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A Novel Isoquinoline Bioactive Compound 5-Amino-4-Methoxyisoquinolin-1(2H)-One From Desert Actinobacterium Streptomyces Fragilis Strain Da7-7: Isolation, Characterization, And Molecular Docking Analysis

Nithya Krishnasamy[1*], S. Akilandeswari[2], Muthukumar Chinnasamy[3*]

[1*]Department of Pharmaceutical Chemistry, School of Pharmacy, Dhanalakshmi Srinivasan University, Samayapuram, Tiruchirappalli - 621 112, Tamil Nadu, India. Email; nithyak.sop@dsuniversity.ac.in.
[2]Dean, School of Pharmacy, Dhanalakshmi Srinivasan University, Samayapuram, Tiruchirappalli - 621 112, Tamil Nadu, India
[3*]PG & Research Department of Botany, National College (Autonomous), Tiruchirappalli – 620 001, Tamil Nadu, India.
Email:cmkumar1979@gmail.com.

Abstract:
A broad spectrum bioactive compound producing actinobacterium *Streptomyces fragilis* strain DA7-7 was isolated from the desert soil sample of Riyadh Province, Saudi Arabia. The bioactive compounds were extracted using ethyl acetate, separated, and purified. The purified active compound was characterized and identified as 5-amino-4-methoxyisoquinolin-1(2H)-one ($C_{10}H_{10}N_2O_2$, 191.07 m/z). The compound possessed the lowest minimum inhibitory concentration 15.6 µg/mL against the pathogenic bacteria *Escherichia coli* ATCC 10536 and *E. coli* CIB 008, while 31.32 µg/mL against pathogenic fungus *Crytococcus neoformans* ATCC 90113. *In silico* antibacterial mechanism and the preclinical evaluation revealed that 5-amino-4-methoxyisoquinolin-1(2H)-one is able to bind efficiently with Undecaprenyl Diphosphate Synthase (-5.02 kcal/mol) when compared to DNA gyrase-Topoisomerase (-4.82 kcal/mol) and IARS (-4.43 kcal/mol) target proteins. Based on the merits of in vitro antimicrobial potential, *in silico* antibacterial mechanism, and the preclinical evaluations, the biologically active compound could be used as a commercial antimicrobial drug in the near future for human welfare.

Keywords: Actinobacteria, *Streptomyces*, 5-amino-4-methoxyisoquinolin-1(2H)-one, minimum inhibitory concentration, molecular docking.

Introduction

The screening of microbial secondary metabolites is becoming successful aspirants for innovation of antibiotic and non-antibiotic compounds. The microorganisms are widely accepted and virtually unlimited sources for the production of novel substances. Among them, actinobacteria embraced a prominent position owing to their diversity and also renowned candidates for the production of new substances. In actinobacteria, the genus *Streptomyces* is recognized one of the best candidates have delivered many important bioactive metabolites with high commercial values.[1]

The discovery of new molecules from actinobacteria has marked an epoch in antibiotic research as most of its antibiotics are too complex to be synthesized by combinatorial chemistry. Nearly two-third of antibiotics occurring from natural resources has been discovered from *Streptomyces*. The number of antimicrobial compounds obtained from this genus increased every year exponentially between 1960 and 1970, subsequently at a slower pace during the years 1980 and 1990.[2] Although, *Streptomyces* derived secondary metabolites have attracted much attention from the community as they possess diverse bioactivities such as antibacterial, antifungal, antitumor, and antioxidant.[3,4,5,6,7,8]

The rate of discovery of new compounds from terrestrial actinobacteria has decreased, whereas the rate of re-isolation of known compounds has increased. The search of underexplored ecological niches has revealed that the need to explore new groups of actinobacteria from such habitats as a source of novel bioactive secondary metabolites. Baltz has estimated that less than one part of the earth's soil surface has been screened for actinobacteria.[9] Only 10 % of natural products from screened strains and just 1 % of molecules from the global consortium of microbial producers may have been discovered by researchers.[10]

The increase in antibiotic resistance bacteria, and decrease in the rate of discovery of new antimicrobial compounds draws the attention of scientist to try to investigate unexplored habitats for novel actinobacteria as possible candidates of new antimicrobials. The current status and discovery of secondary metabolites from extremophilic actinobacteria suggest that,

these organisms could add a new dimension to microbial natural products search.[11,12,13] In Saudi Arabia, the desert habitats are still unexplored in the actinobacterial diversity and also their potential applications. Only fewer studies are made in actinobacterial research in this environment.[8,14,15,16,17] In this study, *Streptomyces* strain DA7-7 was isolated from desert soil of Riyadh Province and the bioactive compound was isolated and identified, and also the antimicrobial potential of the compound was evaluated by *in vitro* and *in silico* analysis.

2. MATERIALS AND METHODS

2.1 Isolation of actinobacteria and primary screening

The potential isolate DA7-7 was isolated from the desert soil samples of Riyadh province, Saudi Arabia. The detailed sampling procedure and isolation protocol have been described in our previous article.[18] The primary screening was performed by the cross streak method.[19] The strain DA7-7 was streaked across the diameter on modified nutrient glucose agar (MNGA) medium and incubated at 28 ºC for 7 days. Pathogenic bacteria and fungi were streaked perpendicular to the central strip of actinobacterial culture. The inoculated plates were incubated and the zone of inhibition was measured. Based on the results of the cross streak method, the potential isolate DA7-7 was selected for further studies.

