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Evaluation of novel nicotine analogues for their anti-bacterial and anti-fungal activity

Abstract

Microbes like bacteria and fungi are known to cause several human illnesses. Amongst those various diseases, certain bacterial and fungal infections are more common because of their tendency to develop new strains under any circumstances by developing resistance against the existing drugs and decontamination methods currently employed. This alarms the scientists for continuously exploring alternate molecules to control these illnesses. Several nicotinic derivatives were evaluated for their antibacterial and anti-fungal activity. The nicotinic acid moiety with characterized de-addiction property exhibited antimicrobial activity against various pathogens of clinical importance. One compound (5-(4-fluorophenyl)nicotinoyl)-1-methylpyrrolidin-2-one developed in the present study was found very effective against *Candida albicans*, a highly pathogenic opportunistic fungus responsible for 80-95% vaginal infection in humans and has developed resistance to several antifungals. Therefore, this class of compounds could be a good starting point to develop new lead compounds for handling this pathogenic fungus. In addition, the broad spectrum anti-microbial action of nicotine analogues developed in the present study may find immense applications in formulating new disinfection or decontamination strategies against widely spreading pathogens of clinical significance.

Keywords: anti-bacterial, anti-fungal, nicotinic acid derivatives, de-addiction, *Candida albicans*

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Introduction

Infectious diseases have increased in recent years, causing a serious public health threat despite of significant advances in anti-microbial therapies. It is estimated that up to 5% of all the infections are known to be caused by fungi worldwide. Fungal infections in high risk patients are known to rapidly multiply and would make the treatment difficult.[1] The emergence of bacterial resistance to common antibiotics always warrants the necessity of exploring new antibacterial and anti-fungal therapies. Consequently, there is always a huge demand for developing new drugs with fewer toxic side effects, improved pharmacokinetics properties and broad spectrum activity against Gram-positive and Gram-negative bacteria, including resistant strains.[2–5] Nicotinic acids and their analogues are less explored and with limited studies on anti-bacterial, anti-oxidant, anti- tuberculosis, anti-diabetic, anti-inflammatory and anti-carcinogenic activities.[6] However, nicotinamide and nicotinic acid have been in use for over six decades due to their unusual broad spectrum antimicrobial actions. Several nicotinic acid derivatives in their form metallic complex forms have been shown to possess antimicrobial activities against various bacteria, fungi, and yeast species.[7–9] But, addiction is the major drawback attributed to Nicotine for their therapeutic application. If nicotine analogues can be designed with de addiction property, they can be converted into excellent molecules for curing many ailments. It is known that the pyridine nucleus within the nicotine structure is responsible for wide range of activities and it is present in many products such as drugs, vitamins, food, flavorings, plants, dyes, adhesives, insecticides, rubber products and herbicides.[10] They also have anti-bacterial, anti-oxidant, anti-carcinogenic and anti-inflammatory activities, with therapeutic potential for osteoarthritis, granuloma annulare and mycobacterium infections.[11,12] With this background, the present study was aimed to develop series of nicotinic acid derivatives with different substituted groups or atoms and different heterocycle moieties with de-addiction characteristics based on the molecular docking study for de-addiction. The compounds were synthesized and tested for antibacterial and antifungal activity.

Materials and methods

Synthesis of nicotine analogues

Twenty-five compounds labelled as ARP100101-ARP100125 as listed in Table 1 were synthesized from Chemveda Life Sciences India Pvt Ltd, Hyderabad, India as described below. The 1H and NMR spectral data of the purified compounds were recorded on 300 MHz Bruker NMR spectrometer.

Table 1 List of Nicotine analogues synthesized in the present study

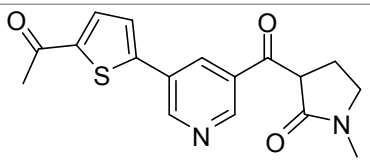
Compound ID	Compound structure	Purity (Tautomers ratio)
ARP100101		98.94% HPLC (93.43+5.41)

