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Antibacterial activities of novel nicotinic acid hydrazides and their conversion into *N*-acetyl-1,3,4-oxadiazoles

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Dedicated to Professor Abdel-Rahman S. Ferwanah on the occasion of his retirement

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ABSTRACT

Synthesis of a series of novel *N*-acylhydrazones of nicotinic acid hydrazides **3a–j** via condensation of nicotinic acid hydrazide **1** with the corresponding aldehydes and ketones is described. The series **3a–j** was evaluated for in vitro antibacterial activity against two gram negative (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and two gram positive (*Streptococcus pneumoniae* and *Staphylococcus aureus*) bacteria. The zone of inhibition was measured using the disk diffusion method, and in vitro minimum inhibitory concentration indicating that compounds **3a** and **3e** were effective against *P. aeruginosa* with MICs of 0.220 and 0.195 μg respectively.

Emerging multi-drug resistant (MDR) pathogens have become increasingly difficult to treat with existing antibiotics, and the future effectiveness of antimicrobial agents remains in doubt.[1] It is imperative that a global strategy be implemented to prevent further microbial resistance and that new antimicrobial agents be discovered.[2] Cases of infection with MDR-bacteria have increased to alarming levels[3] and isolates of *Mycobacterium tuberculosis*, the causative agent in tuberculosis (TB), are now frequently found to express genes for antibiotic resistance.[4]According to a World Health Organization (WHO) report published in 2013, around 1.3 million fatalities in 2012 were attributable to TB, including 320,000 deaths amongst HIV-sufferers.[5]

The hydrazone function, $R_1R_2C = NR_3–NR_4$ (R = alkyl, aryl or H) is an important pharmacophore in a variety of drugs.[6] Related hydrazide-hydrazones, $R_1R_2C = NR_3–NR_4COR_5$ have been shown to exhibit significant antibacterial, antifungal,[7,8] anticonvulsant,[9,10] anti-inflammatory,[11,12] antimalarial[13,14] and antitubercular activity.[15,16] Hydrazide–hydrazones are also synthetic precursors to pharmacologically active 1,3,4-oxadiazoles.[17,18] A 1,3,4-oxadiazole, raltegravir, was the first integrase inhibitor approved for HIV therapy by the FDA (adults, 2007, paediatric therapy, 2011).[19] Oxadiazoles currently in late stage clinical trials include zibotentan, (1,3,4-oxadiazole) an anticancer agent and ataluren, (1,2,4-oxadiazole) a potential treatment for cystic fibrosis.[20,21]

N-Acylhydrazones, which are also precursors of 1,3,4-oxadiazoles, can be synthesized by condensation of an *N*-acylhydrazide with an aldehyde or ketone, either in the presence or absence of an acid catalyst.[22,23] Cyclization of *N*-acylhydrazides to give 1,3,4-oxadiazoles can be achieved under a variety of dehydrating conditions.[24–27] Herein, we describe the synthesis and antimicrobial activity of novel synthetic *N*-acylhydrazones derived from nicotinic acid hydrazide and their conversion into *N*-acetyl-1,3,4-oxadiazoles.

Compounds **3a–j** and **4a–j** were prepared by condensation of nicotinic acid hydrazide **1** with aliphatic ketones **2a–e** or with either aliphatic aldehyde **2f** or aromatic aldehydes **2g–j** under