2.2 Cultural, biochemical and molecular characterization of the isolate DA7-7

The cultural, biochemical and molecular characterization of the isolate DA7-7 was already reported in our previous article.[18]

2.3 Test Microorganisms

The pathogenic microorganisms bacteria such as *Enterococcus faecalis* ATCC 49532, *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 10536, *Klebsiella pneumoniae* ATCC 13882, *Pseudomonas aeruginosa* ATCC 27883, *Proteus vulgaris* ATCC 33420, *Salmonella typhimurium* ATCC 13311, and fungi *Candida albicans* ATCC 2091, *Saccharomyces cerevisiae* ATCC 9763, and *Cryptococcus neoformans* ATCC 90113 were procured from American Type culture collection (ATCC),VA USA. The clinical bacterial strains such as *Escherichia coli* CIB008, *Proteus vulgaris* CIB016, *Staphylococcus aureus* CIB053, and fungal strains including *Candida albicans* CIF005*, Candida albicans* CIF009*, Candida* sp. CIF017 were obtained from K.A.P. Viswanatham Government Medical College, Tiruchirappalli 620 001, TN, India.

2.4 Extraction of bioactive metabolites and antimicrobial activity

Five fermentation media including yeast extract and malt extract (ISP 2), fermentation medium (M6), modified nutrient glucose (MNG), sabouraud dextrose (SD) and yeast peptone glucose (YPG) media were used for this study. The strain DA7-7 was inoculated and incubated at 28 ºC with 120 rpm for 8 days. The antimicrobial activity of fermented broth was performed against pathogenic microorganism by agar well diffusion method.

Based on the media selection, MNG medium was found suitable media for the production of antimicrobial metabolites. Hence, the inoculums (5%) of DA7-7 were inoculated into 700 mL of MNG medium and incubated for 8 days. The broth was centrifuged and the supernatant was collected, and an equal volume of ethyl acetate (v/v) was added, two layers were formed, the organic layer which contained the secondary metabolites separated by using separating funnel. The organic phase was dried over anhydrous Na_2SO_4 and concentrated, and the crude extract was collected and stored at 4ºC for further analysis.

The antimicrobial activity of crude extract of DA7-7 was performed by disc diffusion method.[20] The sterile disc (Hi-media, India), filled with 0.5 mg/disc of crude extract DA7-7 was prepared using DMSO. The discs were placed over the pathogenic microorganism inoculated plates and incubated for 37°C for bacteria and 28°C for fungi. The zone of inhibition was recorded for each microbe separately. Streptomycin (10 µg/disc) and Ketocanozole (25 µg/disc) were used as the positive control for bacteria and fungi, respectively, while DMSO was used as the negative control.

2.5 Purification of bioactive compound

The purification of DA7-7 crude compound, the solubility was evaluated with solvent systems including ethyl acetate, chloroform, pyridine, acetone, 1-butanol, ethanol, hexane, methanol and water. The solvents chloroform and methanol was selected based on thin layer chromatography (TLC). Two gram of crude extract DA7-7 was purified by column chromatography with silica gel column (35 x 5 cm), silica gel (60 mesh size, Hi-media, India) and gradient solvent systems ($CHCl_3$: MeOH) at 8:2, 7:3, 6:4, 5:5, 4:6, 3:7 and 2:8. The fractions obtained from column chromatography was assessed the antibacterial potential against *E. coli* by disc diffusion method.[20] The active fractions are further purified by HPLC (Shimadzu chromatograph with DL-C_{18} column, 5.0 µm, 250 mm × 4.6 mm, and mobile phase of methanol and water 80:20 with a flow rate of 0.5 mL/min at 265 nm UV detectors). The resulting fractions (C_1, C_2 and C_3) were subjected to antibiogram using disc diffusion method.

2.6 Spectroscopic analysis of bioactive compound

The potent fraction C_3 was subjected to spectroscopic analysis. UV-visible spectra were recorded using T80 spectrophotometer (PG instrument, UK), and Infrared spectrum was obtained on a FT-IR Alpha II with AT-XT Golden gate accessories (Bruker, USA). ^{1}H NMR and ^{13}C NMR were recorded in DMSO *d*6 using GSX-400 spectrophotometer (JEOL, Japan) at 400 MHZ for ^{1}H and 300 MHZ for ^{13}C, and mass spectrum was recorded on a Finnigan MAT 8230.

2.7 Minimal inhibitory concentration (MIC) of bioactive compound

The MIC of the purified compound C_3 was assessed by micro dilution plate assay.[21] The compound dissolved in DMSO (500 µg/ml) and a two-fold dilution series (250, 125, 62.5, 31.25, 15.6, 7.8, and 3.9 µg/mL) was prepared, and used for MIC against pathogenic bacteria and fungi. The lowest concentration of bioactive compound exhibiting antimicrobial activity against the test pathogens was taken as the MIC of the bioactive compound. Streptomycin and Ketocanozole were used as the positive controls for bacteria and fungi, respectively, and DMSO was used as the negative control.

2.7 Molecular Docking analysis

In silico antibacterial mechanisms was carried out using Auto dock tool 4.2 between the bioactive compound C_3 and target proteins of test bacterial pathogen (*E. coli*). Auto dock estimates the binding affinity of the drug molecule and predicts ligand conformations accurately by atom affinity potentials pre-calculated using Autogrid 4 on grid maps Preclinical evaluation study about selection of bacterial proteins as receptors such as DNA gyrase-Topoisomerase II (topoisomerase IV) (PDB ID 1AB4), Undecaprenyl Diphosphate Synthase (PDB ID 4H2M) and IARS (PDB ID 1JZQ) were fetched from Protein Data Bank. Druglikeness property was investigated with the help of Lipinski drug filter from Superconducting Facility for Bioinformatics and Computational Biology; and pharmacokinetic and toxicity properties (mutagenicity, carcinogenicity, reproductive effective and irritant assay) were performed through web based pre-ADMET tool for the bioactive compound C_3.[22,23,24]

3. RESULTS AND DISCUSSION

3.1 Extraction and antimicrobial activity of DA7-7 extract

The desert soil actinobacterium *Streptomyces fragilis* strain DA7-7 was exhibited good antimicrobial potential in the preliminary screening (Figure 1).

