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Pyrido[1,2-*a*]benzimidazole-Based Agents Active Against Tuberculosis (TB), Multidrug-Resistant (MDR) TB and Extensively Drug-Resistant (XDR) TB

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Abstract

The struggle against tuberculosis (TB) is still far from over. TB, caused by *Mycobacterium tuberculosis*, is one of the deadliest infections worldwide. Co-infection with human immunodeficiency virus (HIV) and the emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) strains have further increased the burden for this disease. Herein, we report the discovery of 2-(4-chlorobenzyl)-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile as an effective antitubercular agent and the structural modifications of this molecule that have led to analogues with improved potency and lower toxicity. A number of these derivatives were also active at sub-micromolar concentrations against resistant TB strains and devoid of apparent toxicity to Vero cells, thereby underscoring their value as novel scaffolds for the development of new anti-TB drugs.

Keywords

benzimidazole derivatives; drug design; drug discovery; drug resistance; tuberculosis

Introduction

The resurgence of tuberculosis (TB) is one of the most serious public health concerns worldwide, and the causative pathogen, *Mycobacterium tuberculosis* (Mtb), is frequently observed in immuno-compromised individuals suffering from human immunodeficiency

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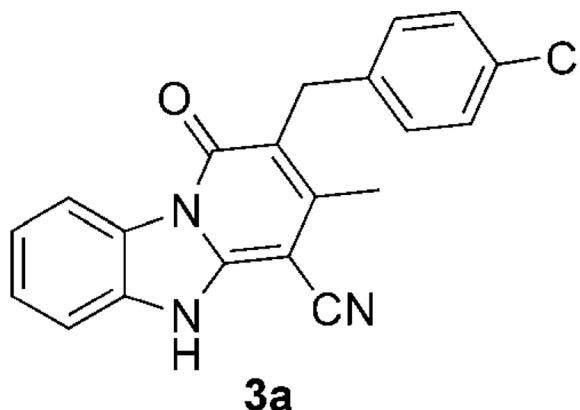
virus (HIV).^[1] The World Health Organization (WHO) has reported that Mtb was responsible for 9.2 million new cases of infection and 1.78 million deaths in 2008, and that one-third of the world's population is estimated to be infected by latent TB.^[2] The treatment of TB must be continued for 6–12 months in order to purge both the active bacteria, as well as the bacteria present in a quiescent state.^[3] However, interconnected factors, including the high cost of drugs, the slow expansion of directly observed treatment, short course (DOTS), poor patient compliance with the treatment regimen and physician errors, often result in relapses and the emergence of drug resistance.^[4, 5] These resistant strains, in turn, often demand a prolongation of the therapeutic regimen, with the use of additional drugs that leads to an increase in treatment costs.

The number of new cases of drug-resistant TB has increased in the recent years, with 440 000 cases of multidrug-resistant TB (MDR-TB) that have led to an estimated 150 000 deaths in 2008.^[6] By definition, MDR-TB strains lose their susceptibility to two of the first-line drugs used in therapy, namely isoniazid (INH) and rifampin (RIF), while extensively drug-resistant tuberculosis (XDR-TB) strains are in addition resistant to at least one fluoroquinolone and one of the injectable anti-TB drugs, such as amikacin, kanamycin (KAN), or capreomycin.^[7] Even more problematic is the recent isolation of strains resistant to all of the standard first- and second-line drugs used in TB therapy,^[8] giving rise to the likely threat of a virtually incurable infection.

Despite its global impact on world health, TB is considered a neglected disease and no new anti-TB therapeutics have been introduced into the market over the last half century. Although a number of novel agents are fueling the TB pipeline,^[9, 10] RIF, used to treat TB since 1966,^[11] still represents the last novel class of anti-TB drugs. The foregoing facts strongly highlight the need for the identification and development of agents capable of allowing for a shorter treatment regimen while interacting with a unique biological target, thereby avoiding cross resistance with existing drugs. Many research efforts to identify new antibiotics have made it apparent that a whole-cell phenotypic high-throughput screening (HTS) approach is more likely to yield lead candidates for further advancement than the isolated target approach.^[12] Indeed, compounds TMC207^[13] and PA824,^[14] two of the most promising anti-TB agents in clinical trials, are the result of the improvement of hit compounds identified after a random anti-TB screening of in-house chemical libraries at Johnson & Johnson and Ciba-Geigy, respectively.

In our own efforts to discover novel anti-TB chemotypes,^[15, 16] phenotypic screening of a library of ~6000 compounds was carried out affording a number of new hits with anti-TB activity deemed of interest for further development. Among these structures, compound **3a**, containing a pyrido[1,2-*a*]benzimidazole core, was chosen for further investigation based on its activity, relatively low Vero cell toxicity, and the ease of analogue synthesis. These structure–activity relationship (SAR) elucidation efforts have led to the identification of molecules having both an improved anti-TB potency and selectivity index. Some of the molecules were also investigated for their chemical stability in human liver microsomes, thus providing some insights into the metabolism of these compounds. Of greatest note is our finding that compound **3h** was able to maintain good activity when tested against a panel

of MDR-TB and XDR-TB strains, thus making these pyrido[1,2-*a*]benzimidazoles attractive leads for further development in the quest for improved anti-TB drugs.



Results and Discussion

Chemistry

The target compounds (**3a–w**, **4–6**, Figure 1) were synthesized by employing a straightforward and efficient protocol described previously (Scheme 1).^[17] Benzimidazole-2-acetonitrile was reacted with an equimolar amount of a suitable α -substituted ethyl acetoacetate (**2a–w**) in the presence of ammonium acetate either under neat conditions or in anhydrous toluene at 150°C. When not commercially available, α -substituted ethyl acetoacetate derivatives **2** were synthesized by reacting ethyl acetoacetate with a substituted bromomethyl- or bromoethylbenzene, using potassium *tert*-butoxide as a base in anhydrous tetrahydrofuran (THF) at 70°C.^[18] Compound **2u** was synthesized by reacting 4-(2-pyridinyl)benzaldehyde with ethyl acetoacetate in the presence of the Me₃SiCl/NaI/CH₃CN reagent from room temperature to 60°C.^[19] Oxidation of **3j** with tellurium dioxide/hydrogen peroxide resulted in the sulfoxide **4** in good yields, while the sulfone **5** was obtained using *meta*-chloroperoxybenzoic acid (mCPBA) as an oxidizing agent in acetone. Demethylation of **3h** with boron tribromide in anhydrous dichloromethane at 0°C to room temperature produced the hydroxy derivative **6** in good yield. Product **3b** was further examined through 2D-NMR spectroscopy in order to assess which one of the possible tautomeric forms (figure S1 in the Supporting Information) predominates in solution.

In vitro biological studies

All of the target compounds synthesized were first tested against the Mtb H37Rv strain to obtain the minimum inhibitory concentration (MIC) as detected using the microplate Alamar Blue assay (MABA), that is, the lowest concentration leading to at least a 90% reduction of bacterial growth (MIC_{MABA}). In addition, for compounds with low MIC_{MABA} values, the MIC was measured using a broth dilution (BD) method, that is the lowest concentration of drug preventing visible bacterial growth in a 5 mL Tween-free Middlebrook broth dilution culture (MIC_{BD}). The apparent cytotoxicity was obtained using Vero cells. Several derivatives were found to be highly active in inhibiting the growth of actively replicating

Mtb with MIC_{MABA} values of less than 1 µg mL⁻¹ (Table 1). For these compounds, the MIC_{BD} values were the same or slightly higher than those of the corresponding MIC_{MABA}, thereby indicating a cidal effect for this class of derivatives.

From the HTS studies, compound **3a** had an MIC_{MABA} value of 0.12 µg mL⁻¹ versus a toxicity toward Vero cells of > 100 µm. However, upon repeat testing, **3a** exhibited a higher MIC_{MABA} value of 0.5 µg mL⁻¹ and showed a sharp decrease in the selectivity index (SI=16) with an IC₅₀ value of only 8 µg mL⁻¹ against the Vero cells, which is not consistent with the data obtained from the initial HTS study. Improvements were thus needed, and the first round of modifications aimed at improving the activity and toxicity profile were made at the site of the benzyl ring, as the pyrido[1,2-*a*]benzimidazole nucleus along with the keto moiety at C1 and the nitrile group at C4 were believed to be important determinants of activity.