Table continued

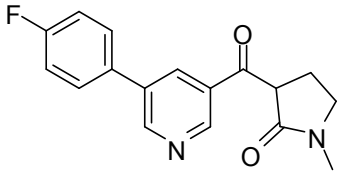
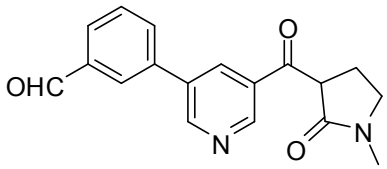
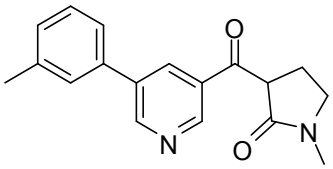
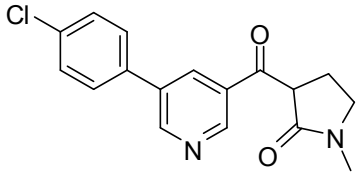
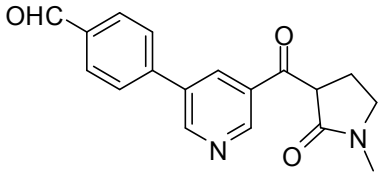
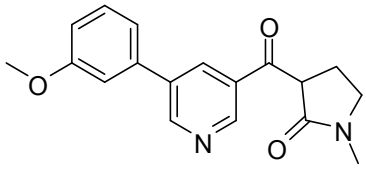
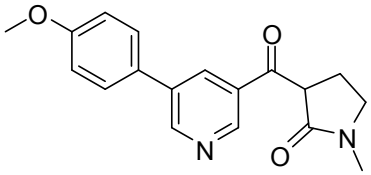
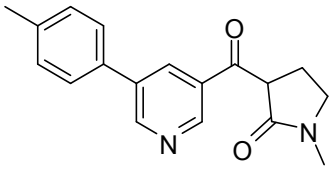
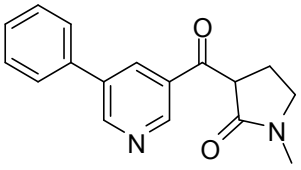
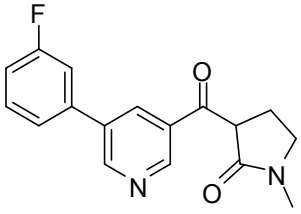
ARP100102		99.47% HPLC (96.42+3.05)
ARP100103		99.74% HPLC (94.07+5.67)
ARP100104		98.21% HPLC (94.14+4.07)
ARP100105		94.68% HPLC (87.72+6.96)
ARP100106		98.93% HPLC (85.05+13.88)
ARP100107		97.30% HPLC
ARP100108		92.50% HPLC (88.30+4.20)
ARP100109		98.39% HPLC (90.37+8.02)
ARP10010		96.50% HPLC (87.17+7.33)
ARP100111		98.25% HPLC (87.89+10.36)

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Table continued

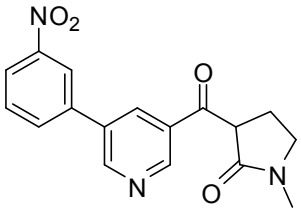
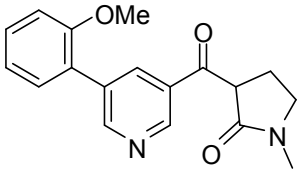
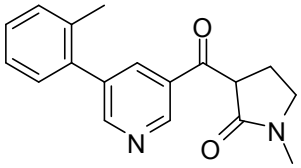
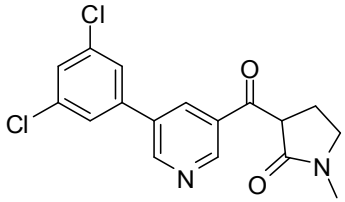
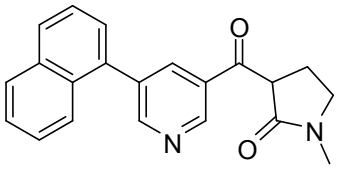
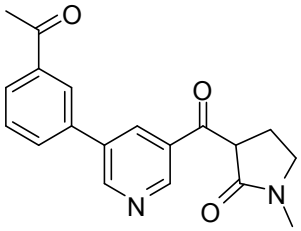
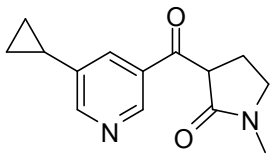
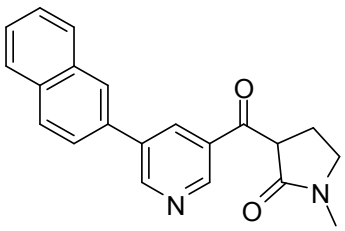
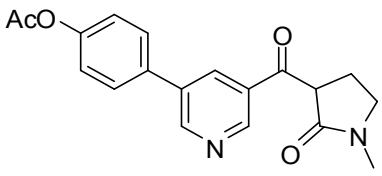
ARP100112		98.13% HPLC (96.70+1.43)
ARP100113		98.13% HPLC (85.74+12.39)
ARP100114		96.36% HPLC (90.09+6.27)
ARP100115		99.18% HPLC (95.93+3.25)
ARP100116		94.58% HPLC (75.54+19.04)
ARP100117		97.12% HPLC (84.20+12.92)
ARP100118		92.72% HPLC (84.83+7.89)
ARP100119		97.40% HPLC (88.06+9.34)
ARP100120		97.08% HPLC (83.55+13.53)

