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2-[4-(4-Methoxyphenylcarbonyloxy)benzylidene]-6-dimethylaminomethyl cyclohexanone hydrochloride: A Mannich base which inhibits the growth of some drug-resistant strains of *Mycobacterium tuberculosis*

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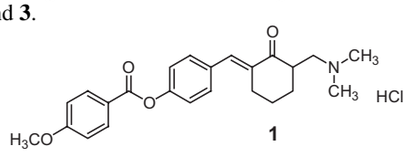
2-[4-(4-Methoxyphenylcarbonyloxy)benzylidene]-6-dimethylaminomethyl cyclohexanone hydrochloride **1** has a MIC value of 0.78 μg/mL towards *Mycobacterium tuberculosis* H_{37}Rv and displays similar or identical MIC figures towards various drug-resistant strains of this microorganism. The enone **1** along with a partial structure 2-dimethylaminomethylcyclohexanone hydrochloride **3** affected respiration in isolated rat liver mitochondria differently which may contribute to the variation in toxicity to both normal cells and *M. tuberculosis*.

Several years ago the discovery of the growth-inhibiting properties of various Mannich bases of 2-benzylidenecyclohexanones towards *Mycobacterium tuberculosis* H_{37}Rv was disclosed (Dimmock et al. 2004). From this study, **1** emerged as a lead molecule having a minimum inhibitory concentration (MIC) value of 0.78 μg/mL. Bearing in mind that the MIC figure of the highly potent antitubercular drug rifampicin is 0.25 μg/mL (Guillon et al. 1998), **1** is considered to be an important lead molecule which is structurally divergent from the contemporary medication used in treating tuberculosis. In an attempt to find the contribution of the groups at positions 2 and 6 of **1** to antitubercular properties, the related partial structures **2** and **3** were evaluated. At a concentration of 12.5 μg/mL, **2** caused a 51% inhibition of the growth of *M. tuberculosis* (Dimmock et al. 2004) and was not considered further. However the MIC value of **3** is 12.5 μg/mL and further investigations were conducted with **1** and **3**.



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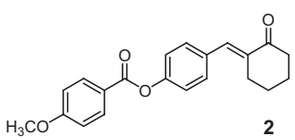

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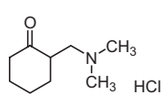

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Ideally an antitubercular agent will have not only significant growth-inhibiting properties towards *M. tuberculosis* but will be well tolerated by mammalian cells. In other words, a greater toxicity to the microorganism than to normal tissues should be displayed. Both **1** and **3** were evaluated against Vero cells and the IC_{50} values for **1** and **3** are 16.4 and 0.05 μg/mL, respectively. The selectivity index (SI) figures, i.e., IC_{50}/MIC, for **1** and **3** are therefore 21 and 0.004, respectively. Consideration was given with a view to understanding the reason for the difference in toxicity between **1** and **3** towards normal cells. A number of Mannich bases interfere with respiration in mitochondria (Dimmock et al. 1983; Hamon et al. 1982). The MIC figure of **1** is 0.78 μg/mL or 1.81 μM. Hence concentrations of 2, 4 and 8 μM were employed to detect any differences in the effect on respiration of rat liver mitochondria. Using concentrations of 2 and 4 μM of **1**, stimulation of respiration by 13 and 107%, respectively, was noted. However at 8 μM inhibition of respiration by 13% occurred. This bimodal effect was in contrast to **3** in which a concentration-dependent increase in stimulation of respiration took place, i.e., by 30, 45 and 65% when 2, 4 and 8 μM, respectively, of **3** was employed. Thus a possible reason for the variation in mammalian toxicity between **1** and **3** is the difference in effects on mitochondrial respiration.

A compound with a SI value of 10 or greater is considered a useful lead molecule (TAACF, 2009). Hence **1**, with a SI value of 21, was investigated further. An enormous clinical problem in treating tuberculosis patients is the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* (Lenaerts et al. 2008). The enone **1** was evaluated against seven strains of *M. tuberculosis* (the MIC figures in μg/mL are in parentheses) which are resistant to isoniazid (1.56), rifampin (1.56), ethionamide (1.56), thioacetazone (0.78), ciprofloxacin (0.78), *p*-aminosalicylic acid (1.56) and kanamycin sulphate (3.13). These MIC values are identical or very similar to the figure generated using the H_{37}Rv strain of *M. tuberculosis*, namely 0.78 μg/mL. Thus these drug-resistant strains do not possess cross resistance to **1** implying that this Mannich base has a different mode of action than a number of antimycobacterial drugs.

In some series of antimycobacterials an increase in potencies is found with a rise in logP values (Patole et al. 2006). The logP value of **1** is 1.84 which is substantially greater than the figure of −1.86 for the related Mannich base **3**. Thus in the future, the insertion of lipophilic groups into the aryl rings of **1** will increase the logP figures which may lead to analogues displaying greater antitubercular potencies than **1**.

In conclusion, 2-[4-(4-methoxyphenylcarbonyloxy)benzylidene]-6-dimethylaminomethyl cyclohexanone hydrochloride **1** displays potent growth-inhibiting properties towards *M. tuberculosis* H_{37}Rv as well as a number of drug-resistant strains of this microorganism. The enone **1** has a good SI figure. A related Mannich base **3** has moderate antitubercular potency but is more toxic to Vero cells than **1**. This variation in bioactivity may be due to differences in the effects on respiration in mitochondria and possibly the divergent logP values of **1** and **3**.

Experimental

Both **1** and **2** were prepared by a literature method (Dimmock et al. 2004) while the synthesis of **3** has been reported previously (Dimmock et al. 1993). A literature procedure was followed in the isolation of mitochondria from rat liver (Kowaltowski et al. 1996). The effect of **1** and **3** on mitochondrial respiration was determined polarographically (Estabrook 1967). In this experiment mitochondria (1 mg protein/mL) were incubated at 30° C in an aqueous buffer pH 7.2 containing succinate (5 mM), magnesium chloride (1 mM), potassium phosphate (5 mM), HEPES (10 mM) and sucrose (125 mM). In the case of **1**, the percentage stimulation of mitochondrial respiration (concentration in μM in parentheses) was

13.06 ± 1.87 (2) and 107.2 ± 4.57 (4) while at 8 μM, respiration was inhibited by $12.50 \pm 1.96\%$. The percentage stimulation of respiration by **3** is 29.89 ± 5.21 (2), 44.77 ± 4.06 (4) and 64.58 ± 0.80 (8).

The evaluation of **1** towards the drug-resistant strains of *M. tuberculosis* was undertaken using the microplate alamar blue assay (Collins and Franzblau, 1997). The cytotoxicity towards Vero cells was carried out using serial dilutions commencing with ten times the concentration of the MIC figure towards *M. tuberculosis* $H_{37}R_v$.

The logP values of the free bases of **1** and **3** were obtained using the Molinspiration WebME Editor 1.16 (Molinspiration Chemoinformatics).

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