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Original Article

Broad spectrum antimicrobial activity of dispirooxindolopyrrolidine fused acenaphthenone heterocyclic hybrid against healthcare associated microbial pathogens (HAMPs)

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ABSTRACT

Background: Healthcare-associated infections (HAI) are prime health task worldwide and issue of patient safety besides intensifying antimicrobial drug resistance. It is essential to formulate structurally fascinating novel, active and cost-effective anti-microbial drugs possessing a peculiar way of action and capable of overcoming the resistance to effectively combat this disease.
Materials and methods: The synthesized spiro-heterocyclic hybrids (SHHs) were elucidated through spectroscopic analysis and were assessed for their *in vitro* antimicrobial activity by agar diffusion method and minimal inhibitory concentration (MIC) value was also determined. In addition, antioxidant potential was also evaluated through DPPH radical scavenging assays.
Results: The novel class of SHHs **4a** and **4b** displayed significant antibacterial activity against selected healthcare associated microbial pathogens (HAMPs). In addition, SHH **4b** showed potent antioxidant properties.
Conclusion: Antibacterial and antifungal activity of dispirooxindolopyrrolidine fused acenaphthenone heterocyclic hybrids were examined. Interestingly, SHH **4b** exhibited potent antimicrobial activity against selected HAMPs. Further, these compounds were also showed potent antioxidant properties. These results revealed that SHH **4b** is a promising lead for the development of new antimicrobial drugs.

Introduction

In accordance with Centre for Disease Prevention and Control (CDC) analysis, healthcare associated infections (HAI) increases morbidity and mortality globally. International reports demonstrated the burden of six main HAIs including "urinary tract infection (UTI), surgical site infection (SSI), bloodstream infection (BSI), laboratory-confirmed bloodstream infection or central-line associated bloodstream infection and pneumonia" [1–3]. Besides, HAIs also exists in bones, joints, central nervous system, cardiovascular system, skin and soft tissues. The HAIs are predominantly caused by healthcare associated microbial pathogens (HAMPs) *viz*., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* sp., *Proteus* sp., *Serratia* sp., *Candida albicans* and *Aspergillus niger* [4,5]. However, commercially available antimicrobial drugs on the market have several limitations including toxicities, low effectiveness, intensifying microbial drug resistance and associated with inappropriate drugs consumption [6,7], parenteral administration, extended hospital stay, latent disability, environmental issues, extra health costs [8,9] and occasionally leading to death [10,11]. In addition, it is an important problem, threatening patient safety and infections attained in the health care facilities. HAIs are not only a regional issue, it become a global problem and its effects are massive in terms of mortality, morbidity, disability, psychosocial effects on humanity. These circumstances stimulate an essential need to find structurally diverse novel group of compounds with persistent action, less toxicity and overpowering the drug resistance besides possessing different mechanisms of action from currently existing antimicrobial therapeutic agents.

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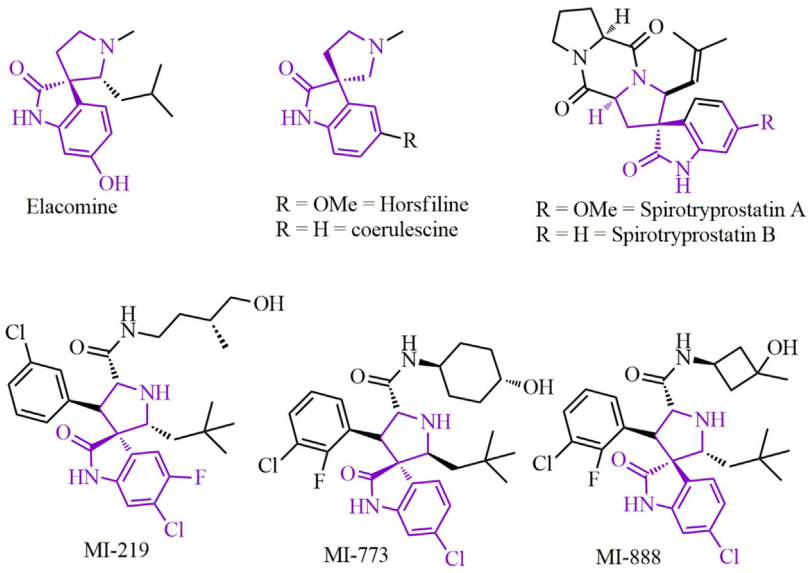


Fig. 1. Biologically relevant spirooxindolo-pyrrolidine analogs.