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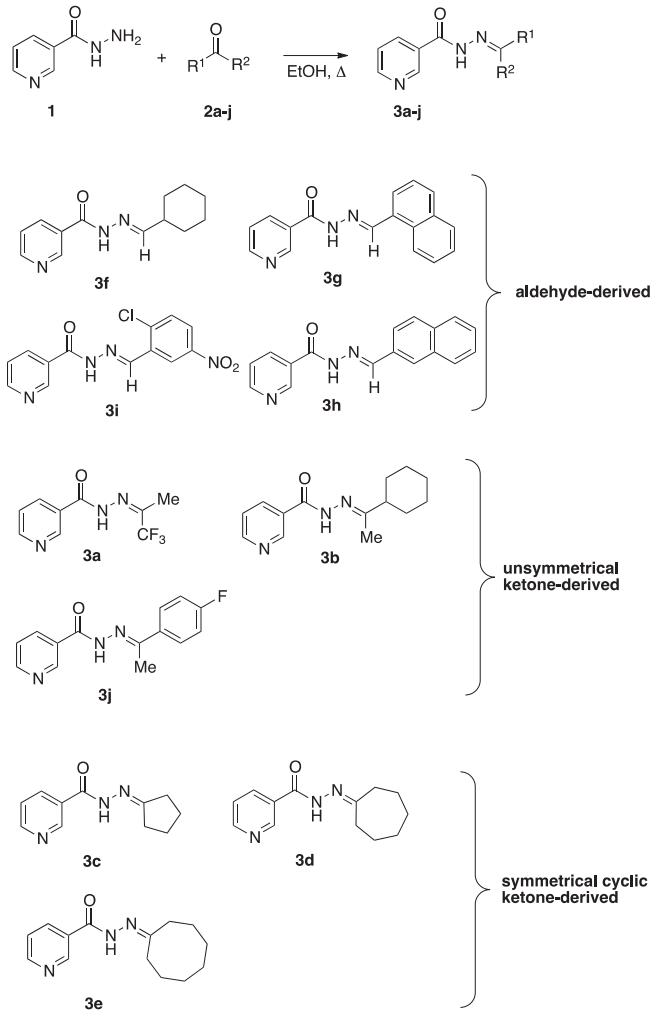
reflux in ethanol to yield the target compounds in 80–90% yield (Scheme 1). No acid catalyst was required to carry out these reactions. The crude products were recrystallised from the minimum amount of ethanol. This provided a range of hydrazones with mono- **3f**, **3g**, **3h**, and **3i** or disubstitution **3a**, **3b**, and **3j**. The use of cyclic ketones provided a group of symmetrically-disubstituted systems **3c**, **3d** and **3e**.

Formation of compounds **3a–j** was evident from the characteristic *N*-acylhydrazone NH IR stretch at ~3190 cm^{-1} and appearance at lower wavenumber of the hydrazinamide carbonyl to ~1650 cm^{-1} (~1680 cm^{-1} in the starting material **1**).

The structure of compounds **3b** and **3i** was further confirmed by X-ray analysis[28] (Fig. 2).

In the ^{1}H NMR the hydrazinamide NH was evident, appearing as a broad singlet at ~δ 9.2 ppm in $CDCl_3$ or at δ 11.6–12.3 ppm in DMSO-d_6. Signals corresponding to the C1 and C5 protons of the 3-substituted pyridine ring were observed as a singlet and a doublet, respectively, at ~δ 9.1–8.9 ppm, downfield of the C3 and C4 protons of the nicotinyl nucleus at ~δ 8.5–7.3 ppm. A singlet resonating in the region 8.3–8.7 ppm indicated formation of the N=CH bond in aldehyde-derived compounds **3f**, **3g**, **3h** and **3i**. The ^{1}H NMR and ^{13}C NMR spectra of compounds **3b**, **3c**, **3d**, **3e** and **3f** (aliphatic mono and di substituted and symmetrically-disubstituted hydrazones) carried out in $CDCl_3$ showed two peaks in ratio of 1:0.7 arising from the two possible rotamers or *E*/*Z* isomers. Two sets of signals were observed for all groups in either the ^{1}H NMR or ^{13}C NMR spectra or both for each compound indicating the possibility of equilibrium and inter-conversion between rotamers (and/or configurational isomers) in solution. An illustrative example is provided by the ^{1}H NMR spectrum for **3b** (Fig. 1) showing two peaks for the hydrazinamide NH at 9.1 for the major diastereomer/rotamer, and 8.9 ppm for the minor one. The C1 proton of the 3-substituted pyridine ring also appeared as two singlet peaks at 9.0 and 8.8 ppm. Two sets of singlets arising from C3 and C5 protons were appeared as doublets at (8.2 and 8.1 ppm) for C3 proton and at (8.65 and 8.61 ppm) for C5 protons. The integration for the C4 proton corresponded to 1.7 protons and appeared as a triplet at 7.3 ppm. It is worth noting that the ratio of duplicate peaks was solvent-dependent. The diastereomeric/rotameric ratio was decreased to 10% when deuterated DMSO was used as NMR solvent.