We first evaluated the effect of removal of the benzyl appendage. As it is apparent from the activity of compound **3w** (MIC_{MABA} > 128 µg mL⁻¹) that the benzyl ring plays an important role in conferring the desired anti-TB activity. We next investigated the role of the chlorine atom and its position on the benzyl ring. Moving the chlorine from the *para* to the *meta* and *ortho* positions (**3b**, **3c**, MIC_{MABA}=128 µg mL⁻¹) also resulted in a loss of the anti-TB activity, while the removal of the halogen atom led to a smaller drop in potency, with **3v** having an MIC_{MABA} value of 8.0 µg mL⁻¹. In the majority of cases, introduction of various substituents on the benzyl ring at positions other than the *para* position resulted in an increase in the MIC_{MABA} value. In particular, *meta* substitution with electron-withdrawing groups, such as CF₃ (**3d**, MIC_{MABA}=128 µg mL⁻¹, X_{CF₃}=3.18)^[20] and OCF₃ (**3f**, MIC_{MABA}=64 µg mL⁻¹, X_{OCF₃}= 3.53),^[20] was less tolerated than small electron-donor groups, such as CH₃ (**3g**, MIC_{MABA}=8 µg mL⁻¹, X_{CH₃}=2.47).^[20] On the other hand, modifications at the *para* position of the benzyl group played a key role in enhancing the anti-TB potency with a variety of substituents being tolerated. Among the analogues synthesized, the *para* methoxy-substituted compound **3h** exhibited one of the best activities in the series with an MIC_{MABA} value of 0.25 µg mL⁻¹, only fivefold higher than that of INH, the most effective drug against actively replicating Mtb.

More importantly, **3h** also had a favorable selectivity index with an IC₅₀ value against Vero cells of greater than 128 µg mL⁻¹ and improved physicochemical parameters (table S2 in the Supporting Information) compared to **3a** such as the ClogP (4.0 vs 4.8), topological polar surface area (TPSA; 70.3 Å² vs 61.1 Å²), and the predicted aqueous solubility (AlogP_S; -3.72 [65 mgL⁻¹] vs -4.08 [29 mgL⁻¹]). Analogue **3j**, bearing a *para* methylthioether group, retained good potency (MIC_{MABA}=0.5 µg mL⁻¹), almost comparable with that of the corresponding methoxy derivative. Oxidation of the sulfur to the sulfoxide **4** (MIC_{MABA}= 2.0 µg mL⁻¹) and the sulfone **5** (MIC_{MABA}=1.0 µg mL⁻¹) caused a small reduction in anti-TB activity. Interestingly, the introduction of a polar *p*-hydroxy group caused a loss of activity, the MIC_{MABA} value of **6** being greater than 128 µg mL⁻¹.

The sharp difference in potency between **6** and the other pyrido[1,2-*a*]benzimidazole analogues may be rationalized by the existence of a hydrophobic pocket in the target binding site, located within the vicinity of the *para* position of the benzyl moiety. This notion is to

some extent further supported by the activity of a small series of derivatives for which the benzyl moiety has been substituted with a methylpyridinyl group. When the nitrogen of the pyridine ring is in the *para* position in relation to the linker (**3n**, MIC_{MABA} > 128 µg mL⁻¹), activity is lost, whereas the anti-TB activities are somehow restored when the nitrogen atom of the pyridine ring is moved around the ring as in derivatives **3o**, **3p** (MIC_{MABA}=4.0 µg mL⁻¹), and **3q** (MIC_{MABA}=0.5 µg mL⁻¹). Replacement of the chlorine atom with a smaller fluorine atom (**3i**, MIC_{MABA}=2.0 µg mL⁻¹) did not significantly affect the potency. However, compounds containing two or three halogen atoms, namely the fluorine containing analogues **3k** (MIC_{MABA}=0.5 µg mL⁻¹) and **3l** (MIC_{MABA}=0.25 µg mL⁻¹), and the dichloro derivative **3m** (MIC_{MABA}=0.25 µg mL⁻¹), were found to be more potent than the corresponding monosubstituted derivatives. Interestingly, the biological data of compounds **3a**, **3b** and **3m** support the notion that the activity is dependent upon the presence of a lipophilic substituent at the *para* position of the benzyl ring.

Next, bulkier substituents were introduced at the same attachment point so as to obtain further insights regarding the hypothesized lipophilic pocket and its size. The *tert*-butyl derivative **3r** retained excellent activity, with an MIC_{MABA} value of 0.5 µg mL⁻¹, whereas compound **3u**, bearing a 2-pyridyl ring as the substituent, was found to be less active with an MIC_{MABA} value of 4.0 µg mL⁻¹. These findings give further support to the hypothesis of the presence of a hydrophobic pocket in the target binding site in proximity to the *para* position of the benzyl ring. Likely, this pocket is important for the interaction with the present molecules and is able to accommodate a range of substituents mainly of a nonpolar character. Finally, we investigated the effect of the methylene bridge on the anti-TB activity. Removal of the methylene linker (**3s**, MIC_{MABA}= 8.0 µg mL⁻¹) did not have any apparent effect on the potency (**3v** vs **3s**), whereas the elongation of the linker with an additional methylene group caused the complete loss of the anti-TB activity (**3t**, MIC_{MABA} > 128 µg mL⁻¹).

For selected compounds, MIC_{BD} values were determined, which further confirmed the promising activity of these molecules with SAR consistent with that of the MIC_{MABA} values. Some basic considerations regarding the structure–toxicity relationships for this class of derivatives can be surmised from the small set of compounds synthesized. Compounds such as **3a**, **3i**, **3l** and **3m**, bearing halogen substituents on the benzyl ring, although exhibiting high anti-TB potency, were found to be toxic to Vero cells. Compounds such as **3r** and the lead **3h**, bearing a *tert*-butyl and a methoxy group, respectively, did not show any apparent toxicity in the same cell line (Table 1). Thus, it might be hypothesized that the electron-withdrawing nature of the substituent on the benzyl ring is responsible for the cytotoxicity, while electron-donating groups afford compounds with potent anti-TB activity but lacking toxicity to Vero cells.

Although phenotypic screening is a powerful tool in the discovery of new antibacterial leads, in the majority of cases it fails to provide any insights into the mechanism of action of the hits. Some of the pyrido[1,2-*a*]benzimidazoles that we have identified here have previously been reported to inhibit the biosynthesis of β-1,6-glucan, a key component of the mycetes cell wall.^[21] This fact, along with the bactericidal nature of these compounds, leads to the hypothesis that a mycobacterial orthologue of the fungal β-1,6-glucan synthases might

be the target for these molecules. Although the presence of potential β -glucan synthase orthologue(s) in Mtb cannot be excluded, it is known that the mycobacterial genome does not appear to contain genes encoding the enzymes traditionally associated with β -glucan synthesis,^[22] and, in addition, it is debatable whether or not glucans are crucial components of the mycobacterial cell wall, thus leaving open the question, what is the actual biosynthetic pathway inhibited by these compounds?

The most promising compound of the series, i.e., **3h**, was also tested for its ability to inhibit the growth of selected MDR-TB and XDR-TB strains, namely KZN494, V2475^[23] (MDR-TB), TF-274,^[23] and R-506^[23] (XDR-TB). It was extremely encouraging to find that **3h** maintained the same activity, as found for the susceptible strain H37rv, in three of the mutated Mtb strains (KZN494, TF-274, and R-506), whereas it failed to show appreciable activity against the MDR strain V2475 (Table 2). Although this may be considered a weakness for the further advancement of **3h** and related analogues, this information can be used to better understand the molecular target(s) of these molecules, which is(are) as yet undetermined. However, the data at present would appear to suggest that the activity of this novel class of antimycobacterial agents might depend on a novel mechanism of action, different from those of the most commonly used drugs.

Selected compounds, namely **3a**, **3d**, **3e**, **3v** and **3w**, were also tested for their metabolic stability. After 30 min incubation in the presence of mouse liver microsomes, the half life and the ratio of predicted clearance to plasma flow were derived and calculated based on the well-stirred model (Table 3; see also table S4 in the Supporting Information). Although the set of derivatives tested is modest, basic conclusions about the metabolism of these compounds can be made. The presence of electron-withdrawing groups in the *para* position leads to a shorter half life, as does the unsubstituted benzyl group. Substituents in the *meta* position are somehow able to enhance the stability of these compounds, especially in the case of a strong electron-withdrawing group. The methyl derivative **3w** was found to be the most stable member of the set of compounds tested, leading to the conclusion that the benzyl moiety is crucial for the anti-TB activity, but also constitutes the metabolic “softspot” for this series of compounds.