The combination of more than one biologically significant operational motifs in a single molecule might accomplish multi-drug resistance and this approach has already been demonstrated with admirable outcomes in drug discovery research [12]. In this context, spiro-compounds gains more attention in the view of their wide range of biological and pharmaceutical applications. In particular, spirooxindolopyrrolidine is an attractive structural unit in drug discovery and exists in various inherent alkaloids like elacomine [13] and horsfiline [14] extracted from *Eleagnus commutata* [14] and *Horsfieldia superba* respectively. The spirotryprostatins A and B (Fig. 1) was isolated from *Aspergillus fugimatus* and effectively inhibit G2/M cell cycle of mammalian cells [15,16]. Rhynchophylline and isorhynchophylline isolated from *Uncaria* species were used as antipyretic, anti-hypertensive and anticonvulsant, vertigo and epilepsy [17] besides acting as noncompetitive antagonists of the NMDA receptor [18]. Apart from natural origin, many more synthetic spirooxindolopyrrolidine hybrids possessed various pharmaceutical activities *viz*., anticancer [19,20], antimycobacterial [21], anti-inflammatory, anti-microbial [22], analgesic [23], local anesthetic [24] and AChE and BChE inhibition activities [25–27]. Incidentally, some of the spiroacenaphthenone comprising pyrrolidine/pyrrolizidine heterocyclic hybrids are known to display antimicrobial, anticancer, anti-mycobacterium and cholinesterase inhibitory activities [28–30]. The importance of the aforementioned biologically active scaffolds, prompted us to synthesize the target molecules comprising spirooxindolopyrrolidine and acenaphthenone moieties in a single molecule as this molecular hybridization approach would be of great interest in innovative antimicrobial drug research.

In addition, antioxidants played a vital role in food as a health-protecting factor. The scientific reports recommend that antioxidants diminish the risk of chronic diseases such as cancer and heart diseases. In general, most of the antioxidant compounds in a classic diet is originated from plants and it belongs to different types of compounds with wide variety of physio-chemical features. Further, the absence of more effective antioxidants commercially, prompted the current research to focus and unveil novel heterocyclic units with significant antioxidant properties in the process of drug development [31–33].

In recent past years, several libraries of spiro-heterocyclic hybrids (SHHs) have been synthesized and evaluated for their anticancer, antimycobacterial, antimicrobial and cholinesterase inhibitory activities. Some of these spiro-compounds were found to possess comparable even superior activity than the standard drug. The above precedents encouraged us in the present study to explore the synthesis of novel SHHs including dispirooxindole, pyrrolidine and acenaphthenone motifs employing one-pot three component [3 + 2] cycloaddition reaction and their antimicrobial activity against selected and/or more prevalent healthcare associated bacterial pathogens. Further, the antioxidant activity of compounds has also been investigated.

Materials and methods

*Synthesis of dispirooxindolopyrrolidine fused acenapthenone heterocyclic hybrids **4a**/**4b***

A mixture of electron deficient alkene, (Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene) indolin-2-one (1 mmol), acenaphthylene-1,2-dione (1 mmol), 2-amino-3-phenylpropanoic acid (1 mmol) in methanol was refluxed for 30 min. After completion of the reaction as evidenced by TLC analysis, the solvent was removed under reduced pressure and the crude products were purified by flash column chromatography.