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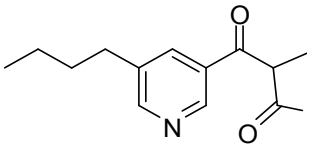
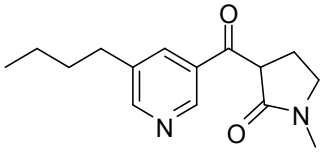
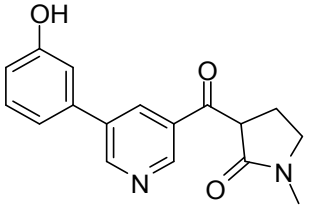
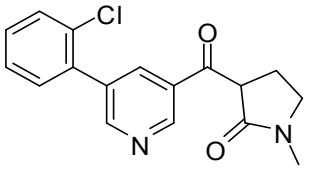
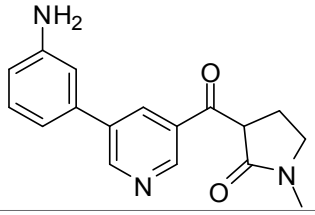
Table continued

ARP100121		98.97% HPLC (94.35+4.62)
ARP100122		96.64% HPLC (65.73+30.91)
ARP100123		93.59 HPLC (85.95+7.64)
ARP100124		94.97% HPLC (86.72+8.25)
ARP100125		96.51% HPLC (89.80+6.71)

General synthetic scheme: Ice cold solution of 5-bromonicotinic acid in methanol under nitrogen atmosphere was mixed with concentrated H_2SO_4 drop wise. The mixture was slowly heated to reflux and continued for 15 h, after completion of the reaction; volatiles were removed under reduced pressure. The resulting solid was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution; the combined organic layer was dried over with sodium sulfate, filtered and evaporated under reduced pressure to obtain methyl 5-bromonicotinate intermediate (Figure 1).

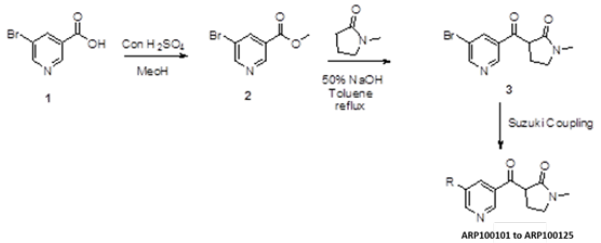


Figure 1

^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.12 (s, 1H), 8.84 (s, 1H), 8.43 (s, 1H), 3.96 (s, 3H); Mass (m/z): 216 $(M+H)^+$, 218 $(M+2H)^+$.

General procedure for synthesis of 3-(5-bromonicotinoyl)-1-methylpyrrolidin-2-one: A suspension of NaH (60% in oil, 1.84g, 46 mM) was washed with toluene (3X10ml) under nitrogen atmosphere. The resulting slurry in 50 ml of toluene was refluxed for 30 minutes under nitrogen and to it was added slowly a toluene (25ml) solution of methyl 5-bromonicotinate (5g, 23.26mM) and 1-methyl-2-pyrrolidone (4.58g, 46.5mM) for 45 minutes. Then the reaction mixture was refluxed for 10h. After completion of the reaction cooled to room temperature and added 10ml of saturated ammonium chloride. The separated organic layer was dried over with sodium sulfate, filtered and evaporated under reduced pressure to obtain crude residue which was purified by silica-gel column chromatography using EtOAc/hexane (60:40) as an eluent to afford an off white solid (1.6 g, 24.4% yield).

^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.21 (s, 1H), 8.84 (s, 1H), 8.54 (s, 1H), 4.38 (q, 1H), 3.58 (m, 1H), 3.43 (m, 1H), 2.86 (s, 3H), 2.72 (m, 1H), 2.25 (m, 1H); Mass (m/z): 216 $(M+H)^+$, 218 $(M+2H)^+$.

General procedure for synthesis of Nicotine analogues ARVP100101-ARVP100125: A mixture of 3-(5-bromonicotinoyl)-1-methylpyrrolidin-2-one (1 equiv), Boronic acid (1.2 equiv), 1,1'-bis(diphenylphosphino)ferrocene) dichloro palladium (II) complex with DCM (0.1 equiv) and 2M Na_2CO_3 (4 equiv) in toluene/1,4-dioxane (4:1, 15 volumes) was degassed and filled with nitrogen. Then the reaction mixture was heated to 85°C and maintained for 2h. After completion of the reaction filtered through Celite bed and washed with ethyl acetate. The combined organic layer was dried over with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to obtain crude residue which was purified by silica-gel column chromatography using 2% MeOH/DCM as an eluent to afford 45-50% yield. 1H and NMR spectral data were recorded in

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$CDCL_3$ or Acetone-d6 on a 300 MHz Bruker NMR spectrometer. The following compounds were prepared as per the above general procedure.

