(4-(piperidin-1-yl)phenyl)prop-2-en-1-one) (**3v**) as a lead endowed with high antitubercular activity. This study also shows the potential of combining stereoelectronic property analysis with synthetic approaches and biological evaluation in developing improved nitroaromatics as antitubercular agents. Further structure–activity relationship and mechanistic studies are under progress in our laboratory.

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