Fig 1. Preliminary screening of *Streptomyces fragilis* strain DA7-7 against pathogenic microorganisms

1, K. pneumonia ATCC 12882; 2, E. coli ATCC 49532; 3, E. faecalis ATCC 49532; 4, P. vulgaris CIB 016; 5, C. albicans CIF005; 6, S. cerevisiae ATCC 9763; 7, C. neoformans ATCC 90113; 8, P. aeruginosa ATCC 27883; 9, C. albicans ATCC 2091; 10, S. aureus CIB 053.

Among the five different fermentation media tested, MNG was a highly suitable medium for the production of antimicrobial compounds for the isolate DA7-7. The isolate DA7-7 was mass cultured using MNG medium and the broth was extracted using ethyl acetate as solvent. The crude extract DA7-7 (0.5 mg/disc) exhibited good antimicrobial activity against bacterial pathogens with the zone of inhibition 12 to 22 mm, and against pathogenic fungi with the zone of inhibition 11 to 16 mm (Figure 2).

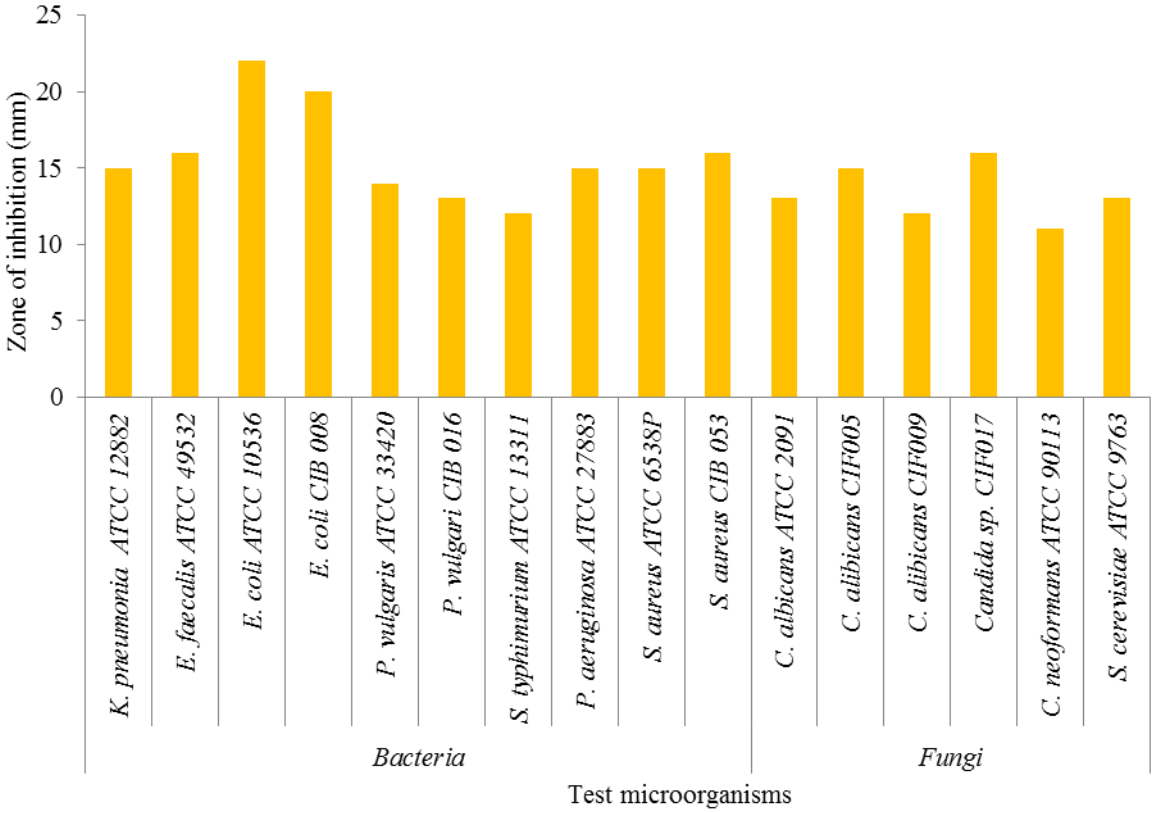


Fig 2. Antimicrobial activity of ethyl acetate crude extract of strain DA7-7 against pathogenic microorganisms

Most of the secondary metabolites and antibiotics produced by actinobacteria are extracellular in nature.[12] In the present study, the potent isolate DA7-7 produced antimicrobial metabolites, which were extracted using ethyl acetate as a solvent and exhibited respectable antimicrobial activity against the tested pathogenic bacteria and fungi. Many researchers have studied and reported that ethyl acetate is a suitable solvent for extracting antimicrobial compounds from actinobacteria, especially, from the genus of *Streptomyces*.[8,12,25]

3.2 Purification and characterization of bioactive compound

The crude bioactive compound derived from the isolate DA7-7 broth, which was extracted using ethyl acetate. The extracted compound was purified by column chromatography. A total of 13 fractions were obtained, in which four fractions were active against pathogenic bacteria. These four fractions were pooled together and subjected to analytical and preparative high performance liquid chromatography at 265 nm. Based on the maximum area percentages, three compounds were selected and categorized as C_1, C_2, and C_3, which were subjected to an antibiogram. Compound C_3 was exhibited good antimicrobial activity against pathogenic bacteria *E.coli* ATCC 10536 (26 mm) and against pathogenic fungi *C. albicans* ATCC 2091 (20 mm), whereas the fractions C_1 and C_2 exhibited poor antimicrobial activities against pathogenic bacteria and fungi. Hence, the fraction C_3 was selected for compound characterization and structural elucidation.