Synthesis of 3-(5-(5-acetylthiophen-2-yl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100101): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.27 (s, 1H), 9.06 (s, 1H), 8.66 (s, 1H), 7.61 (d, 1H), 7.19 (d, 1H), 4.46 (t, 1H), 3.60 (m,1H), 3.41 (m,1H), 2.87 (s, 3H), 2.78 (m, 1H), 2.58 (s, 3H), 2.26 (m, 1H); Mass (m/z): 329.2 $(M+H)^+$.

Synthesis of 3-(5-(4-fluorophenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100102): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.26(s, 1H), 8.97 (s, 1H), 8.60 (s, 1H), 7.7 (d, 1H), 7.46 (d, 1H), 4.45 (t, 1H), 3.60 (m, 1H), 3.42 (m, 1H), 2.86 (s, 3H), 2.78 (m, 1H), 2.58 (s, 3H), 2.24 (m, 1H); Mass (m/z): 299.2 $(M+H)^+$.

Synthesis of 3-(5-(1-methyl-2-oxopyrrolidine-3-carbonyl) pyridin-3-yl) benzaldehyde (ARP100103): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 10.11 (s, 1H), 9.05 (s, 1H), 8.68 (s, 1H), 8.15 (s, 1H), 7.94 (m, 2H), 7.69 (t, 1H), 4.51 (t, 1H), 3.61(m, 1H), 3.42 (m, 1H), 2.88 (s, 3H), 2.78 (m, 1H), 2.58 (s, 3H), 2.28 (m, 1H); Mass (m/z): 309.2 $(M+H)^+$.

Synthesis of 1-methyl-3-(5-m-tolylnicotinoyl) pyrrolidin-2-one (ARP100104): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.26 (s, 1H), 9.00 (s, 1H), 8.60 (s, 1H), 7.38-7.42 (m, 3H), 7.22 (s, 1H), 4.49 (t, 1H), 3.61 (m, 1H), 3.44 (m, 1H), 2.88 (s, 3H), 2.74 (m, 1H), 2.44 (s, 3H), 2.28 (m, 1H); Mass (m/z): 295.2 $(M+H)^+$.

Synthesis of 3-(5-(4-chlorophenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100105): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.15 (d, 2H), 8.64 (s, 1H), 7.87 (d, 2H), 7.61 (d, 1H), 4.90 (t, 1H), 3.40-3.43 (m, 2H), 2.74 (s, 3H), 2.21 (s, 2H); Mass (m/z): 315.2 $(M+H)^+$.

Synthesis of 4-(5-(1-methyl-2-oxopyrrolidine-3-carbonyl) pyridin-3-yl) benzaldehyde (ARP100106): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 10.09 (s, 1H), 9.34 (s, 1H), 9.06 (s, 1H), 8.71 (s, 1H), 8.03 (d, 2H), 7.84 (d, 2H), 4.50 (q, 1H), 3.63 (m, 1H), 3.47 (m, 1H), 2.89 (s, 3H), 2.80 (m, 1H), 2.30 (m, 1H); Mass (m/z): 309.2 $(M+H)^+$.

Synthesis of 3-(5-(3-methoxyphenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100107): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.28 (s, 1H), 9.01 (s, 1H), 8.61 (s, 1H), 7.42 (t, 1H), 7.26 (s, 1H), 7.24 (d, 1H), 6.99 (d, 1H), 4.50 (t, 1H), 3.88 (s, 3H), 3.62 (m, 1H), 3.45 (m, 1H), 2.88 (s, 3H), 2.76 (m, 1H), 2.30 (m, 1H); Mass (m/z): 311.2 $(M+H)^+$.

Synthesis of 3-(5-(4-methoxyphenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100108): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.22 (s, 1H), 8.98 (s, 1H), 8.59 (s, 1H), 7.59 (d, 2H), 7.03 (d, 2H), 4.48 (t, 1H), 3.87(s, 3H), 3.62 (m, 1H), 3.42 (m, 1H), 2.88 (s, 3H), 2.78 (m, 1H), 2.26 (m, 1H); Mass (m/z): 311.2 $(M+H)^+$.

Synthesis of 1-methyl-3-(5-p-tolylnicotinoyl) pyrrolidin-2-one (ARP100109): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.25 (s, 1H), 9.00 (s, 1H), 8.61 (s, 1H), 7.53 (d, 2H), 7.31 (d, 2H), 4.5 (t, 1H), 3.63 (m, 1H), 3.46 (m, 1H), 2.89 (s, 3H), 2.77 (m, 1H), 2.42 (s, 3H), 2.28 (m, 1H); Mass (m/z): 295.3 $(M+H)^+$.