Although the synthesis of these pyrido[1,2-*a*]benzimidazoles has been described earlier, the question as to which one of the possible tautomers is present in solution has not been addressed. Therefore, compound **3b** was investigated in order to assign the exchangeable proton at δ_{H} 13.47 ppm and the neighboring protons thereby determining the structure of the molecule (figure S2 in the Supporting Information). We first used a combination of correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) to assign each proton's resonance signal to the corresponding carbon. Once each proton of the structure was characterized (table S3 in the Supporting Information), selective 1D-nuclear Overhauser effect spectroscopy (NOESY) was used to determine each proton's proximity to δ_{H} 13.47 ppm. Briefly, the resonance signal at δ_{H} 13.47 ppm was irradiated with a selective shaped pulse, so as to detect only those protons that are proximal to it. Only one peak, at δ_{H} 7.52 ppm, was revealed in the experiment, thus establishing that the exchangeable proton was attached at the nitrogen of the benzimidazole ring (figure S2 in the Supporting Information), and that the pyridone form is the major tautomer in solution. However, as is

well known, the tautomeric equilibrium can be shifted upon binding of the compound to its molecular target.

Conclusions

Based on data from a phenotypic screening assay of about 6 000 compounds, which identified compound **3a** as a good lead candidate, we constructed a small set of analogous pyrido[1,2-*a*]benzimidazoles. A number of the synthesized compounds were found to exhibit excellent bactericidal activities, with several of them having MIC_{MABA} values lower than 1 µg mL⁻¹ and MIC_{BD} values similar or slightly higher than the corresponding MIC_{MABA}. Modifications of the benzyl moiety led to a range of activities and allowed us to draft a preliminary SAR. Removal of the benzyl group caused a loss in activity, whereas substitution at its *para* position led to improved anti-TB activity, with a variety of substituents being tolerated. Halogens, a methoxy group, an alkyl appendage, and a heteroaromatic ring were found to be suitable substituents, whereas the more hydrophilic hydroxy group caused a loss in activity. The biological results may be consistent with the existence of a hydrophobic region in the target binding site within the proximity of the benzyl ring. However, the introduction of halogens resulted in compounds that are toxic to Vero cells, whereas electron-donating substituents coupled strong activities with an apparent lack of cytotoxicity. Removal of the linker methylene unit did not affect the potency, while elongation of the linker with an additional methylene group (**3t**) led to an inactive compound. More importantly, against the two XDR-TB and one MDR-TB strains tested in this study, **3h** showed equal in vitro activities as found against the susceptible control strain, thereby suggesting potential for use in MDR-TB and XDR-TB chemotherapy. The molecular target, mechanism of action, and the in vivo efficacy of such compounds are currently under investigation. Overall, the high bactericidal potency against drug-susceptible and drug-resistant Mtb strains establishes these pyrido[1,2-*a*]benzimidazole derivatives as a novel promising anti-TB chemotype.

Experimental Section

Biology

The minimum inhibitory concentration (MIC) values were determined using *M. tuberculosis* H37Rv and the Kwa-Zulu Natal strains. Initial MIC screening was done with the microplate Alamar Blue assay (MABA) according to published procedures.^[23b] MIC_{BD} determinations were made by using broth dilution methods. Reported MIC values are an average of three individual measurements. For a description of the biological assays, see the Supporting Information.

Chemistry

¹H NMR and ¹³C NMR spectra were recorded on a Bruker spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. ¹⁹F NMR spectra were recorded on a Bruker spectrometer at 376 MHz with trifluoroacetic acid (TFA) as an external standard. Standard abbreviations indicating multiplicity were used as follows: s=singlet, d= doublet, dd=doublet of doublets, t=triplet, q=quadruplet, m= multiplet and

br=broad. High-resolution mass spectrometry (HRMS) was performed on Q-TOF-2TM (Micromass). TLC was performed with Merck 60 F₂₅₄ silica gel plates. Column chromatography was performed using CombiFlash R_f system with RediSep columns or alternatively using Merck silica gel (40–60 mesh). Preparative HPLC was carried out on a Shimadzu SCL-10A VP instrument with an ACE 5-AQ (21.2 mm×150 mm) column. Analytical HPLC was carried out on Agilent 1100 HPLC system with a Synergi 4 μm Hydro-RP 80 A column, on a variable wavelength detector G1314A. Method 1: Flow rate=1.4 mL min⁻¹; gradient elution over 20 min, from 30% CH₃CN/H₂O to 100% CH₃CN with 0.05% TFA. For 2D-NMR experiments a spectrometer Bruker at 900 MHz was used: **3b** was dissolved in 0.75 mL of [D₆]DMSO (99.96% deuterium) and transferred into a 5 mm NMR tube. A Bruker AV900 spectrometer equipped with a 5 mm ATM BBO probe was used to collect 1H, DEPTQ, COSY, HSQC, HMBC and sel 1D-NOESY data used in resonance assignment. Spectral information was referenced to the corresponding residual solvent peak.

The purity of the target compounds was determined to be > 98% by analytical HPLC (table S1 in the Supporting Information).

General procedure for the synthesis of **2d, f, g, i, j, k, l, m, p, t**

To a suspension of *t*BuOK (1.2 equiv) in anhydrous THF (2 mL mmol⁻¹), ethyl acetoacetate (1.1 equiv) and *t*BuOH (0.1 equiv) were added at 0°C under argon. The resulting clear solution was stirred for 30 min, and then the appropriate benzyl bromide (1 equiv) was added dropwise at 0°C. The reaction mixture was stirred at 70°C until consumption of the starting material as determined by TLC, and then the reaction mixture was quenched with H₂O and saturated aq. NaHCO₃. The aqueous layer was extracted with Et₂O (3×10 mL) and the combined organic extracts were washed with brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified by flash chromatography to obtain the desired product. Compounds **2a, b, c, e, h, o, q, r, s, v, w** were commercially available, while analytical data for compounds **2d**,^[24] **f**,^[24] **i**,^[25] **m**,^[24] **n**,^[26] matched the data previously published.

Ethyl 2-[3-(trifluoromethyl)benzyl]-3-oxobutanoate (**2 d**):^[24]

Purified by column chromatography (EtOAc-hexane 1:4). Yield 67% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.21 (t, *J*=7.2 Hz, 3 H), 2.23 (s, 3H), 3.15–3.25 (m, 2 H), 3.78 (t, *J*=8.0 Hz, 1H), 4.47 (q, *J*= 7.2 Hz, 2H), 7.30–7.55 ppm (m, 4H).

Ethyl 2-[3-(trifluoromethoxy)benzyl]-3-oxobutanoate (**2 f**):^[24]

Purified by column chromatography (EtOAc-hexane 1:4). Yield 87% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.24 (t, *J*=7.2 Hz, 3 H), 2.20 (s, 3H), 2.32 (s, 3 H), 3.12–3.20 (m, 2 H), 3.78 (t, *J*=7.6 Hz, 1H), 4.16 (q, *J*=7.2 Hz, 2H), 6.80–7.00 (m, 3H), 7.15–7.25 ppm (m, 1 H).

Ethyl 2-(3-methylbenzyl)-3-oxobutanoate (**2 g**)

Purified by column chromatography (EtOAc-hexane 1:4). Yield 69% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.22 (t, *J*=7.2 Hz, 3 H), 2.23 (s, 3 H), 3.15–3.25 (m, 2 H), 3.78 (t, *J*=8.0 Hz, 1 H), 4.47 (q, *J*=7.2 Hz, 2 H), 7.30–7.55 ppm (m, 4H).

Ethyl 2-(4-fluorobenzyl)-3-oxobutanoate (2 i):^[25]

Purified by column chromatography (EtOAc-hexane 1:4). Yield 65% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.27 (t, *J*=7.2 Hz, 3 H), 2.26 (s, 3 H), 2.55–2.65 (m, 2 H), 3.46 (t, *J*=7.2 Hz, 1 H), 4.28 (q, *J*=7.2 Hz, 2 H), 7.12 (d, *J*=8.4 Hz, 2 H), 7.22 ppm (d, *J*=8.4 Hz, 2 H).