The UV spectrum of compound C_3 was recorded from 200 to 400 nm and showed absorbance maxima at 265 nm. The FT-IR spectrum of compound C_3 possess two peaks at 3417.16 and 3374.48cm^{-1} showed the presence of primary amine, whereas the strong peak at 2975.33 cm^{-1} undergoes SP^3 hybridization indicating the presence of C-H stretch, a strong peak at 1710.27 cm^{-1}showed the presence of ketone group, peak at 1593.21 cm^{-1} indicates NH bend, peak at 1263.42 cm^{-1} indicates C-N stretch and 752.59 cm^{-1} indicates NH wag. The weak peak at 1489.60 cm^{-1} indicates the -C=C- stretch, 1441.45 cm^{-1}indicates C-C stretch, peak at 1087.12 cm^{-1} showed in plane C-H bending and peak at 878.97 cm^{-1} and 703.95 cm^{-1} showed SP^2 C-H bend (presence of aromatic ring). A peak at 1372.34 cm^{-1} undergoes SP^3 hybridization showed the presence of –C-H bend, peak at 1006.12 cm^{-1} showed the presence of C-O alkoxy group and 1046.45 cm^{-1} indicates the aromatic alkoxy group (Figure 3).

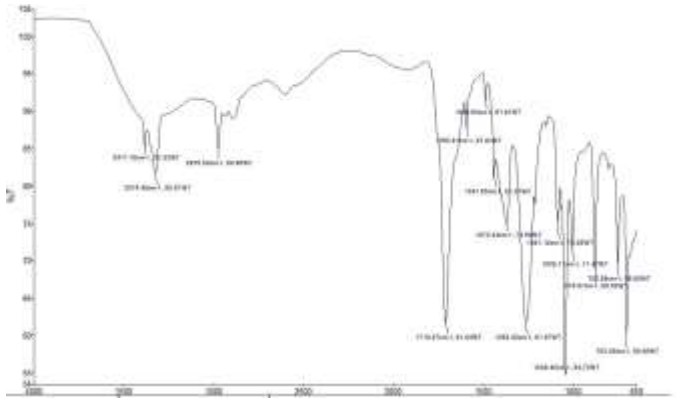


Fig 3. FT-IR spectrum of compound C3 from *Streptomyces fragilis* strain DA7-7

In proton NMR spectrum, the signal at 3.32 ppm indicates the presence of methoxy group and the characteristics signal at 4.92 ppm indicates the presence of aromatic primary amine, whereas the signal 6.64 ppm, 7.08 ppm, 7.11 ppm, and 8.72 ppm shows the presence of aromatic proton and -NH respectively (Figure 4).

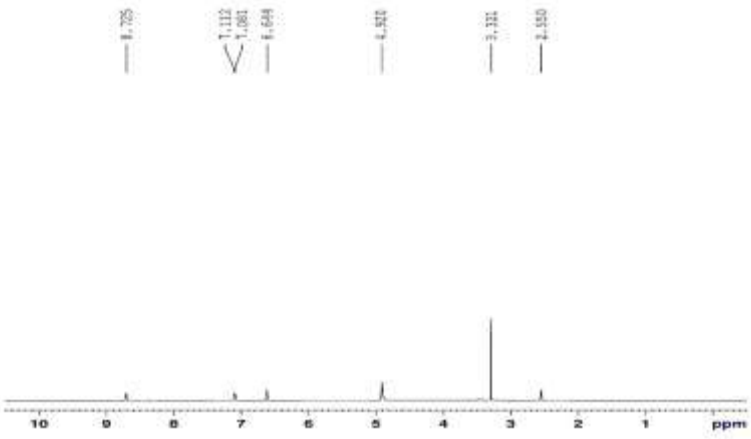


Figure 4. 1H-NMR spectrum of compound C3 from *Streptomyces fragilis* strain DA7-7

Further, in the carbon NMR spectrum, the signal at 56.12 ppm indicate the presence of –C-O-, the signal between 115.13-147.74 ppm showed the presence of –C=C- and -C-C- whereas, the signal at 161.75 ppm showed the presence of –O=C-N- (Figure 5).

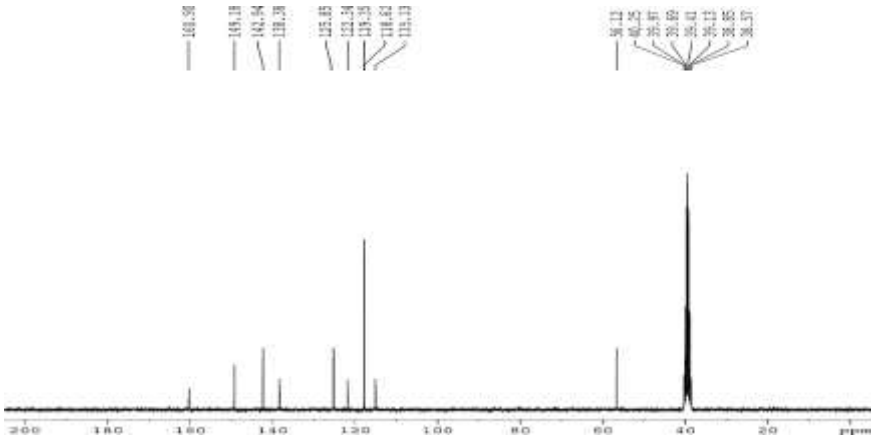


Fig 5. 13C-NMR spectrum of compound C3 from *Streptomyces fragilis* strain DA7-7

ESI-MS spectrum was analyzed the compound C_3, and revealed the expected m/z= [compound + H^+] =191.09, whereas calculated m/z= [compound + H^+] =191.07. Based on the fragmentation pattern, the base peak at m/z 143.0730