Scheme 1. Synthesis of *N*-acylhydrazones.

However, determination of *E*- or *Z*- geometry of C=N bond by ^{1}H NMR remained inconclusive. Fortunately, suitable crystals of compounds **3b** and **3i** enabled X-ray crystal structure determinations and proved the more stable *E* stereochemistry of the C=N to be the most stable *E* isomer. The crystal structure of **3b** (Fig. 2a) is stabilized by NH···O hydrogen bonds (1.88(4) Å), forming a double-chain polymer in the crystal, where the two neighbouring monomers within each chain are oriented anti to one another, forming a 56.6(2)° N3N2N2N3 torsional plane.

The crystal structure of **3i** (Fig. 2b) is stabilized by a combination of intermolecular NO···Cl bonds (3.134(1) Å) forming a parallel double-chain polymer in the crystal lattice and also hydrogen bonding interactions between the molecule and dimethylsulfoxide solvent molecules (Fig. 2a and b). For the sake of clarity, only one of the two DMSO molecules hydrogen bonding to **3i** in the unit cell is depicted. The two DMSO molecules present in the unit cell are hydrogen bond acceptors for NH bonds in two different molecules (Tables S1 and S2, Supplementary data).

The experimental bond distance for N3=C4 is 1.286(3) Å (Fig. 2a) while the corresponding N2–C7 bond is 1.279(2) Å (Fig. 2b), indicating the double bond character of the C=N bonds. In contrast, the N2—N3 bonds are longer, at 1.413(3) Å and 1.379(19) Å in Figure 2a and b, respectively, consistent with a single bond between nitrogen atoms. Geometrical parameters showed that the hydrazone moieties between C1 and C12 in Figure 2a and between C1 and C8 in Figure 2b are effectively planar and that the hydrazone moieties have *E* configurations at the C4=N3 and C7=N2 double bonds, in the two figures, respectively. The pyridinium rings are twisted away from the plane of the hydrazones, with a dihedral angle of 48.8(2)° between N2C1 and N7C10 in Figure 2a and 33.8(2)° between the N3–C8 and N4–C13 planes in (Fig. 2b).

The disk diffusion method was employed to screen DMSO solutions of **3a–j** for antimicrobial activity. A sterile 4 mm filter paper disk was immersed in each solution for 10 s and placed on the surface of a Müller–Hinton Agar plate that had previously been streaked with the test organism. Compounds exhibiting any degree of antimicrobial activity were stained with 2,3,5-triphenyltetrazolium chloride (TTC) using the microbroth dilution method to determine the minimum inhibitory concentration (MIC).

MIC tests were carried out according to the method of Eloff,[29] using a microtitre plate (96 wells). Stock solutions were diluted and transferred into the first well, and serial dilutions were performed. The inoculum was added to all wells and the plates were incubated at 37 °C over 24 h. Antimicrobial activity was detected by adding 20 μL of 0.5% aqueous TTC, supplied by Merck. MIC was defined as the lowest concentration of inoculum that inhibited visible growth, as indicated by TTC staining.