Antibiotic resistance and multiple antibiotic resistance (MAR) index of HAMPs

Antibiotic sensitivity test was accomplished for HAMPs by the Kirby Bauer disc diffusion method as described by Bauer et al. [34]. The list of antibiotics were selected based on microbial pathogens used in the studies as follows; Ampicillin (A) (10 μg), Amoxicillin (Ac) (30 μg), Chloramphenicol (C) (30 μg), Ciprofloxacin (Cf) (5 μg), Clindamycin (Cd) (2 μg), Co-trimoxazole (Co) (1.25 μg), Erythromycin (E) (15 μg), Gentamycin (G), (10 μg), Kanamycin (K) (30 μg), Methicillin (M) (5 μg), Nalidixic acid (Na) (30 μg), Penicillin G (P) (10 units), Streptomycin (S) (10 μg), Tetracycline (T) (30 μg), Vancomycin (V) (30 μg), Fluconazole (FLC) (25 μg), Ketoconazole KT (50 μg), Amphotericin (AP) (20 μg), and Nystatin (NS) (50 μg). A single pure culture was inoculated into 10 ml of nutrient broth (NB) and potato dextrose broth (PDB) and incubated at 37 °C for bacterial pathogens and 30 °C for fungal pathogens, respectively. The broth culture was uniformly inoculated over the Muller Hinton Agar (MHA) plates with sterile cotton swab. Then, each antibiotics disc was positioned in the surface of MHA plates with sterilized forceps permitted for 15 min at room temperature. Later incubation,

the clear zones were measured and diameter of inhibition zone was calculating by mm. All the antibiotic discs and culture medium were procured from HiMedia Pvt Ltd., Mumbai, India.

The multiple antibiotics resistance (MAR) index was estimated for HAMPs against the examined antibiotics according to the method as follow; MAR index = T/(A × P), where T-total number of antibiotic resistant, A-total number of antibiotics used in the study and P-total number of HAMPs. The MAR index value "equal/less than 0.2 was that antibiotics were not used, but if MAR index value was more than 0.2 was considered as an indicator of the high risk of exposure to antibiotics".

*Antimicrobial activity of SHHs **4a** and **4b***

Antibacterial activity of SHH **4a** and **4b** was accomplished by agar well diffusion method described by CLSI, [35]. The selected HAMPs were cultivated in NB were inoculated with microbial culture broth by swabbing using cotton swabs. The compounds **4a** and **4b** of 1 mg/mL was dissolved in Dimethyl Sulfoxide (DMSO). The six mm boreholes were made with help of sterile cork-borer in the inoculated MHA plates. Each well was loaded with different concentration of compounds **4a** and **4b** (15, 25, 50 and 75 μL), positive control (streptomycin 30 μg) and solvent control (DMSO), respectively. All the plates were allowed to imbue for 15 min at room temperature and incubated for 24 h at 37 °C. Later, each MHA plate was examined for the zone formation corresponding to respective tested SHHs. The inhibition zones were noted, recorded in mm. The assay was completed in triplicate.

Antifungal activity

The selected two *C. albicans* and *A. niger* were cultivated in PDB at 30 °C for 36 h. The MHA plates were inoculated with fungal culture by cotton swabbing. The Each 6 mm well was loaded with different concentration of compounds **4a** and **4b** (15, 25, 50 and 75 μL), positive control (nystatin μg) and solvent control (DMSO), respectively. The plates were incubated in 24 h–36 h at 30 °C for *C. albicans* and *A. niger* respectively. After period of incubation, the MHA plates were examined for the zone inhibition against assayed SHHs **4a** and **4b**. The inhibition zones were observed and tabulated. The assay was completed in triplicate.

*Minimum inhibitory concentrations (MIC) determination of SHH **4b***

The MIC was determined by broth micro dilution method according to procedure of CLSI, [36]. Twofold serial dilutions of SHH **4b** was prepared directly into 96 well titer plate comprising Mueller Hinton broth (MHB) to attain different concentrations. The tested HAMPs were inoculated into final concentration of 5×10^5 CFU/mL in each well. Streptomycin and nystatin were used as a positive control for bacterial and fungal pathogens respectively. The sealer enclosed 96 well titer plate was incubated at 37 °C, 18 h for bacterial pathogens and at 30 °C, 24 h for fungal pathogens. After incubations, the wells containing the turbidity of microbial bacterial growth was examined and the MIC was measured as the least volume of the compound **4b** that absolutely constrains the growth of microbial pathogens.

*DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay of hybrid compounds **4a** and **4b***

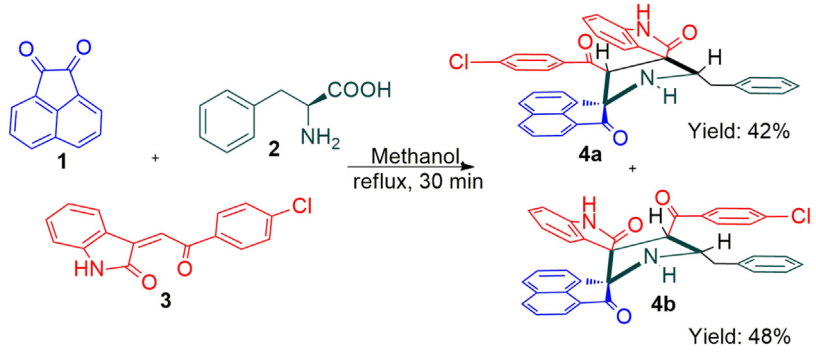
The DPPH free radical scavenging activity of the heterocyclic compounds **4a** and **4b** was determined by DPPH method [37]. Briefly, it was evaluated by absorbance at 516 nm of coloured DPPH. The 10 mg/10 ml concentration of compound **4a** and **4b** was prepared in dimethyl sulfoxide (DMSO). After this, one ml was diluted up to 10 ml. From the stock solution 0.10, 0.20, 0.40, 0.60, 0.80, and 1.00 ml was taken in different tubes, whose concentration was 10, 20, 40, 60, 80, and 100 μg/mL, respectively. 75 μL of DPPH solution (1.3 mg/mL) was added to each test tubes and kept in dark for 30 min, then the absorbance was read at 516 nm with UV–vis spectrophotometer and IC50 was calculated from percentage of inhibition. The ascorbic acid as a reference control (positive control). The DPPH free radical scavenging activity was determined by the formula as follows; % inhibition = [(Control − Sample)/(Control)] × 10. Here, Control – absorbance of DPPH alone, Sample – absorbance of DPPH with sample.

Molecular docking study

Computational molecular docking studies were executed to characterize the molecular interaction on the preferred structural pose on the antimicrobial target protein of outer membrane protein 1IWN. The molecular complex has been predicted the best orientation to form the stable complex to have better interaction Gscore and energy value. Computational ligand interaction calculation was simulated using Schrodinger 12.1 software. The ligand molecule of **4b** structure was drawn in chemdraw software optimized. The selective ligand and macromolecule of protein were minimized using Ligprep and protein preparation module, which can generate stable conformation of molecules based on the molecular mechanics and biochemical optimization. The protein structure flexible docking grid was predicted based on the PDB database existed active binding site with ratio of x = 48A y = 62A z = 65A along with ligand interaction grid ratio between 20A.

Results and discussion

The target dispiropyrrolidine analogs, *viz.* 5-benzyl-4-chlorobezoyl-spiro[2.1′] acenapthyleno-spiro[3.3″]indolinopyrroline



Scheme 1. Synthesis of spirooxindolopyrrolidine fused acenaphthenone heterocyclic hybrids, **4a/4b**.

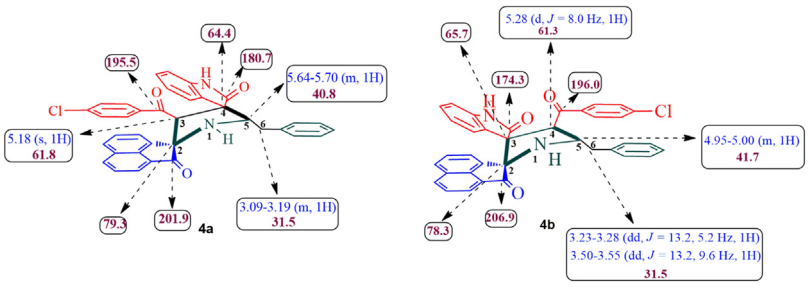
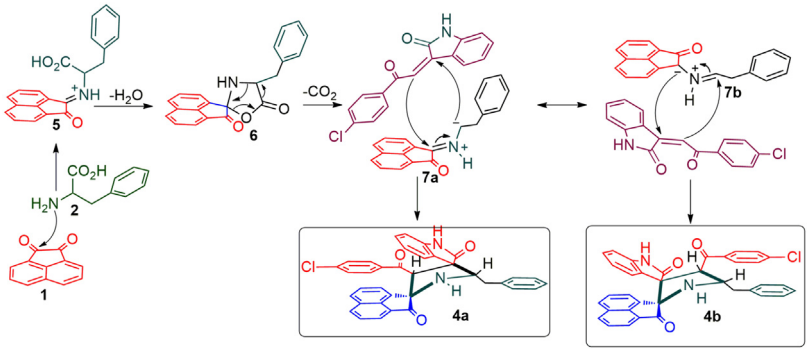


Fig. 2. Selected chemical shift of **4a** and **4b**.