($C_9H_5N_1O_1$) and the characteristic peak at m/z 158.0621 ($C_9H_7N_2O_1$ + H^+) whereas the molecular ion peak at m/z 191 ($C_{10}H_{10}N_2O_2$+ H^+) (Figure 6).

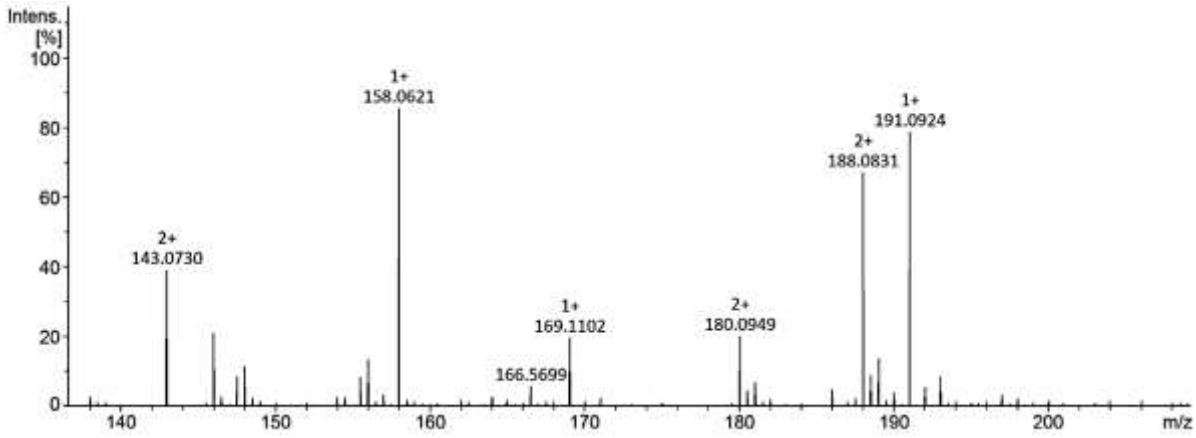


Fig 6. Mass spectrum of compound C3 from *Streptomyces fragilis* strain DA7-7

Thus, the compound C_3 structure was elucidated as 5-amino-4-methoxy isoquinoline-1(2H)-one with molecular formula $C_{10}H_{10}N_2O_2$ and molecular weight as 191.09 (Figure 7).

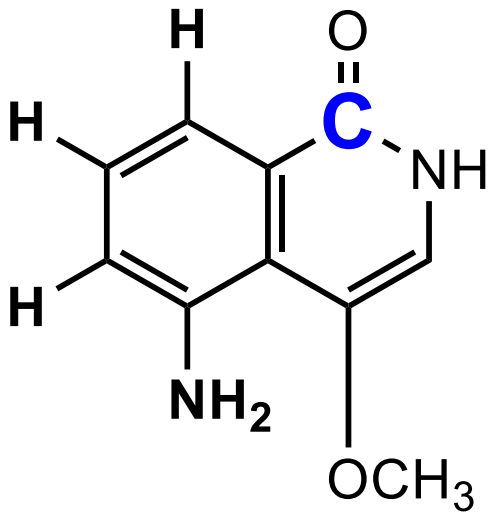


Figure 7. Structure of bioactive compound 5-amino-4-methoxyisoquinolin-1(2H)-one isolated from desert actinobacterium *S. fragilis* strain DA7-7

Further the physical properties of 5-amino-4-methoxyisoquinolin-1(2H)-one was brown in colour, pungent odour, soluble in acetone, ethanol, diethyl ether, poorly soluble in water, melting point 98 °C (Table 1).

Table 1. Characteristics of 5-amino-4-methoxyisoquinolin-1(2H)-one

S.No	Characters	Results
1.	Colour	Pale yellow
2.	Odour	Unpleasant odour
3.	Lustre	Shiny
4.	Stability	Store in dark place
5.	Solubility	Ethanol, methanol, acetone, diethyl ether, Dimethyl sulphoxide

6.	Melting point	27 °C
7.	pH	5-9
8.	Temperature stability	Up to 45°C
9.	IR (cm-1)	3417.16, 3374.48, 2975.33, 1710.27, 1593.21, 1489.60, 1441.45, 1046.45,1372.34, 1263.42, 1087.12, 1006.12, 878.97 752.59, 703.95,
10.	^{1}HNMR (ppm)	3.32, 4.92, 6.64, 7.08, 7.11, 8.72
11.	^{13}CNMR (ppm)	56.12, 115.13, 118.34, 119.35, 122.34, 126.85, 135.02, 142.24, 147.74.
12.	Mass spectrum (m/z)	191.09
13.	Molecular formula	$C_{10}H_{10}N_2O_2$

A number of bioactive compounds have been reported from actinobacteria, especially in the genus *Streptomyces,* including 2-methyl-heptyl isonicotinate;[26] Arylomycins A and B;[27] Himalomycin A and B;[28] 1,5,7-Trihydroxy-3-Hydroxy Methyl Anthraquinone;[29] Methyl substituted □-lactum;[13] 2-Methyl butyl propyl phthalate and Diethyl phthalate;[30] Pyridine-2,5-diacetamide;[8]. Remarkably, isoquinoline alkaloid has recently been reported from *Streptomyces* species.[31,32,33] In addition, the present study also supported that the desert actinobacterium *Streptomyces fragilis* strain DA7-7 produced a novel isoquionline alkaloid analogous compound (5-amino-4-methoxy isoquinoline-1(2H)-one).