Ethyl 2-[4-(methylthio)benzyl]-3-oxobutanoate (2 j)

Purified by column chromatography (EtOAc-hexane 1:4). Yield 62% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.23 (t, *J*=7.2 Hz, 3 H), 2.20 (s, 3 H), 3.09–3.20 (m, 2 H), 3.75 (t, *J*=7.6 Hz, 1 H), 4.47 (q, *J*=7.2 Hz, 2 H), 7.11 (d, *J*=8.4 Hz, 2 H), 7.22 ppm (d, *J*=8.4 Hz, 2H).

Ethyl 2-(2,3,4-trifluorobenzyl)-3-oxobutanoate (2 k)

Purified by column chromatography (EtOAc-hexane 1:4). Yield 81% (white solid); ¹H NMR (400 MHz, CDCl₃): δ=1.23 (t, *J*=7.2 Hz, 3 H), 2.25 (s, 3 H), 3.10–3.22 (m, 2 H), 3.80 (t, *J*=8.0 Hz, 1 H), 4.20 (q, *J*=7.2 Hz, 2 H), 6.85–6.95 ppm (m, 2H).

Ethyl 2-(2,4-difluorobenzyl)-3-oxobutanoate (2 l)

Purified by column chromatography (EtOAc-hexane 1:4). Yield 61% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.22 (t, *J*=7.2 Hz, 3 H), 2.23 (s, 3 H), 3.05–3.15 (m, 2 H), 3.81 (t, *J*=8.0 Hz, 1 H), 4.15 (q, *J*=7.2 Hz, 2 H), 6.70–6.80 (m, 2H), 7.05–7.20 ppm (m, 1 H).

Ethyl 2-(3,4-dichlorobenzyl)-3-oxobutanoate (2m):^[24]

Purified by column chromatography (EtOAc-hexane 1:4). Yield 70% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.22 (t, *J*=7.2 Hz, 3 H), 2.23 (s, 3 H), 3.05–3.20 (m, 2 H), 3.73 (t, *J*=7.6 Hz, 1 H), 4.16 (q, *J*=7.2 Hz, 2 H), 7.03 (dd, *J*₁=2.0 Hz, *J*₂=8.0 Hz, 1 H), 7.25–7.30 (m, 1 H), 7.30–7.35 ppm (m, 1 H).

Ethyl 2-(pyridin-2-yl-methyl)-3-oxobutanoate (2 p)

Purified by column chromatography (EtOAc-hexane 1:4). Yield 92% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.21 (t, *J*=7.2 Hz, 3 H), 2.32 (s, 3 H), 3.25–3.45 (m, 2 H), 4.16 (q, *J*=7.2 Hz, 2H), 4.29 (t, *J*=7.6 Hz, 1 H), 7.10 (m, 1 H), 7.18 (d, *J*=7.6 Hz, 1H), 7.57 (m, 1H), 8.46 ppm (m, 1 H).

Ethyl 2-[(4-chlorophenyl)ethyl]-3-oxobutanoate (2 t)

Purified by column chromatography (EtOAc-hexane 1:4). Yield 26% (colorless oil); ¹H NMR (400 MHz, CDCl₃): δ=1.28 (t, *J*=7.2 Hz, 3H), 2.10–2.20 (m, 2H) 2.21 (s, 3H), 2.55–2.65 (m, 2H) 3.38 (t, *J*=7.2 Hz, 1 H), 4.20 (q, *J*=7.2 Hz, 2H), 7.10 (d, *J*=8.4 Hz, 2H), 7.24 ppm (d, *J*=8.4 Hz, 2 H).

Ethyl 2-[4-(pyridin-2-yl)-benzyl]-3-oxobutanoate (2 u)

To a stirred mixture of (CH₃)₃SiCl (3.44 mL, 27.3 mmol), NaI (4.1 g, 27.3 mmol), ethyl acetoacetate (0.689 mL, 5.45 mmol) and CH₃CN (1 mL mmol⁻¹ of (CH₃)₃SiCl), 4-(pyridin-2-yl)benzaldehyde (1 g, 5.45 mmol) was added at 0°C. The mixture was stirred for

6 h at RT, and then overnight at 60 °C. After cooling, H₂O was added and the organic layer was extracted with EtOAc, washed with aq. Na₂S₂O₃, brine, dried over MgSO₄, filtered, and concentrated in vacuo. The resulted yellow powder was used for the next reaction step without further purification (0.88 g, yield 57%); ¹H NMR (400 MHz, CDCl₃): δ=1.27 (t, *J*=7.2 Hz, 3H), 2.27 (s, 3 H), 3.25–3.45 (m, 2H), 4.16 (q, *J*=7.2 Hz, 2 H), 4.36 (t, *J*=7.6 Hz, 1H), 7.30–7.40 (m, 1 H), 7.72 (d, *J*=8.0 Hz, 2 H), 8.00 (d, *J*=8.4 Hz, 2 H), 8.18 (d, *J*=8.4 Hz, 2H), 8.75 ppm (d, *J*=7.6 Hz, 1 H).

General procedure for the synthesis of 3a–w

Benzimidazole-2-acetonitrile (1 equiv), ammonium acetate (2.2 equiv) and the appropriate substituted ethyl acetoacetate **2a–w** (1.2 equiv) were reacted either in neat or in anhydrous toluene at 150°C for 0.5–1.5 h. After cooling, the solid was washed with a mixture of EtOH/Et₂O (1:1, 3×10 mL), collected by filtration and purified either through HPLC or recrystallized from EtOH/DMF. In the cases of the reactions in toluene, after cooling the solvent was evaporated, and the resulting solid washed with a mixture of EtOH/Et₂O (1:1, 3×10 mL), collected by filtration and purified through HPLC. Analytical data for compounds **3s**^[22] and **3v**^[17] matched the data published previously.

2-(4-Chlorobenzyl)-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (**3 a**)

Neat; purified by preparative HPLC. Yield 73% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.32 (s, 3 H), 3.91 (s, 2 H), 7.22 (d, *J*=8.4 Hz, 2H); 7.26 (d, *J*=8.4 Hz, 2 H), 7.33 (m, 1H), 7.45–7.55 (m, 2H), 8.56 (d, *J*=8.0 Hz, 1 H), 13.47 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.3, 30.6, 69.8, 111.3, 114.0, 116.3, 116.8, 122.2, 126.6, 127.8, 128.2, 129.9, 130.4, 131.7, 139.5, 145.5, 147.4, 158.7 ppm; HRMS (ESI) calcd for C₂₀H₁₄ClN₃O: 348.0898 [*M*+*H*]⁺, found: 348.0896.

2-(3-Chlorobenzyl)-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (**3 b**)

Neat; purified by preparative HPLC. Yield 62% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.33 (s, 3 H), 3.95 (s, 2 H), 7.17–7.26 (m, 4H); 7.34 (m, 1H), 7.51 (m, 2H), 8.58 (d, *J*=8.0 Hz, 1 H), 13.47 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.3, 30.8, 69.8, 111.2, 113.5, 116.2, 116.8, 122.1, 125.8, 126.5, 126.7, 127.7, 127.7, 130.0, 131.6, 132.9, 143.1, 145.5, 147.3, 158.7 ppm; HRMS (ESI) calcd for C₂₀H₁₄ClN₃O: 348.0898 [*M*+*H*]⁺, found: 348.0900.

2-(2-Chlorobenzyl)-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (**3 c**)

Neat; recrystallized from EtOH/DMF. Yield 81% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.26 (s, 3 H), 3.98 (s, 2H), 6.91 (d, *J*=7.2 Hz, 1H), 7.14 (t, *J*=7.2 Hz, 1 H), 7.21 (t, *J*=7.2 Hz, 1H), 7.35 (m, 1H), 7.45 (d, *J*=8.0 Hz, 1H), 7.46–7.55 (m, 2H), 8.55 (d, *J*=8.0 Hz, 1H), 13.57 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO) δ=18.3, 29.0, 70.0, 111.3, 112.0, 116.3, 116.9, 122.3, 126.7, 127.3, 127.7, 127.8, 128.5, 129.1, 131.8, 133.2, 137.1, 145.8, 148.1, 158.7 ppm; HRMS (ESI) calcd for C₂₀H₁₄ClN₃O: 348.0898 [*M*+*H*]⁺, found: 348.0898.