Synthesis of 1-methyl-3-(5-phenylnicotinoyl) pyrrolidin-2-one (ARP1001010): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.28 (s, 1H), 9.02 (s, 1H), 8.64 (s, 1H), 7.65 (d, 2H), 7.42-7.52 (m, 3H), 4.51 (q, 1H), 3.63 (m, 1H), 3.45 (m, 1H), 2.89 (s, 3H), 2.76 (m, 1H), 2.30 (m, 1H); Mass (m/z): 281.2 $(M+H)^+$.

Synthesis of 3-(5-(3-fluorophenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100111): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.29 (s, 1H), 8.96 (s, 1H), 8.61 (s, 1H), 7.46-7.25 (m, 3H), 7.12 (t, 1H), 4.42 (m, 1H), 3.60 (m,1H), 3.44 (m,1H), 2.87 (s, 3H), 2.78 (m, 1H), 2.26 (m, 1H); Mass (m/z): 299.3 $(M+H)^+$.

Synthesis of 1-methyl-3-(5-(3-nitrophenyl) nicotinoyl) pyrrolidin-2-one (ARP100112): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.35 (s, 1H), 9.05 (s, 1H), 8.68 (s, 1H), 8.50 (s, 1H), 8.30 (d, 1H), 7.96 (d, 1H), 3.61 (m, 1H), 3.42 (m, 1H), 3.35 (m, 1H), 2.87 (s, 3H), 2.28 (m, 1H); Mass (m/z): 326.3 $(M+H)^+$.

Synthesis of 3-(5-(2-methoxyphenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100113): ^{1}HNMR (500 MHz, $CDCl_3$): δppm: 9.23 (s, 1H), 8.95 (s, 1H), 8.55 (s, 1H), 7.25-7.36 (m, 2H), 7.05 (m, 2H), 4.47 (m, 1H), 3.82 (s, 3H), 3.60 (m, 1H), 3.42 (m, 1H), 2.87 (s, 3H), 2.70 (m, 1H), 2.15 (m, 1H); Mass (m/z): 311.3 $(M+H)^+$.

Synthesis of 1-methyl-3-(5-o-tolylnicotinoyl) pyrrolidin-2-one (ARP100114): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.28 (s, 1H), 8.76 (s, 1H), 8.39 (s, 1H), 7.25-7.31 (m, 4H), 4.46 (q, 1H), 3.62 (m, 1H), 3.42 (m, 1H), 2.87 (s, 3H), 2.74 (m, 1H), 2.30 (s, 3H), 2.28 (m, 1H); Mass (m/z): 295.3 $(M+H)^+$.

Synthesis of 3-(5-(3,5-dichlorophenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100115): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.32 (s, 1H), 8.95 (s, 1H), 8.56 (s, 1H), 7.42-7.50 (m, 3H), 4.45 (t, 1H), 3.60 (m, 1H), 3.44 (m, 1H), 2.90 (s, 3H), 2.76 (m, 1H), 2.26 (m, 1H); Mass (m/z): 349.1 $(M+H)^+$.

Synthesis of 1-methyl-3-(5-(naphthalen-1-yl) nicotinoyl) pyrrolidin-2-one (ARP100116): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.37 (s, 1H), 8.93 (s, 1H), 8.54 (s, 1H), 7.93 (d, 2H), 7.80 (d, 2H),7.40-7.60 (m, 6H), 4.48 (t, 1H), 3.59 (m, 1H), 3.42 (m, 1H), 2.88 (s, 3H), 2.75 (m, 1H), 2.44 (m, 1H), Mass (m/z): 331.2 $(M+H)^+$.

Synthesis of 3-(5-(3-acetylphenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100117): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.32 (s, 1H), 9.06 (s, 1H), 8.66 (s, 1H), 8.22 (s, 1H), 8.00 (d, 1H), 7.82 (d, 1H), 7.61 (t, 1H), 4.50 (m, 1H), 3.62 (m, 1H), 3.42 (m, 1H), 2.86 (s, 3H), 2.80 (s, 3H),2.30 (m, 1H); Mass (m/z): 323.2 $(M+H)^+$.

Synthesis of 3-(5-cyclopropylnicotinoyl)-1-methylpyrrolidin-2-one (ARP100118): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.06 (s, 1H), 8.58 (s, 1H), 7.98 (s, 1H), 4.40 (q, 1H), 3.60 (m, 1H), 3.42 (m, 1H), 2.85 (s, 3H), 2.72 (m, 1H), 2.28 (m, 1H); Mass (m/z): 245.1 $(M+H)^+$.

Synthesis of 1-methyl-3-(5-(naphthalen-2-yl) nicotinoyl) pyrrolidin-2-one (ARP100119): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.30 (s, 1H), 9.16 (s, 1H), 8.75 (s, 1H), 8.08 (s, 1H), 7.70-7.95 (m, 4H), 7.50 (d, 2H), 4.52 (q, 1H), 3.61 (m, 1H), 3.42 (m, 1H), 2.86 (s, 3H), 2.78 (m, 1H), 2.28 (m, 1H); Mass (m/z): 331.4 $(M+H)^+$.