3.3 MIC of 5-amino-4-methoxyisoquinolin-1(2H)-one

MIC of 5-amino-4-methoxyisoquinolin-1(2H)-one was determined, the compound exhibited activity against all the test pathogenic bacteria, with MIC values ranging from 15.6 to 62.5 µg/mL. The lowest MIC value was obtained against *E. coli* ATCC 10536 and *E. coli* CIB 008 (15.6 µg/mL) followed by *K. pneumonia* ATCC 12882 (31.2 µg/mL), *E. faecalis* ATCC 49532 (31.2 µg/mL), *P. vulgaris* ATCC 33420 (31.2 µg/mL), *P. vulgaris* CIB 016 (31.2 µg/mL), *P. aeruginosa* ATCC 27883 (31.2 µg/mL), whereas, the highest MIC value was obtained against *S. aureus* ATCC 6538P and *S. aureus* CIB 053 at 62.5 µg/mL. Though, the compound also exhibited activity against pathogenic fungi, the lowest MIC value against *C. neoformans* ATCC 90113 at 31.2 µg/mL, and the rest of other fungi tested, the compound's exhibited the MIC at 62.5 µg/mL (Table 2).

Table 2. Minimum inhibitory concentrations (MIC) of 5-amino-4-methoxyisoquinolin-1(2H)-one against pathogenic microorganisms

Test organisms	**MIC (µg/mL)**	
	5-amino-4-methoxyisoquinolin-1(2H)-one	**Control**
Bacteria		**Streptomycin**
K. pneumonia ATCC 12882	31.2	25
E. faecalis ATCC 49532	31.2	25
E. coli ATCC 10536	15.6	50
E. coli CIB 008	15.6	25
P. vulgaris ATCC 33420	31.2	25
P. vulgaris CIB 016	31.2	25
S. typhimurium ATCC 13311	62.5	50
P. aeruginosa ATCC 27883	31.2	50
S. aureus ATCC 6538P	62.5	25
S. aureus CIB 053	125	25
Fungi		Ketaconazole
C. albicans ATCC 2091	62.5	25
C. alibicans CIF005	62.5	25

C. alibicans CIF009	62.5	50
Candida sp. CIF017	62.5	25
C. neoformans ATCC 90113	31.2	50
S. cerevisiae ATCC 9763	62.5	25

New antimicrobial compounds such as mutamycin C and mutamycin PR have been reported from Algerian desert actinobacterium *Saccharothrix* sp., and these two compounds showed antimicrobial activities against certain gram-positive bacteria in *in-vitro* condition.[34] Saquayamycins A and C have also been reported from *Streptomyces* sp. PAL114 isolated from a Saharan soil of Algeria. MIC of the compounds showed the strongest activities against *Candida albicans* M3 and *Bacillus subtilis* ATCC 6633.[35] In this study, the MICs of 5-amino-4-methoxyisoquinolin-1(2H)-one exhibited the strongest activities against pathogenic bacterium *E. coli* ATCC 10536 and fungus *C. neoformans* ATCC 90113 and also lowest MIC at 15.6 µg/mL and 31.2 µg/mL, respectively were achieved when compared to the standard antibiotics such as streptomycin and ketoconazole.

3.4 Molecular Docking analysis of 5-amino-4-methoxyisoquinolin-1(2H)-one

The antibiotics and the antimicrobial compounds can be classified based on their mechanism of the action, they are; inhibitors of cell wall synthesis, inhibitors of protein synthesis, inhibitors of nucleic acid synthesis and antimetabolites. In general, antibiotics inhibit these routes by interacting with specific cell proteins, usually responsible for defined activity.[36] The docking procedure implicates the prediction of ligand confirmation and binding within the protein. In this study, the binding free energies of the DNA gyrase- Topoisomerase II, undecaprenyl Diphosphate Synthase and IARS with 5-amino-4-methoxyisoquinolin-1(2H)-one was determined by autodock were -4.43, -5.02 and -4.82 kcal/mol, respectively. On the other hand, the binding free energies of streptomycin to DNA gyrase- Topoisomerase II, undecaprenyl Diphosphate Synthase and IARS as -6.13, -3.45 and -5.85 kcal/mol, respectively. The binding free energy difference indicates that 5-amino-4-methoxyisoquinolin-1(2H)-one is able to bind efficiently with undecaprenyl Diphosphate Synthase when compare than that of streptomycin (Table 3).