3-Methyl-1-oxo-2-[3-(trifluoromethyl)benzyl]-1*H*,5*H*-pyrido[1,2-*a*]-benzimidazole-4-carbonitrile (3 d)

Neat; recrystallized from EtOH/DMF. Yield 83% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.35 (s, 3H), 4.05 (s, 2 H), 7.30–7.40 (m, 1H); 7.40–7.55 (m, 5 H), 7.60 (s, 1 H), 8.58 (d, *J*=8.4 Hz, 1H), 13.51 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.4, 31.1, 69.9, 99.6, 111.4, 113.5, 116.3, 122.3, 123.7 (q, *J*=270 Hz), 124.6 (q, *J*=3.2 Hz), 126.7, 127.9, 128.9, 129.2, 129.4 (q, *J*=40 Hz), 131.8, 132.1, 142.1, 145.7, 147.5, 158.8 ppm; HRMS (ESI) calcd for C₂₁H₁₄F₃N₃O: 380.1016 [*M*+H]⁺, found: 380.1035.

3-Methyl-2-(3-methoxybenzyl)-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 e)

Neat; purified by preparative HPLC. Yield 83% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.32 (s, 3 H), 3.68 (s, 3H), 3.92 (s, 2H), 6.70–6.85 (m, 3H); 7.10–7.15 (m, 1 H), 7.33 (m, 1 H), 7.45–7.55 (m, 2 H), 8.58 (d, *J*=8.4 Hz, 1 H), 13.60 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.3, 31.2, 54.9, 69.8, 110.9, 111.3, 114.0, 114.3, 116.2, 116.9, 120.2, 122.4, 126.5, 127.9, 129.3, 131.8, 142.1, 145.6, 147.2, 158.8, 159.4 ppm; HRMS (ESI) calcd for C₂₁H₁₇N₃O₂: 344.1394 [*M*+H]⁺, found 344.1395.

3-Methyl-1-oxo-2-[3-(trifluoromethoxy)benzyl]-1*H*,5*H*-pyrido[1,2-*a*]-benzimidazole-4-carbonitrile (3 f)

Neat; purified by preparative HPLC. Yield 63% (grey powder); ¹H NMR (400 MHz, [D₆]DMSO): δ= 2.34 (s, 3H), 4.01 (s, 2H), 7.15–7.40 (m, 5H); 7.45–7.55 (m, 2H), 8.58 (d, *J*=8.4 Hz, 1H), 13.66 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.4, 30.9, 69.9, 111.3, 113.5, 116.3, 116.9, 118.2, 120.5, 121.4, 122.3, 126.7, 127.0, 127.9, 130.1, 131.8, 143.5, 145.7, 147.5, 148.5, 158.8 ppm; HRMS (ESI) calcd for C₂₁H₁₄F₃N₃O₂: 398.1088 [*M*+H]⁺, found 398.1099.

3-Methyl-2-(3-methylbenzyl)-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 g)

Neat; recrystallized from EtOH/DMF. Yield 91% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.22 (s, 3 H), 2.34 (s, 3 H), 3.92 (s, 2H), 6.90–7.10 (m, 3 H), 7.11 (m, 1H); 7.36 (m, 1H), 7.45–7.55 (m, 2H), 8.60 (d, *J*=8.0 Hz, 1 H), 13.49 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.4, 21.1, 31.1, 69.8, 111.3, 114.5, 116.3, 117.0, 122.2, 125.0, 126.5, 126.6, 127.9, 128.2, 128.6, 131.8, 137.3, 140.4, 147.2, 158.8 ppm; HRMS (ESI) calcd for C₂₁H₁₇N₃O: 328.1444 [*M*+H]⁺, found 328.1450.

3-Methyl-2-(4-methoxybenzyl)-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 h)

Neat; purified by preparative HPLC. Yield 74% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.33 (s, 3H), 3.67 (s, 3 H), 3.87 (s, 2H), 6.79 (d, *J*=8.0 Hz, 2H); 7.03 (d, *J*=8.0 Hz, 2 H), 7.33 (m, 1 H), 7.45–7.55 (m, 2 H), 8.58 (d, *J*=8.0 Hz, 1 H), 13.61 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.3, 30.3, 55.0, 69.6, 111.2, 113.7, 114.9, 116.3, 116.9, 122.2, 126.6, 127.9, 129.0, 131.8, 132.3, 145.5, 146.9, 157.4, 158.8 ppm; HRMS (ESI) calcd for C₂₁H₁₇N₃O₂: 344.1394 [*M*+H]⁺, found 344.1395.

2-(4-Fluorobenzyl)-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 i)

Neat; recrystallized from EtOH/DMF. Yield 91% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.34 (s, 3 H), 3.93 (s, 2 H), 7.04 (m, 2H); 7.20–7.30 (m, 2H), 7.35 (m, 1H), 7.45–7.55 (m, 2H), 8.58 (d, *J*=8.0 Hz, 1H), 13.50 ppm (s, 1H); ¹⁹F NMR (376 MHz, [D₆]DMSO): δ=−116.2; HRMS (ESI) calcd for C₂₀H₁₄FN₃O: 332.1194 [*M*+*H*]⁺, found 332.1203.

3-Methyl-1-oxo-2-[4-(thiomethyl)benzyl]-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 j)

Neat; purified by preparative HPLC. Yield 81% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.32 (s, 3H), 2.39 (s, 3 H), 3.89 (s, 2H), 7.12 (d, *J*=8.4 Hz, 2H); 7.15 (d, *J*=8.4 Hz, 2 H), 7.35 (m, 1 H), 7.45–7.53 (m, 2 H), 8.57 (d, *J*=8.0 Hz, 1 H), 13.67 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=15.1, 18.4, 30.6, 69.7, 111.3, 114.4, 116.3, 116.9, 122.3, 126.4, 126.7, 127.9, 128.7, 131.8, 134.9, 137.4, 145.5, 147.1, 158.8 ppm; HRMS (ESI) calcd for C₂₁H₁₇N₃OS: 360.1165 [*M*+*H*]⁺, found 360.1153.

3-Methyl-1-oxo-2-(2,3,4-trifluorobenzyl)-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 k)

Neat; purified by preparative HPLC. Yield 50% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.33 (s, 3 H), 3.93 (s, 2 H), 6.90 (m, 1H), 7.11 (m, 1H); 7.33 (m, 1 H), 7.45–7.55 (m, 2H), 8.53 (d, *J*=8.0 Hz, 1 H), 13.54 ppm (s, 1H); ¹⁹F NMR (376 MHz, [D₆]DMSO): δ=−161.0 (app t, *J*=24 Hz), −137.5 (dd, *J*₁=4.0 Hz, *J*₂=16.0 Hz), −137.2 ppm (dd, *J*₁=4.0 Hz, *J*₂=16.0 Hz); HRMS (ESI) calcd for C₂₀H₁₂F₃N₃O: 366.0884 [*M*+*H*]⁺, found 366.0874.

2-(2,4-Difluorobenzyl)-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 l)

Neat; recrystallized from EtOH/DMF. Yield 75% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.33 (s, 3H), 3.90 (s, 2H), 7.10–7.20 (m, 2 H), 7.33 (m, 1 H), 7.45–7.55 (m, 2 H), 8.55 (d, *J*=8.4 Hz, 1 H), 13.54 ppm (s, 1H); ¹⁹F NMR (376 MHz, [D₆]DMSO): δ=−112.4 (d, *J*=4.0 Hz), −112.1 ppm (d, *J*=8.0 Hz); HRMS (ESI) calcd for C₂₀H₁₃F₂N₃O: 350.1088 [*M*+*H*]⁺, found 350.1092.

2-(3,4-Dichlorobenzyl)-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3m)

Neat; purified by preparative HPLC. Yield 51% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.36 (s, 3H), 3.98 (s, 2H), 7.20–7.25 (m, 1 H), 7.36 (m, 1 H), 7.45–7.55 (m, 4 H), 8.59 (d, *J*=8.0 Hz, 1H), 13.61 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.4, 30.4, 69.9, 99.6, 111.3, 113.3, 116.3, 116.9, 122.3, 126.7, 127.8, 128.5, 129.9, 130.4, 130.8, 131.7, 141.9, 145.7, 147.6, 158.8 ppm; HRMS (ESI) calcd for C₂₀H₁₃Cl₂N₃O: 380.0363 [*M*−*H*]⁺, found 380.0375.