Synthesis of 4-(5-(1-methyl-2-oxopyrrolidine-3-carbonyl) pyridin-3-yl) phenyl acetate (ARP100120): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.31 (s, 1H), 9.04 (s, 1H), 8.67 (s, 1H), 8.08 (d, 2H), 7.75 (d, 1H), 4.49 (q, 1H), 3.62 (m, 1H), 3.44 (m, 1H), 2.88 (s, 3H), 2.80 (m, 1H), 2.42 (s, 3H), 2.26 (m, 1H); Mass (m/z): 323.3 $(M+H)^+$.

Synthesis of 3-(5-(1-methyl-2-oxopyrrolidine-3-carbonyl) pyridin-3-yl) benzaldehyde (ARP100121): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:10.11 (s, 1H), 9.32 (s, 1H), 9.06 (s, 1H), 8.67 (s, 1H), 8.14 (s, 1H), 7.94 (m, 2H), 7.68 (d, 1H) 4.49 (t, 1H), 3.61 (m, 1H), 3.44 (m,

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1H), 2.86 (s, 3H), 2.76 (m, 1H), 2.44 (s, 3H), 2.28 (m, 1H); Mass (m/z): 309.2 $(M+H)^+$.

Synthesis of 3-(5-butylnicotinoyl)-1-methylpyrrolidin-2-one (ARP100122): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.36 (s, 1H), 8.98 (s, 1H), 8.88 (s, 1H), 8.67 (s, 1H), 8.50-8.80 (m, 2H), 4.45 (q, 1H), 3.60 (m, 1H), 3.45 (m, 1H), 3.10 (m, 1H), 2.80-2.95 (m, 5H), 2.30 (m, 1H), 1.70 (m, 2H), 1.42 (m, 2H), 1.00 (t, 3H); Mass (m/z): 261.3 $(M+H)^+$.

Synthesis of 3-(5-(3-hydroxyphenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100123): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.22 (s, 1H), 8.96 (s, 1H), 8.56 (s, 1H), 7.12-7.29 (m, 3H), 6.90 (d, 1H), 4.50 (q, 1H), 3.62 (m, 1H), 3.44 (m, 1H), 2.90 (s, 3H), 2.74 (m, 1H), 2.28 (m, 1H); Mass (m/z): 297.3 $(M+H)^+$.

Synthesis of 3-(5-(2-chlorophenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100124): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.31 (s, 1H), 8.88 (s, 1H), 8.52 (s, 1H), 7.35-7.52 (m, 4H), 4.49 (q, 1H), 3.61 (m, 1H), 3.43 (m, 1H), 2.87 (s, 3H), 2.74 (m, 1H), 2.26 (m, 1H); Mass (m/z): 315.3 $(M+H)^+$.

Synthesis of 3-(5-(3-aminophenyl) nicotinoyl)-1-methylpyrrolidin-2-one (ARP100125): ^{1}HNMR (500 MHz, $CDCl_3$): δppm:9.25 (s, 1H), 9.00 (s, 1H), 8.60 (s, 1H), 7.28 (m, 1H), 7.02 (d, 1H), 6.95 (s, 1H), 6.78 (d, 1H), 4.48 (q, 1H), 3.62 (m, 1H), 3.42 (m, 1H), 2.88 (s, 3H), 2.74 (m, 1H), 2.28 (m, 1H); Mass (m/z): 296.3 $(M+H)^+$.

In vitro pharmacological binding assays for de addiction property

To confirm the de-addiction property of the designed Nicotine analogues, the compounds were randomly selected and subjected to *in vitro* binding assay at $1x10^{-8}$M and $1x10^{-7}$M concentrations in duplicates as previously described using human recombinant α4β2 agonist radio ligand derived from neuronal SH-SY5Y cells.[13] Nicotine was used as a reference compound in the binding assay.

In vitro anti-microbial studies

Bacterial cultures and media preparation: Twenty-five chemically synthesized nicotine analogues (ARP10001 to ARP100125) were evaluated for their antibacterial activities against a panel of gram positive, gram negative and common pathogenic fungi. The bacterial cultures Gram positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*), Gram negative bacteria (*E. coli and, Pseudomonas aeruginosa*) and fungal cultures (*Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus*) were procured from MTCC, Chandigarh, India. All the required media were prepared as per manufacturer's instructions.