Scheme 2. Mechanism for the formation of dispirooxindolopyrrolidine fused acenaphthenone hybrids.

4a and 5-benzyl-4-chlorobezoyl-spiro [2.1′]acenapthyleno-spiro[4.3″]indolinopyrroline **4b** were synthesized employing a one-pot three component cycloaddition methodology as outlined in Scheme 1. The required dipolarophile, (Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene) indolin-2-one **3** was prepared from isatin and 1-(4-chlorophenyl) ethanone in presence of a base according to the literature report [38]. The three component [3 + 2]-cycloaddition reaction of **3** with azomethine ylide generated *in situ* from non-enolizable ketone and α-amino acid afforded the target compounds in good yields. Thus, an equimolar mixture of (Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene) indolin-2-one **3,** acenapthenequinone **1** and 2-amino-3-phenylpropanoic acid **2** in methanol afforded the functionalized spirooxindolo-pyrrolidine fused acenaphthenones **4a** and **4b** (Scheme 1). The progress of the reaction was monitored by TLC intermittently. After completion of the reaction (30 min), as evident from TLC, the solvent was removed under reduced pressure and the crude product was purified by column chromatography to afford compounds **4a** and **4b** in good yields. The non-stabilized 1,3-dipole component derived from acenapthenequinone **1** and 2-amino-3-phenylpropanoic acid **2** is relatively less explored in the literature. The structure of spirooxindolopyrrolidine tethered acenaphthenone regioisomers was confirmed by ^{1}H, ^{13}C NMR spectroscopic, mass spectrometry and elemental analysis. A pictographic representation of the ^{1}H and ^{13}C-NMR chemical shifts of **4a** and **4b**is described in Fig. 2.

The plausible reaction pathway for the formation of spirooxindolopyrrolidine fused acenaphthenone hybrids **4a/4b** has been described in Scheme 2. Firstly, the amino acid **2** attacks the non-enolizable ketone **1** to form the intermediate imine **5** which upon spontaneous elimination of H_2O molecule affords spirooxazolidinone intermediate **6**. The spirooxazolidinone **6** generates *in situ the* 1,3-dipole components **7a** and **7b** by elimination of CO_2. The 1,3-dipole **7a** reacts with the activated double bond of dipolarophile **3** to form compound **4a**. Similarly, other possible 1,3-dipole **7b** attacks compound **3** to furnish compound **4b**.

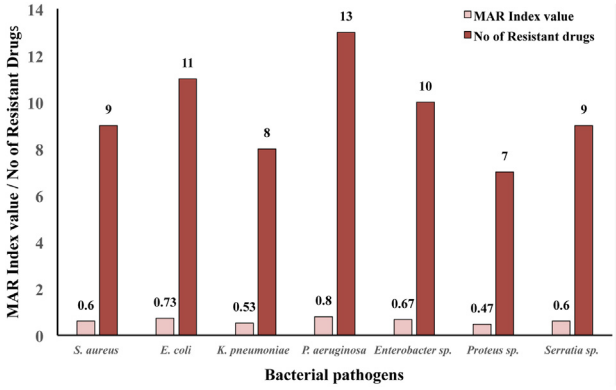


Fig. 3. MAR index value and no. of resistant antibiotics against bacterial pathogens.

Multiple antibiotic resistance (MAR) index of HAMPs

A total of seven bacterial and two fungal clinical pathogens were tested for multiple antibiotics resistance. The HAMPs showed different sensitivity patterns to antimicrobial drugs. Remarkably, among the tested nine microbial pathogens, four isolates were resistant to more than nine antibiotics, while the one strain *P. aeruginosa* showed resistant to thirteen tested antibiotics. In the case of fungal pathogens, *C. albicans* exhibited resistant to three antifungal drugs. The MAR index was calculated and presented in Table 1 and Fig. 3. The results showed that high level of drug resistance existed among all the HAMPs against different types of antimicrobial drugs. In general, the results propose that HAMPs be a reservoir for multiple antibiotics resistance owing to the pervert of drugs among patients [39]. These reports are in accordance with previous studies that MDR strains were exists more recurrently acquired from South West Nigeria tertiary hospitals [40].