3-Methyl-1-oxo-2-[(pyridin-4-yl)methyl]-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 n)

Neat; recrystallized from EtOH/DMF. Yield 78% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.33 (s, 3 H), 3.98 (s, 2H), 7.22 (m, 2H), 7.34 (m, 1 H), 7.45–7.55 (m, 2 H), 8.40, (m, 2H), 8.56 (d, *J*=7.6 Hz, 1 H), 13.50 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.4, 30.7, 70.0, 99.6, 111.4, 112.5, 116.3, 122.2, 123.6, 126.6, 127.9, 132.0, 145.8, 147.7, 149.4, 149.8, 158.8 ppm; HRMS (ESI) calcd for C₁₉H₁₄N₄O: 315.1240 [M+H]⁺, found 315.1245.

3-Methyl-1-oxo-2-[(pyridin-3-yl)methyl]-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 o)

Toluene; purified by preparative HPLC. Yield 85% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.39 (s, 3 H), 3.99 (s, 2 H), 7.27 (m, 1H), 7.36 (m, 1H), 7.45–7.55 (m, 2 H), 7.61 (d, *J*=8.0 Hz, 1H), 8.37, (s, 1 H), 8.50 (s, 1H), 8.60 (d, *J*=8.0 Hz, 1 H), 13.52 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.8, 29.1, 70.3, 111.8, 113.6, 116.6, 117.4, 122.5, 123.9, 127.0, 128.3, 132.6, 135.9, 136.5, 146.2, 147.5, 147.7, 149.8, 159.2 ppm; HRMS (ESI) calcd for C₁₉H₁₄N₄O: 315.1240 [M+H]⁺, found 315.1254.

3-Methyl-1-oxo-2-[(pyridin-2-yl)methyl]-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 p)

Toluene; purified by preparative HPLC. Yield 71% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.41 (s, 3 H), 4.10 (s, 2 H), 7.18 (m, 1 H), 7.24 (d, *J*=8.0 Hz, 1 H), 7.35 (m, 1 H), 7.45–7.55 (m, 2 H), 7.65 (t, *J*=7.6 Hz, 1 H), 8.44, (m, 1 H), 8.58 (d, *J*=8.0 Hz, 1H), 13.52 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=19.0, 34.6, 70.2, 111.8, 113.5, 116.6, 117.4, 121.7, 122.6, 122.8, 127.0, 128.3, 132.4, 137.0, 146.1, 148.1, 149.2, 159.1, 160.0 ppm; HRMS (ESI) calcd for C₁₉H₁₄N₄O: 315.1240 [M+H]⁺, found 315.1255.

2-[(6-Chloropyridin-3-yl)methyl]-3-methyl-1-oxo-1*H*,5*H*-pyrido-[1,2-*a*]benzimidazole-4-carbonitrile (3 q)

Toluene; purified by preparative HPLC. Yield 88% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.37 (s, 3H), 3.94 (s, 2 H), 7.35–7.37 (m, 2H), 7.45–7.55 (m, 2 H), 7.67 (d, *J*=8.0 Hz, 1H), 8.31 (s, 1 H), 8.56 (d, *J*=8.0 Hz, 1 H), 13.51 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.8, 28.3, 70.3, 111.7, 113.4, 116.6, 117.2, 122.7, 124.3, 127.0, 128.2, 132.1, 136.1, 139.3, 146.0, 147.8, 148.2, 149.8, 159.0 ppm; HRMS (ESI) calcd for C₁₉H₁₃ClN₄O: 349.0851 [M+H]⁺, found 349.0867.

3-Methyl-1-oxo-2-[(4-*tert*-butyl)benzyl]-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 r)

Neat; purified by preparative HPLC. Yield 79% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=1.22 (s, 9H), 2.36 (s, 3 H), 3.91 (s, 2H), 7.13 (d, *J*=8.0 Hz, 2H); 7.24 (d, *J*=8.0 Hz, 2 H), 7.34 (m, 1 H), 7.44–7.55 (m, 2 H), 8.59 (d, *J*=8.0 Hz, 1 H), 13.48 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.4, 30.7, 31.2, 34.1, 69.6, 99.6, 111.3, 114.7, 116.3, 117.0, 122.2, 125.0, 126.6, 127.7, 127.9, 131.8, 137.4, 145.5, 146.9, 148.0, 158.8 ppm; HRMS (ESI) calcd for C₂₄H₂₃N₃O: 370.1914 [M+H]⁺, found 370.1915.

3-Methyl-1-oxo-2-phenyl-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 s):^[25]

Neat; purified by preparative HPLC. Yield 81% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.18 (s, 3 H), 7.17 (t, *J*=7.6 Hz, 1H); 7.25–7.35 (m, 3 H), 7.35–7.45 (m, 3H), 7.50 (d, *J*=8.0 Hz, 1H), 8.49 (d, *J*=8.0 Hz, 1H), 13.48 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=19.7, 70.9, 113.5, 115.7, 120.0, 125.2, 126.4, 127.8, 129.4, 131.5, 137.1, 146.5, 158.8, 172.1 ppm; HRMS (ESI) calcd for C₁₉H₁₃N₃O: 300.1131 [*M*+H]⁺, found 300.1132.

2-[(4-Chlorophenyl)ethyl]-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 t)

Toluene; purified by preparative HPLC. Yield 78% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.22 (s, 3 H), 2.70 (m, 2H), 2.78 (m, 2H), 7.24 (d, *J*=8.4 Hz, 2H), 7.25–7.40 (m, 5H), 7.45–7.55 (m, 2 H), 8.59 (d, *J*=8.0 Hz, 1H), 13.57 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=17.8, 28.4, 33.5, 69.3, 111.2, 114.6, 116.2, 117.0, 122.1, 126.5, 127.7, 128.2, 130.3, 130.5, 131.7, 140.8, 145.3, 146.2, 158.3 ppm; HRMS (ESI) calcd for C₂₁H₁₆ClN₃O: 362.1055 [*M*+H]⁺, found 362.1039.

3-Methyl-1-oxo-2-[4-(pyridin-2-yl)benzyl]-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 u)

Toluene; purified by preparative HPLC. Yield 52% (yellow powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.34 (s, 3H), 4.01 (s, 2 H), 7.20–7.40 (m, 4H), 7.45–7.55 (m, 2 H), 7.80–8.00 (m, 4H), 8.10 (m, 1 H), 8.50–8.60 (m, 3H), 13.50 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.1, 31.0, 69.8, 111.3, 114.1, 116.3, 116.9, 121.0, 122.3, 122.9, 126.7, 126.9, 127.9, 128.6, 131.8, 135.0, 138.9, 142.3, 145.6, 147.4, 148.2, 155.2, 158.9 ppm; HRMS (ESI) calcd for C₂₅H₁₈N₄O: 391.1540 [*M*+H]⁺, found 391.1539.

2-Benzyl-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3 v):^[18]

Neat; recrystallized from EtOH/DMF. Yield 90% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.35 (s, 3 H), 3.97 (s, 2 H), 7.10–7.30 (m, 5H), 7.35 (m, 1 H), 7.45–7.55 (m, 2H), 8.60 (d, *J*=8.0 Hz, 1 H), 13.49 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.3, 31.1, 69.6, 111.2, 114.4, 116.2, 116.9, 122.2, 125.8, 126.5, 127.8, 128.0, 128.3, 131.7, 140.5, 145.5, 147.0, 158.7 ppm; HRMS (ESI) calcd for C₂₀H₁₅N₃O: 314.1288 [*M*+H]⁺, found 314.1301.

2,3-Dimethyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (3w)

Neat; recrystallized from EtOH/DMF. Yield 73% (white powder); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.01 (s, 3 H), 2.30 (s, 3 H), 7.32 (bd s, 1H), 7.45–7.55 (m, 2H), 8.55 (d, *J*=6.8 Hz, 1 H), 13.33 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=11.7, 18.5, 69.3, 111.0, 111.2, 116.2, 117.2, 122.1, 126.5, 127.8, 131.8, 145.3, 145.9, 158.6 ppm; HRMS (ESI) calcd for C₁₄H₁₁N₃O: 238.0975 [*M*+H]⁺, found 238.0973.