Compounds **3a–j** were evaluated for their antibacterial activity against gram positive and gram negative bacteria. Only **3a**, **3c**, **3d** and **3e** showed antibacterial activities by the disk diffusion

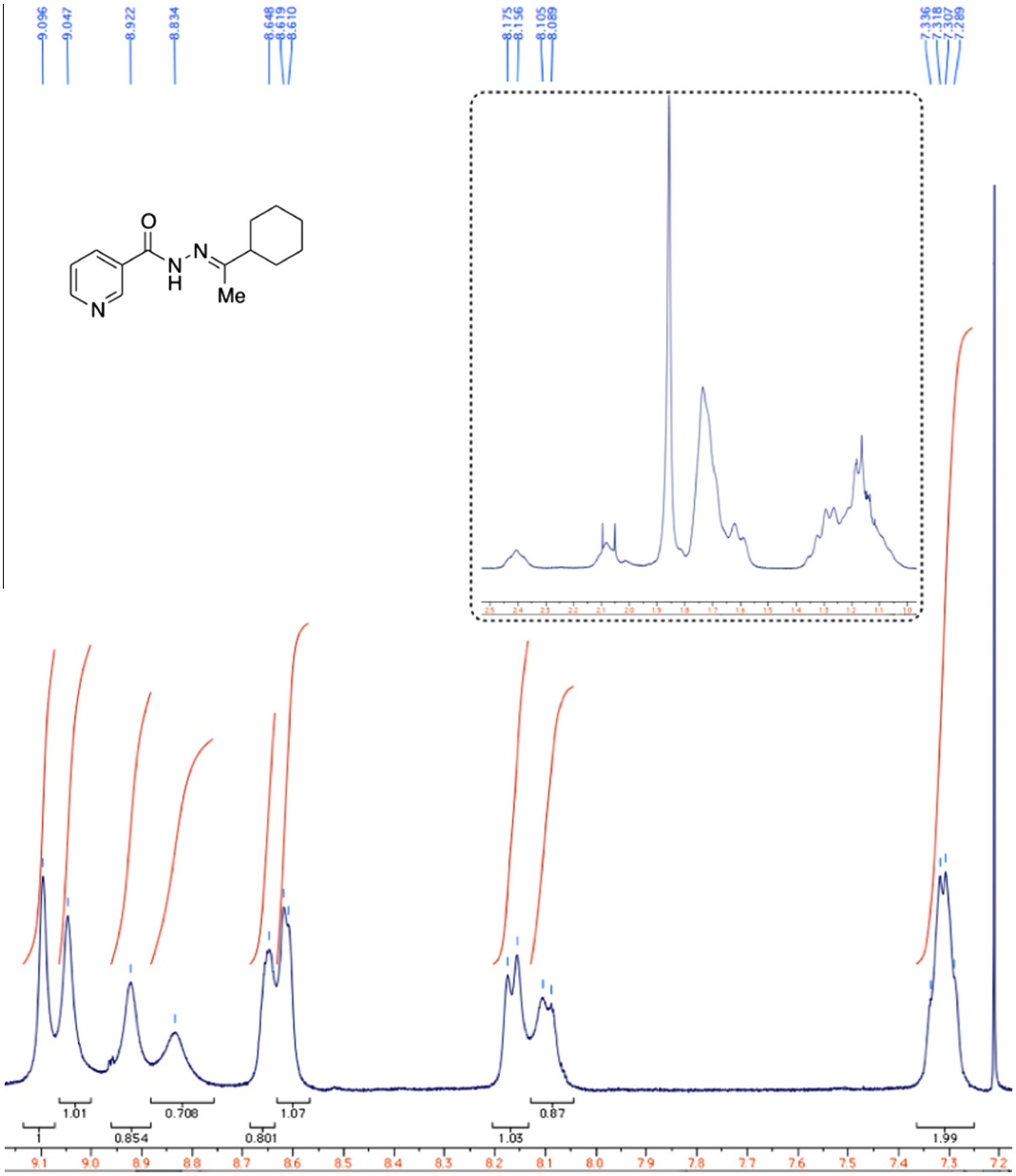


Figure 1. [1]H NMR for **3b** proving the existence of mixture of rotamers.

method. MIC results are summarized in Table 1 for compounds that exhibited antibacterial activity against the two gram negative (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and two gram positive (*Streptococcus pneumoniae* and *Staphylococcus aureus*) bacteria. Zones of inhibition around three compounds (**3a**, **3d** and **3e**) is clearly visible in are shown in (Fig. S3, Supplementary data). The red colour from reduction of 2,3,5-triphenyltetrazolium chloride was then used as an indicator of bacterial metabolism (Fig. S4, Supplementary data).