Preparation of stock solutions of drugs/compounds: Stock solutions of Nicotine analogues (5000µg /mL) were prepared in Dimethyl Sulfoxide (DMSO). Working stock solutions (100µg/mL, 50µg/mL, 25µg/mL 5µg/mL) were prepared from stock solutions. Commercially available standard drugs viz, Ciprofloxacin, Clindamycin, Amikacin, Fluconazole and Triclosan were used as reference standards for comparative evaluation of Nicotine analogues. Ciprofloxacin (5µg/disc, Batch No. 0000131030) standard susceptibility test discs, Amikacin (30µg/disc, Batch No. 0000131028), Clindamycin (25µg/disc, Batch No.0000130591) and Fluconazole (25µg/disc, Batch No. 0000124939) were procured from Hi-Media, Mumbai, India. Triclosan was procured from Kumar Organics Private Ltd, Bangalore, India. For negative controls, Disks impregnated with DMSO were used. The culture media were procured from Hi-Media, Mumbai, India and the chemicals were from Sigma Aldrich, Bangalore, India.

***In vitro* evaluation of anti-bacterial and anti-fungal activity of nicotine analogues:** The Kirby-Bauer disc diffusion method with modification was used to determine the antimicrobial activity of nicotine analogues.[14] The accuracy and reproducibility of the test procedure was validated by the mean values of triplicates.

Preparation of bacterial and fungal culture: The standard bacterial and fungal inoculum for the disc diffusion method was prepared using broth suspension cultures of the standard cultures. At least three to five well-isolated colonies of the same morphological features of the test organism was selected from the agar plate culture. The single colony representing homologous microbial population was aseptically transferred into a tube containing 4 to 5ml of the appropriate broth medium. The broth culture was incubated at 37±1°C until uniform turbidity is obtained. The turbidity of the broth culture was adjusted to match the 0.5 McFarland standard with sterile saline or broth.

Inoculation of test plates: Optimally, within 15 minutes after adjusting the turbidity of the inoculum, a sterile cotton swab was dipped into the inoculum, soaked and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The dried surface of a sterile agar plate was inoculated by streaking the swab over the entire agar surface, rotating the plate approximately 60 each time to ensure an even distribution of inoculum. After the inoculum was absorbed on the agar surface, filter paper discs were impregnated with different concentrations of either test compound or the standard drug was applied within 15 minutes. The discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface with adequate distance between the discs. The plates were incubated in an incubator at 37±1°C for 24h bacteria and at 24±1°C for 48-72h for fungi within 15 minutesafter dispensing the discs.

Results

The NMR spectral data of the purified compounds were verified for their identity (data available upon request). The de-addiction property of the designed Nicotine analogues revealed less than 5% binding to its receptor α4β2 subunit of the nicotine acetylcholine receptor (data not shown).

The compounds evaluated for their anti-microbial and anti-fungal activities via disk diffusion assay. After incubation, each plate was examined for the zone of inhibition. The diameter of the zone of complete inhibition was measured in millimeter (mm), including the diameter of the disc. The diameter of inhibition zone of different concentrations of test item was compared with the inhibition zones of reference standards. Of twenty-five compounds tested, compounds ARP100103, ARP100107, ARP100109, ARP100110, ARP100112-ARP100118, ARP100123, ARP100124 and ARP100125 did not show any antimicrobial or antifungal activity and hence were excluded. Compounds ARP100105, ARP100106, ARP100111 had antibacterial activity up to 50µg/mL concentration with mean diameter of inhibition zone ranging from 12-9mm (Table 2) along with the standard reference drugs. The Negative control did not show any detectable inhibitory zone. Compounds ARP100101, ARP100102, ARP100104, ARP100105, ARP100106, ARP100108, and ARP100119- ARP100122 exhibited antifungal activities ranging from 5µg/mL to 100µg/mL concentration with mean diameter of inhibition zone ranging from 15-9mm (Table 3) along with the standard reference drugs. The Negative control had no detectable inhibition.

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Table 2 Anti-bacterial activity: Mean zone of inihibition (mm) by disc diffusion method for nicotine analogues, Positive Control 1, 5µg Ciprofloxacin (Hi-Media); Positive Control 2, 30µg Amikacin (Hi-Media); Negative control, Diluent (DMSO); '- ' indicates no zone of inhibition; mm, millimeter

Compound ID	Name of the Bacteria	100µg	50µg	25µg	5µg	Negative control	Positive control 1	Positive control 2
ARP100105	*Staphylococcus aureus*	12	10	-	-	-	27	30
	Enterococcus faecalis	-	-	-	-	-	30	24
	Escherishia. coli	12	-	-	-	-	32	25
	Pseudomonas aeruginosa	-	-	-	-	-	38	25
ARP100106	*Staphylococcus aureus*	-	-	-	-	-	27	30
	Enterococcus faecalis	10	-	-	-	-	30	24
	Escherishia. coli	-	-	-	-	-	32	25
	Pseudomonas aeruginosa	-	-	-	-	-	38	25
ARP100111	*Staphylococcus aureus*	-	-	-	-	-	24	30
	Enterococcus faecalis	9	-	-	-	-	30	24
	Escherishia. coli	12	-	-	-	-	38	25
	Pseudomonas aeruginosa	-	-	-	-	-	38	25