3-Methyl-2-[4-(methylsulfinyl)benzyl]-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]-benzimidazole-4-carbonitrile (4)

To a flask containing **3j** (61 mg, 0.17 mmol) suspended in MeOH (2 mL) were added TeO₂ (27 mg, 0.17 mmol), 30% H₂O₂ (0.1 mL), and a drop of concd HCl. The mixture was stirred at RT for 72 h. Water (10 mL) was then added, and the resulting precipitate was collected by filtration. The precipitate was washed with H₂O (3×5 mL) and purified by preparative HPLC to afford **4** as a white solid (42 mg, 66%); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.37 (s, 3H), 2.68 (s, 3 H), 4.08 (s, 2H), 7.35 (m, 1 H), 7.42 (d, *J*=8.0 Hz, 2H); 7.45–7.53 (m, 2H), 8.59 (d, *J*=8.0 Hz, 1H), 13.54 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.8, 31.5, 43.6, 70.2, 111.7, 114.2, 116.7, 117.3, 122.7, 124.1, 127.1, 127.5, 128.2, 129.2, 132.2, 144.0, 146.0, 147.8, 159.2 ppm; HRMS (ESI) calcd for C₂₁H₁₇N₃O₂S: 374.0969 [*M*-H]⁺, found 374.0983.

3-Methyl-2-[4-(methylsulfonyl)benzyl]-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]-benzimidazole-4-carbonitrile (5)

To a solution of **3j** (50 mg, 0.14 mmol) in acetone (3 mL), a solution of *m*-CPBA (80 %, 36 mg, 0.21 mmol) in acetone (1 mL) was added at 0 °C, and the reaction mixture was stirred at RT for 3 h. Water (7 mL) was then added, the solvent concentrated, and the resulting precipitate was collected by filtration. The precipitate was washed with H₂O (3×5 mL), Et₂O (3×5 mL) and purified by preparative HPLC to afford **5** as a white solid; ¹H NMR (400 MHz, [D₆]DMSO): δ=2.41 (s, 3H), 3.15 (s, 3 H), 4.08 (s, 2 H), 7.36 (m, 1 H), 7.42 (d, *J*=8.0 Hz, 1H), 7.45–7.55 (m, 4 H), 7.79 (d, *J*=8.4 Hz, 1H), 8.59 (d, *J*=8.0 Hz, 1 H), 13.54 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ=18.8, 31.9, 43.6, 70.2, 112.9, 114.9, 116.7, 117.3, 123.5, 124.0, 127.1, 127.5, 128.2, 130.2, 132.2, 144.0, 146.1, 147.8, 159.2 ppm; HRMS (ESI) calcd for C₂₁H₁₇N₃O₃S: 390.0918 [*M*-H]⁺, found 390.0933.

2-(4-Hydroxybenzyl)-3-methyl-1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitrile (6)

To a suspension of **3h** (100 mg, 0.29 mmol) in anhydrous CH₂Cl₂ (8 mL), BBr₃ (2.32 mL, 2.33 mmol) was added dropwise at 0 °C and the reaction mixture was stirred for 2 h at RT. Upon completion, the reaction mixture was quenched with H₂O (5 mL), and the organic solvent was evaporated. The solid resulted was collected and purified by preparative HPLC to give **6** as white solid (75 mg, 79%); ¹H NMR (400 MHz, [D₆]DMSO): δ=2.34 (s, 3 H), 3.84 (s, 2H), 6.62 (d, *J*=8.0 Hz, 2H); 7.00 (d, *J*= 8.0 Hz, 2 H), 7.35 (m, 1 H), 7.45–7.55 (m, 2 H), 8.60 (d, *J*=8.0 Hz, 1 H), 9.10 (s, 1 H), 13.46 ppm (s, 1H); ¹³C NMR (400 MHz, [D₆]DMSO): δ= 18.3, 30.2, 69.6, 111.3, 115.1, 116.3, 117.0, 122.3, 126.7, 127.8, 128.9, 130.5, 131.7, 145.5, 146.9, 155.3, 158.8 ppm; HRMS (ESI) calcd for C₂₀H₁₅N₃O₂ : 330.1237 [*M*+H]⁺, found 330.1225.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

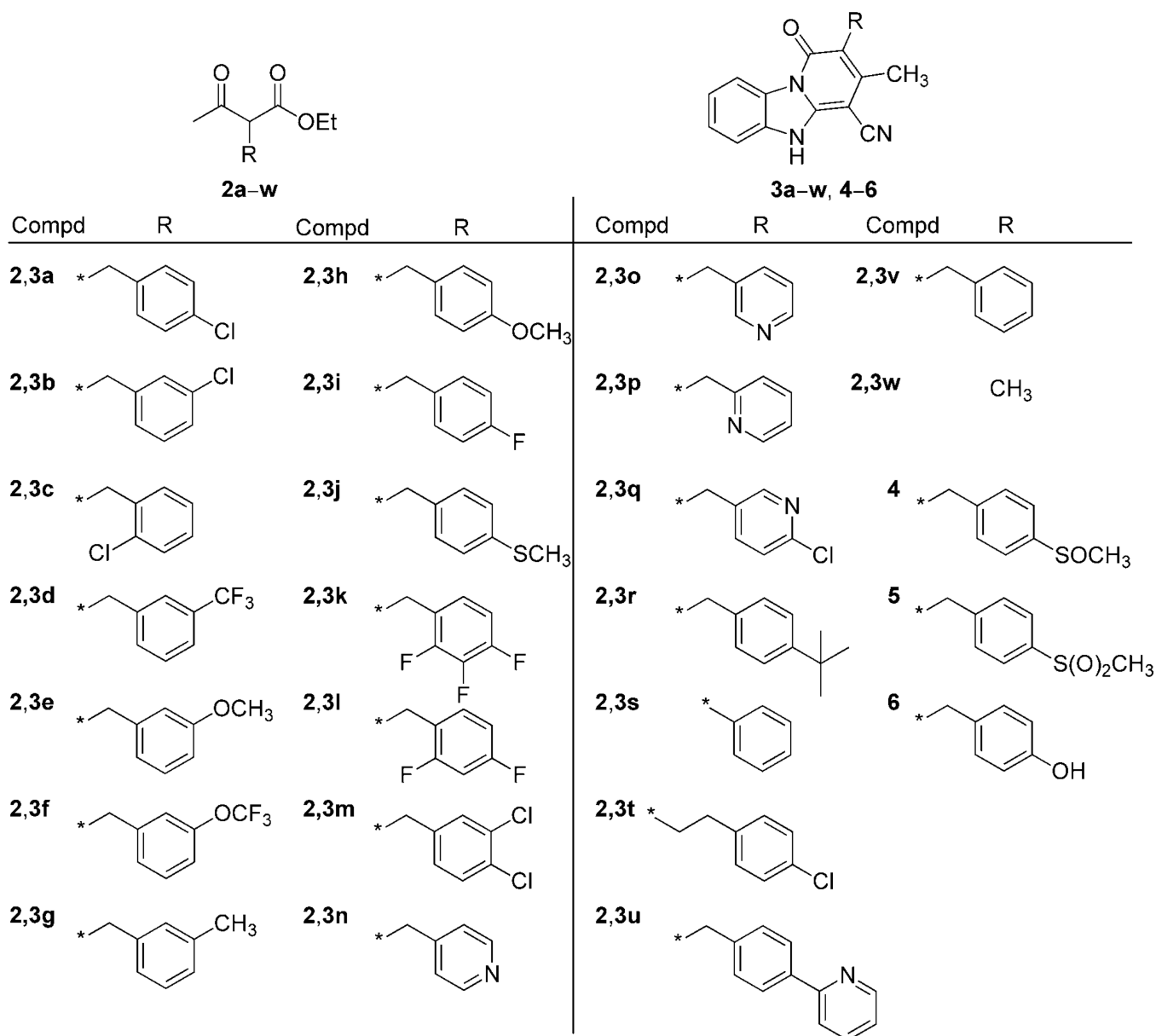
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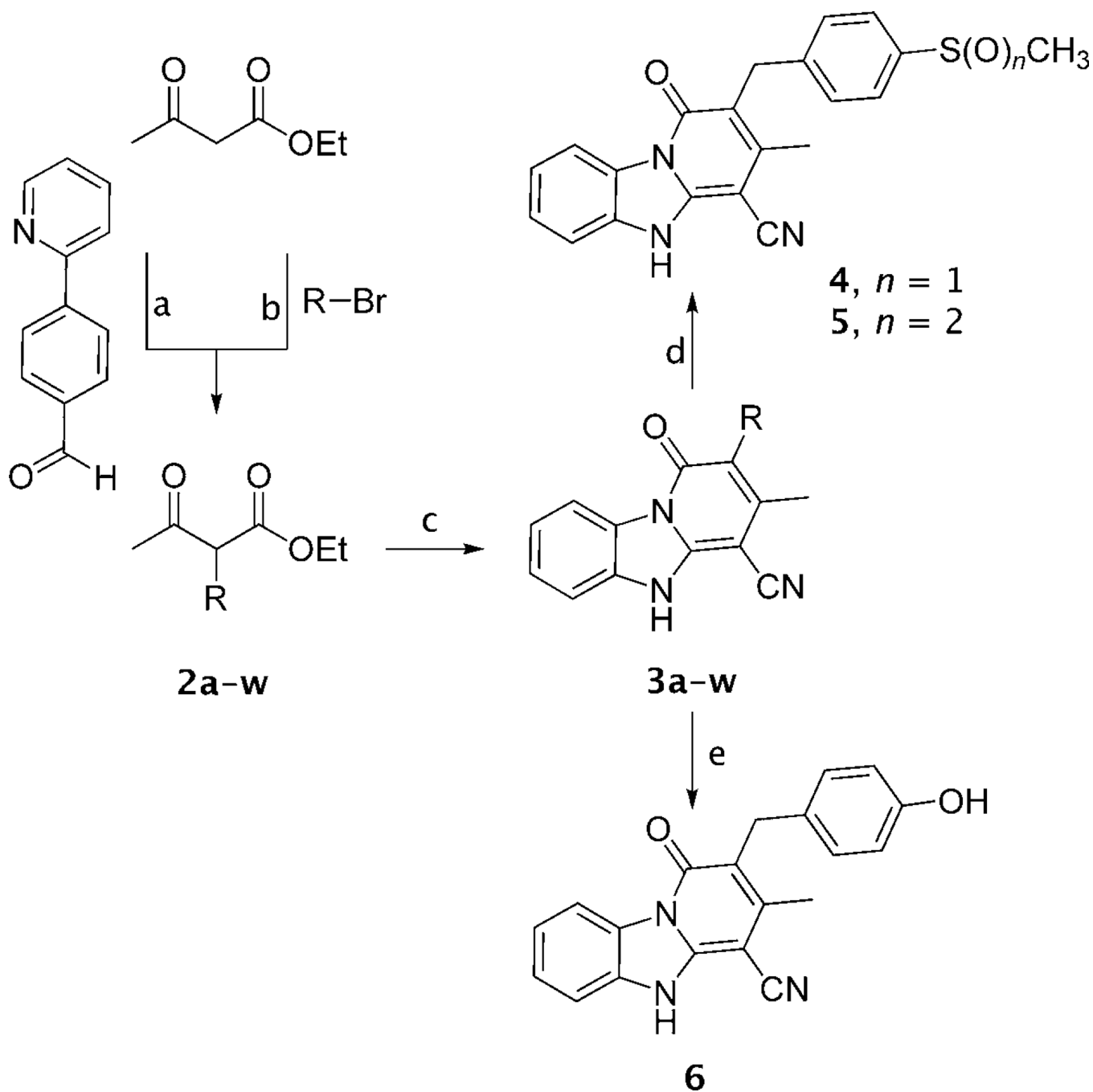
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**Figure 1.**