Table 3. Molecular interaction of 5-amino-4-methoxyisoquinolin-1(2H)-one with target protein obtained from protein data bank (PDB)

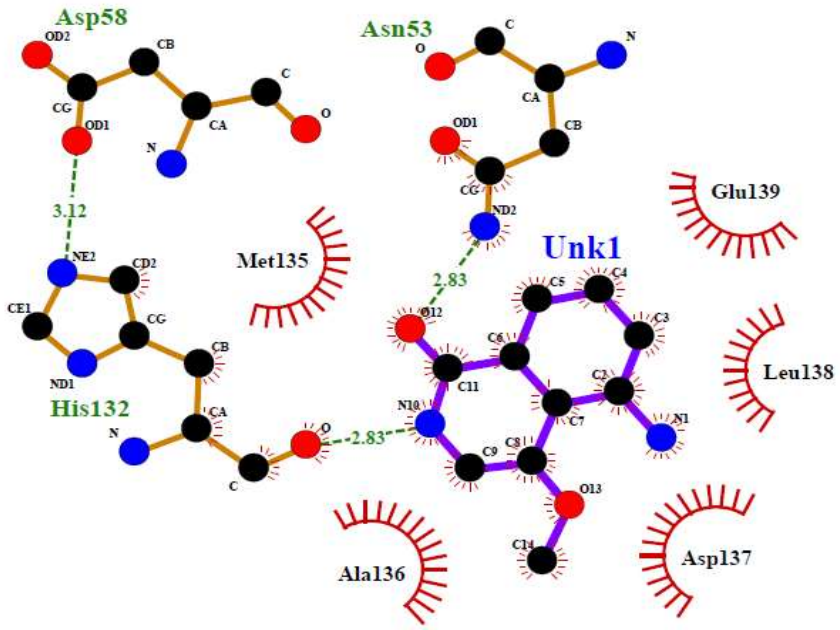
Organism	**Receptors**	**Target**	**PDB ID**	**Binding free energy Kcal/mol**	
				5-amino-4-methoxyisoquinolin-1(2H)-one	**Streptomycin**
Escherichia coli	Undecaprenyl Diphosphate Synthase	Inhibitors of cell wall biosynthesis	4H2M	-5.02	-3.45
	IARS	Inhibitors of protein synthesis	1JZQ	-4.82	-5.85
	DNA gyrase – Topoisomerase II (*E. coli* topoisomerase IV)	Inhibitors of nucleic acid synthesis	1AB4	-4.43	-6.13

Further, LIGPLOT analysis was carried out to study the possible reasons for the decreased binding affinity of 5-amino-4-methoxyisoquinolin-1(2H)-one with DNA gyrase- Topoisomerase II and IARS target structures. The dotted lines and red arc represents the hydrogen and hydrophobic contacts in the complex structures as shown in the figure 5a-c. Undecaprenyl diphosphate Synthase is able to maintain four hydrogen bonds with 5-amino-4-methoxyisoquinolin-1(2H)-one whereas DNA gyrase- Topoisomerase II and IARS is able to maintain only three and one hydrogen bond respectively. On the other hand, more hydrogen bond interaction is involved in the efficient binding process of 5-amino-4-methoxyisoquinolin-1(2H)-one with undecaprenyl Diphosphate Synthase.

Duraipandiyan et al.[29] stated that molecular docking analysis of 1,5,7-trihydroxy-3-hydroxy methyl anthraquinone with DNA topoisomerase IV receptor showed good binding energy of -6.98 (kcal/mol) followed by -5.80 (kcal/mol), -5.27 (kcal/mol) as TtgR and AmpC β-lactamase receptors, respectively. The binding profile of the 1,5,7-trihydroxy-3-hydroxy methyl anthraquinone docked with microbial antigen DNA topoisomerase IV showed 3-CH_2 of ligand interacted with amino acid HIS 120, 5-OH of ligand interacted with amino acid SER 124, 7-OH of ligand interacted with amino acid ASP

78 and 10C=O of ligand interacted with two amino acid SER 124 and ASN 51. In the present study revealed that binding profile of 5-amino-4-methoxyisoquinolin-1(2H)-one docked with DNA gyrase – Topoisomerase II exhibited NH of ligand interacted with two amino acids His132 and Asp 58 and 11C=O of ligand interacted with amino acid Asn53. Whereas, the binding profile of 5-amino-4-methoxyisoquinolin-1(2H)-one docked with Undecaprenyl Diphosphate Synthase revealed NH_2 and OCH_3 of two ligands interacted with one amino acid Vall89, NH of ligand interacted with amino acid Asn 178 and 11C=O of ligand interacted with amino acid Arg 148 (Figure 8a-c).

a)



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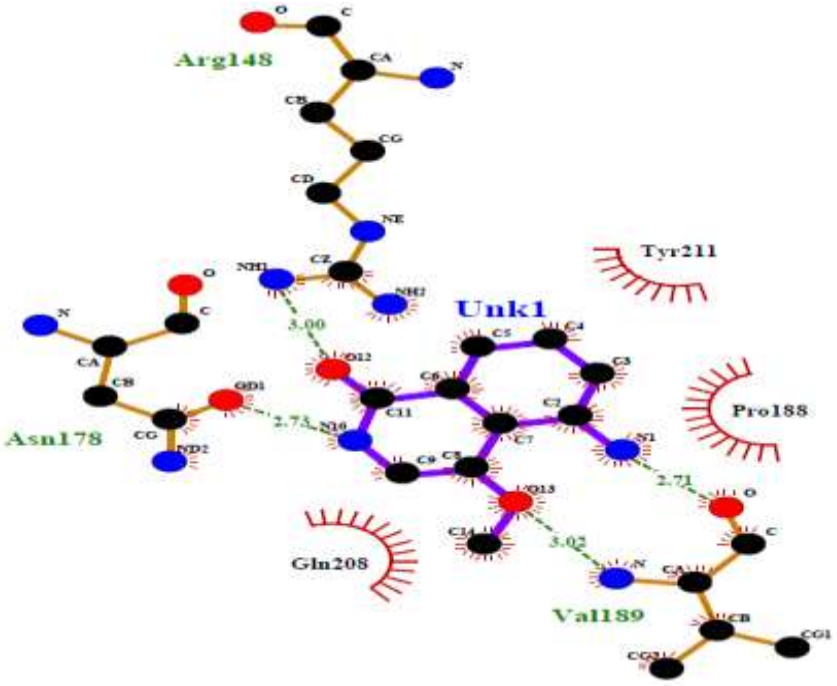