Most strains of *Pseudomonas aeruginosa* are significantly more resistant, even in the absence of R plasmids, to antimicrobial agents, including beta-lactams, tetracycline, chloramphenicol, and fluoroquinolones, than most other gram-negative rods. This broad spectrum resistance has so far been assumed to be mainly due to the low permeability of the *P. aeruginosa* outer membrane.[30] Due to the increase in MDR and even potentially pandrug-resistant (PDR) strains of *P. aeruginosa* and the adverse side effects associated with last line drugs, there is a need to find new and effective antimicrobial treatments.[31]The MICs of the macrolide antibiotics for two *P. aeruginosa* isolates are 512, 1,024 and 512 μg/ml for azithromycin (AZM), erythromycin (EM), roxithromycin (RXM), and rokitamycin (RKM), respectively. The MICs of AZM and EM for 15 other strains of *P. aeruginosa* exceed >64 μg/ml, while the MICs of OFLX, GM, and CAZ for *P. aeruginosa* B16 have been reported as 1.0, 4.0, and 4.0 μg/ml, respectively.[32] In a recent study Adibi et al. reported the synthesis of a different series of hydrazide–hydrazone derivatives of 3-pyridine carboxylic acids and aryl aldehydes, showing activity against mycobacterial tuberculosis with the best MIC reported being 40 μg/mL.[33]

We also sought to demonstrate that our novel range of hydrazones could be converted into 1,3,4-oxadiazoles. Thus, refluxing compounds **3a–j** in acetic anhydride for 30–40 min provided an efficient entry to the corresponding 1,3,4-oxadiazoles **4a–j** (Scheme 2). The crude products were purified by flash chromatography to give pure 1,3,4-oxadiazole derivatives **4a–j** in 55–70% yield. The structures of the products were confirmed by IR, HRMS, [1]H NMR and

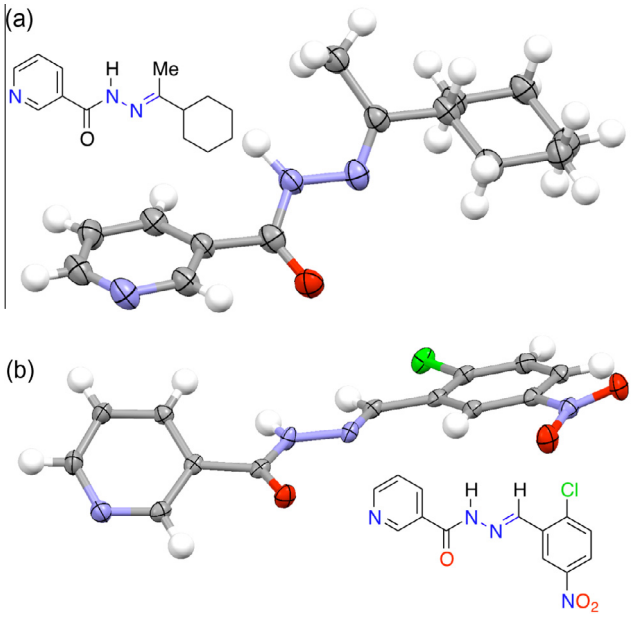
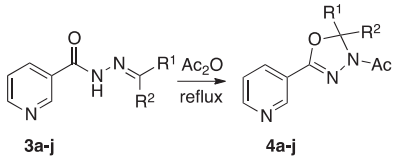


Figure 2. X-ray structures of **3b** and **3i**.