Structures of intermediates **2a-w** and target compounds **3a-w, 4-6**. * indicates the point of attachment.

**Scheme 1.**

Reagents and conditions: a) $(\text{CH}_3)_3\text{SiCl}/\text{NaI}/\text{CH}_3\text{CN}$, CH_3CN , $\text{RT} \rightarrow 60^\circ\text{C}$, 24 h, 57%; b) $t\text{BuOK}$, $t\text{BuOH}$, THF, 70°C , 1–2.5 h, 26–92 %; c) Benzimidazole-2-acetonitrile, $\text{CH}_3\text{COONH}_4$, neat or toluene, 150°C , 0.5–1.5 h, 51–91 %; d) for **4**: $\text{TeO}_2/\text{H}_2\text{O}_2$, $\text{MeOH}/\text{H}_2\text{O}$, RT , 72 h, 66%; for **5**: $m\text{-CPBA}$, acetone, RT , 3 h; e) BBr_3 , CH_2Cl_2 , 0°C ! RT , 2 h, 79%. For complete structures see Figure 1.

Table 1

Anti-TB activity of compounds **3a–w**, **4–6** against *M. tuberculosis* strain H37Rv.

Compd	MIC _{MABA} ^[a] [µg mL ⁻¹]	MIC _{BD} ^[b] [µg mL ⁻¹]	IC ₅₀ Vero cells ^[c] [µg mL ⁻¹]	Compd	MIC _{MABA} [µg mL ⁻¹]	MIC _{BD} [µg mL ⁻¹]	IC ₅₀ Vero cells [µg mL ⁻¹]
3a	0.5	1.0	8.0	3n	>128	nt	nt
3b	>128	>128	nt ^[d]	3o	4.0	nt	nt
3c	>128	>128	nt	3p	4.0	nt	nt
3d	>128	nt	nt	3q	0.5	nt	nt
3e	>128	nt	nt	3r	0.5	1.0	>64
3f	64	nt	nt	3s	8.0	nt	nt
3g	8.0	nt	nt	3t	>128	nt	nt
3h	0.25	0.25	>64	3u	4.0	nt	nt
3i	2.0	2.0	16	3v	8.0	nt	nt
3j	0.5	nt	nt	3w	>128	nt	nt
3k	0.5	nt	nt	4	2.0	nt	nt
3l	0.25	1.0	8.0	5	1.0	nt	nt
3m	0.25	0.5	4.0	6	>128	nt	nt
INH ^[e]	0.04	nt	nt				

^[a] The lowest concentration of drug leading to at least a 90% reduction of bacterial growth signal by microplate Alamar Blue assay (MABA); values reported are the average of three individual measurements;

^[b] The lowest concentration of drug preventing visible bacterial growth in a 5 mL Tween-free Middlebrook broth dilution (BD) culture; values reported are the average of three individual measurements;

^[c] Cytotoxicity against Vero cells;

^[d] Not tested;

^[e] Isoniazid.

Table 2

Comparative anti-TB activity of compounds 3h and selected antitubercular drugs against susceptible, MDR and XDR strains of *M. tuberculosis*.

Compd	V4207 (DS) ^[a]	TF274 (XDR) ^[b]	R506 (XDR) ^[b]	KZN494 (MDR) ^[c]	V2475 (MDR) ^[c]
MIC _{MABA} ^[d] [$\mu\text{g mL}^{-1}$]					
3h	0.5–2	0.5	0.5	0.5	16
INH ^[e]	0.04	4–8	8	16	16
RIF ^[f]	0.063	>128	>128	128	2–4
LEV ^[g]	0.125	1	2	nt ^[l]	nt
OFL ^[h]	0.5	4–8	4	0.5	0.5
KAN ^[i]	2–4	>128	>128	2	2

^[a] A drug-susceptible strain of *Mtb*;

^[b] An extensively drug-resistant strain of *Mtb*;

^[c] A multidrug resistant strain of *Mtb*;

^[d] The lowest concentration of drug leading to at least a 90% reduction of bacterial growth signal by microplate Alamar Blue assay (MABA); values reported are the average of three individual measurements;

^[e] Isoniazid;

^[f] Rifampin;

^[g] Levofloxacin;

^[h] Ofloxacin;

^[i] Kanamycin;

^[l] Not tested.

Table 3

Metabolic stability of selected compounds in mouse liver microsomes.

Compd	Half life [min]	$Q_{p,h}$ [%] ^[a]
3a	6.50	95.4
3d	14.96	90.0
3e	9.43	93.5
3v	7.82	94.5
3w	51.21	72.5

^[a] Percentage ratio of predicted clearance over plasma flow.

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