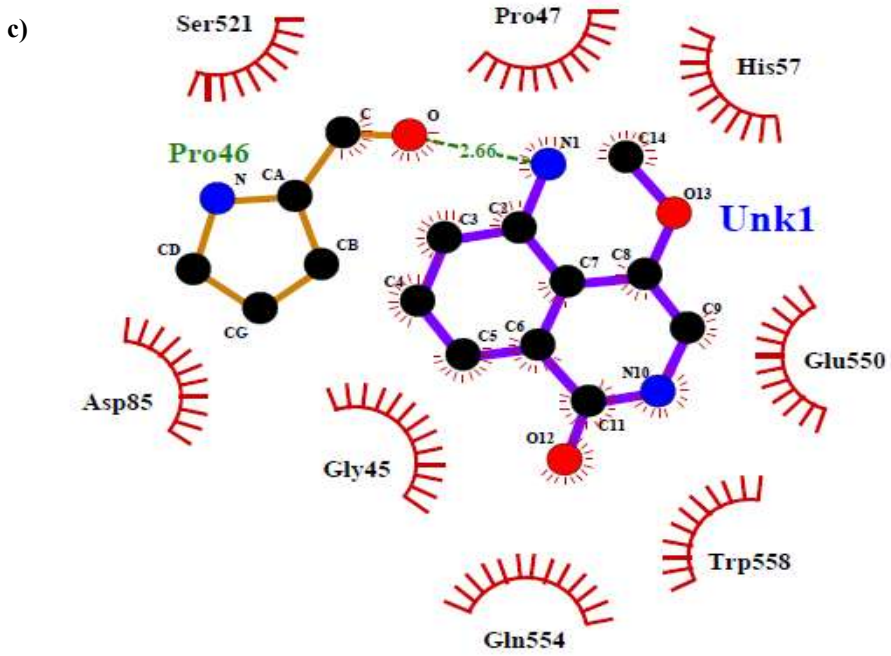

Figure 8. Intermolecular interaction of compound 5-amino-4-methoxy isoquinoline-1(2H)-one with target proteins from pathogenic bacterium E. coli.

a, DNA gyrase-Topoisomerase II (topoisomerase IV); b, (PDB ID 1AB4), Undecaprenyl Diphosphate Synthase (PDB ID 4H2M); c, IARS (PDB ID 1JZQ)

Lipinski rule of 5 for 5-amino-4-methoxyisoquinolin-1(2H)-one was predicated by Lipinski drug filter. The Lipinski rule includes molecular mass less than 500 Da, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, the octanol-water partition coefficient log P not greater than 5 and no more than one number of a violation. The results revealed that 5-amino-4-methoxyisoquinolin-1(2H)-one obeys Lipinski rule of 5 and can be strongly recommended as a drug. The mutagenic rate of the bioactive compound was high compared to streptomycin, even though, there was no carcinogenic effect, reproductive effect and also irritant effect (Table 4).

Table 4. Toxicity analysis of 5-amino-4-methoxyisoquinolin-1(2H)-one

Compound name	Druglikeness	Mutagenic	Tumorigenic	Reproductive Effective	Irritant
5-amino-4-methoxyisoquinolin-1(2H)-one	0.89188	High	None	None	None
Streptomycin	0.83042	None	None	None	High

The milog P of the bioactive compound was found below 5, which indicates that the compound showed good permeability across the cell membrane. TPSA below 160, the number of violation was <1 revealed that the bioactive compound also easily bind to the receptors (Table 5).

Table 5. ADME analysis of 5-amino-4-methoxyisoquinolin-1(2H)-one

Compound name	milogP	TPSA	Natoms	MW	nON	nOHNH	nviolations	nrotb	volume
5-amino-4-methoxyisoquinolin-1(2H)-one	1.14	68.12	14	190.20	4	3	0	1	168.84
Streptomycin	-5.35	336.45	40	581.58	19	16	3	9	497.25

4. CONCLUSION

A novel biologically active compound 5-amino-4-methoxyisoquinolin-1(2H)-one was isolated from desert actinobacterium *Streptomyces fragilis* strain DA7-7 which showed the strongest antibacterial and antifungal properties. The *in silico* antibacterial mechanism and preclinical evaluation revealed that 5-amino-4-methoxyisoquinolin-1(2H)-one is able to bind efficiently with the target protein. Based on the results of *in vitro* antimicrobial potential, *in silico* antibacterial mechanism and the preclinical evaluations, the biologically active compound 5-amino-4-methoxyisoquinolin-1(2H)-one is new natural products from the desert soil ecosystem which could be probed new dimension to antimicrobial natural product research.

DECLARATIONS

Author contributions

Nithya Krishnasamy: Investigation, Data curation, formal analysis, manuscript draft preparation. S. Akilandeswari: Supervision, manuscript editing. Muthukumar Chinnasamy: Conceptualization, writing-review and editing, data curation, formal analysis.

Acknowledgement

The authors KN and SA acknowledge to the management of Dhanalakshmi Srinivasan University to afford the necessary facility.

Conflict of interest statement

The authors declare that no conflict of interest.

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