Table 3 Anti-fungal activity: Mean zone of inihibition (mm) by disc diffusion method for nicotine analogues, Positive control 1, 25µg Fluconazole (Hi-Media); Positive control 2, Triclosan 50µg (Hi-media); Negative control, Diluent (DMSO); '- ' indicates no zone of inhibition; mm, millimeter; NT, Not tested

Compound ID	Name of the Fungi	100µg	50µg	25µg	5µg	Negative control	Positive control 1	Positive control 2
ARP100101	*Candiada albicans*	10	8	-	-	-	30	NT
	Cryptococcus neoformans	-	-	-	-	-	25	NT
ARP100102	*Candiada albicans*	13	12	10	10	-	30	NT
	Cryptococcus neoformans	-	-	-	-	-	25	NT
ARP100104	*Candiada albicans*	10	-	-	-	-	30	NT
	Cryptococcus neoformans	13	-	-	-	-	25	NT
ARP100105	*Candiada albicans*	15	-	-	-	-	30	NT
	Cryptococcus neoformans	15	-	-	-	-	25	NT
ARP100106	*Candiada albicans*	12	-	-	-	-	30	NT
	Cryptococcus neoformans	12	-	-	-	-	25	NT
ARP100108	*Candiada albicans*	15	-	-	-	-	30	NT
	Cryptococcus neoformans	11	-	-	-	-	25	NT
ARP100119	*Candiada albicans*	10	9	-	-	-	30	20
	Cryptococcus neoformans	12	11	-	-	-	25	23
ARP100120	*Candiada albicans*	9	-	-	-	-	30	20
	Cryptococcus neoformans	9	-	-	-	-	25	23
ARP100121	*Candiada albicans*	12	10	-	-	-	30	20
	Cryptococcus neoformans	-	-	-	-	-	25	23
ARP100122	*Candiada albicans*	13	-	-	-	-	30	20
	Cryptococcus neoformans	-	-	-	-	-	25	23

Discussion

Nicotine derivatives are known for their potential anti-bacterial and other therapeutic biological activities. Nicotinic acid belongs to water soluble vitamin B complex is known for hyperlipidemia and to lower triglycerides and cholesterol.[15] As pyridine nucleus within the nicotine structure is responsible for wide range of activities[10] including anti-bacterial, anti-oxidant, anti-carcinogenic and anti-inflammatory activities, with therapeutic potential for osteoarthritis, granuloma annulare and mycobacterium infections,[10–12] we were curious to study the biological activity of the nicotine derivatives with different substituted groups or atoms and different heterocycle moieties. These derivatives were mainly designed to get rid of the undesirable addiction property of nicotine that is known to suppress

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the possible beneficial activities of this wonder molecule. It was worthwhile to undertake the synthesis of the twenty-five compounds out of which three compounds had moderate anti-bacterial property and ten compounds had anti-fungal activities. Two nicotine analogues in the present study (ARP100105 and ARP100106) have broad spectrum anti-microbial action against both bacteria and fungi. From the present study, one compound ARP100102 (3-(5-(4-fluorophenyl) nicotinoyl)-1-methylpyrrolidin-2-one) was more effective even at the lowest concentration tested (5µg/mL) in inhibiting the growth of *Candida albicans*, a diploid fungus which is the causative agent of 85-95 % genital infections in humans.[16-18] *C. albicans* have emerged as important causes of morbidity and mortality in immunocompromised patients with AIDS or under cancer chemotherapy, organ or bone marrow transplantation. The biofilms formed by *C. albicans* on the surface of implantable medical devices and the hospital-acquired infections by *C. albicans* have posed major health concerns. A Nicotine analogue 3-(5-(4-fluorophenyl) nicotinoyl)-1-methylpyrrolidin-2-one developed in the present study exhibited a significant activity when compared with reference drugs. Our data suggest that Nicotine analogue may be selectively targeted to fungal growth, also considering the broad spectrum antibacterial and anti-fungal activity, these compounds may find immense applications in formulating new disinfection or decontamination strategies against widely spreading pathogens of clinical significance.

Conclusion

In general, nicotinic acid derivatives exhibited anti-bacterial and anti-fungal activities against several Gram positive, Gram negative bacteria and fungi of clinical importance. The possessed reasonable to fine antimicrobial effectiveness of Nicotine derivatives sets up a necessary platform for further in-depth *in vitro* and *in vivo* characterization of these compounds against microbial infections. Considering the fact that the designed, nicotine analogues have the de addiction properties and are non-toxic in animal models far above their effective concentrations, they it could be a good starting point to find new lead compounds against several bacterial and fungal pathogens, formulating multipurpose anti-disinfection liquids/sterilizer for wide practical applications.

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None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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