Table 1
The MIC results of compounds that exhibited antibacterial activity (μg/mL)

	P. aeruginosa	*K. pneumoniae*	*S. pneumoniae*	*S. aureus*
3a	0.220 μg/mL	14.000 μg/mL	ND	7.030 μg/mL
3c	1.170 μg/mL	37.500 μg/mL	ND	ND
3d	0.490 μg/mL	15.620 μg/mL	15.600 μg/mL	ND
3e	0.195 μg/mL	3.120 μg/mL	3.120 μg/mL	ND



a R^1=Me R^2=CF_3
b R^1=Cychex R^2=Me
c R^1=R^2= -$(CH_2)_4$-
d R^1=R^2= -$(CH_2)_6$-
e R^1=R^2= -$(CH_2)_7$-
f R^1=Cychex R^2= H
g R^1=1-Naphth R^2=H
h R^1=2-Naphth R^2=H
i R^1=2-Cl,5-NO_2-Ph R^2=H
j R^1=2-F-Ph R^2=Me

Scheme 2. Synthesis of *N*-acetyl-2,5-disubstituted-1,3,4-oxadiazole derivatives.

^{13}C NMR. A series of mono-substituted 1,3,4-oxadiazoles (R^1 = H, R^2 = Ph, *o*-ClPh, *o*-NO_2Ph) was recently reported using similar dehydrating conditions.[34]

Synthesis of the 1,3,4-oxadiazole derivatives **4a–j** was evident from the IR spectrum by the disappearance of the hydrazinamide N–H (3190 cm^{-1}) and from replacement of the hydrazinamide carbonyl by an acetamide carbonyl stretch at 1690 cm^{-1}. Additionally, the appearance of two new peaks at 1300 cm^{-1} and 1130 cm^{-1} was consistent with formation of the oxadiazole ring at 1300 cm^{-1} and 1130 cm^{-1}, attributed to asymmetric and symmetric stretches, respectively, for the C–O–C group. Formation of the *N*-substituted 1,3,4-oxadiazole ring was also confirmed by disappearance of the downfield (9.2 ppm) hydrazinamide NH ^{1}H NMR resonance. By ^{13}C NMR, formation of the oxadiazole ring was confirmed by the appearance of a characteristic quaternary carbon for the oxadiazole ring at 96–106 ppm and the appearance of the CH_3 group at 21 ppm. Doubling of peaks in the ^{1}H and/or ^{13}C spectrum or both for compounds **4a**, **4b**, **4c**, **4f**, **4h**, **4i** and **4j** was observed. The doubling of peaks was attributed to restricted rotation about the exocyclic amide bond of the *N*-acetyl group (Fig. 3). The ^{13}C NMR spectrum for compound **4b** is consistent with the existence of a rotameric mixture (Fig. S5, Supplementary data).

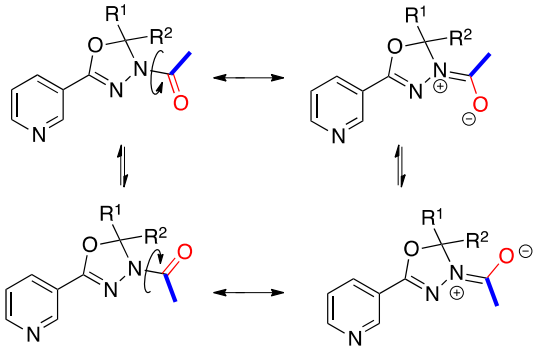


Figure 3. *N*-acetyl 1,3,4-oxadiazole rotamers leading to ^{13}C NMR differentiation.

In conclusion; synthesis of a range of new nicotinic hydrazides is described along with solid-state structural confirmation and insights. Antibacterial evaluation has identified several new hydrazides which show significant antibacterial activity against *P. aeruginosa* in particular. This thus provides an important category of lead compound for development of agents targeting this high profile target. The synthesis here shows that this skeleton can be diversified through various aldehyde and ketone types and would thus be suitable for diverse fragment modifications. Additionally, we show that these are also readily converted into novel 1,3,4-oxadiazoles, an important category of heterocycle.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.029. These data include MOL files and InChiKeys of the most important compounds described in this article.

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