Table 1
Multiple antibiotics resistance index of the HAMPs.

Microbial pathogens	MAR[a] index value	No of resistant antibiotics	Name of the resistant antibacterial antibiotics[b] (T = 15)
Bacterial pathogens			
S. aureus	0.60	9	A, Ac, C, Cd, Cf, G, M, P, V
E. coli	0.73	11	A, Ac, C, Cd, E, G, K, M, P, T, V
K. pneumoniae	0.53	8	A, C, Co, E, K, P, S, V
P. aeruginosa	0.80	13	A, Ac, C, Cd, Cf, E, G, K, M, P, S, T, V
Enterobacter sp.	0.67	10	A, Ac, C, E, G, K, M, P, T, V
Proteus sp.	0.47	7	Ac, C, E, K, P, T, V
Serratia sp.	0.60	9	A, C, E, G, K, M, P, S, V
Fungal pathogens			Name of the resistant antifungal antibiotics[c] (T = 4)
C. albicans	0.75	3	FLC, KT, NS
A. niger	0.50	2	FLC, KT

[a] MAR index value near 1 indicates the antibiotics are resistant.
[b] Antibacterial antibiotics used: Ampicillin (A) (10 μg), Amoxicillin (Ac) (30 μg), Chloramphenicol (C) (30 μg), Ciprofloxacin (Cf) (5 μg), Clindamycin (Cd) (2 μg), Cotrimoxazole (Co) (1.25 μg), Erythromycin (E) (15 μg), Gentamycin (G), (10 μg), Kanamycin (K) (30 μg), Methicillin (M) (5 μg), Nalidixic acid (Na) (30 μg), Penicillin G (P) (10 units), Streptomycin (S) (10 μg), Tetracycline (T) (30 μg), Vancomycin (V) (30 μg).
[c] Antifungal antibiotics used: Fluconazole (FLC) (25 μg), Ketoconazole KT (50 μg), Amphotericin (AP) (20 μg), Nystatin (NS) (50 μg).

Table 2
Antimicrobial activity of dispiropyrrolidines **4a** and **4b** against HAMPs.

Microbial pathogens	Concentration (μg)/zone of inhibition (mm)						
	Compound 4a[a]		Compound 4b				S[b] (10 μg)
	50	75	15	25	50	75	
Bacterial pathogens							
S. aureus	12.00 ±0.10	16.00 ±0.50	9.00 ±0.15	13.00 ±0.10	18.00 ±0.10	21.00 ±0.10	24.00
E. coli	15.00 ±0.05	21.00 ±0.70	11.00 ±0.08	15.00 ±0.10	20.00 ±0.10	24.00 ±0.10	28.00
K. pneumoniae	13.00 ±0.03	17.00 ±0.40	10.00 ±0.20	14.00 ±0.10	19.00 ±0.10	22.00 ±0.10	19.00
P. aeruginosa	–	12.00 ±0.08	7.00 ±0.07	10.00 ±0.10	14.00 ±0.10	17.00 ±0.10	18.00
Enterobacter sp.	–	13.00 ±0.10	8.00 ±0.30	11.00 ±0.10	15.00 ±0.10	18.00 ±0.10	21.00
Proteus sp.	11.00 ±0.04	18.00 ±0.20	11.00 ±0.09	12.00 ±0.10	17.00 ±0.10	19.00 ±0.10	24.00
Serratia sp.	12.00 ±0.15	15.00 ±0.50	10.00 ±0.25	14.00 ±0.10	18.00 ±0.10	21.00 ±0.10	19.00
Fungal pathogens							AP[c] (20 μg)
C. albicans	8.00 ±0.08	11.00 ±0.40	9.00 ±0.15	13.00 ±0.15	18.00 ±0.15	22.00 ±0.15	24.00
A. niger	–	10.00 ±0.15	10.00 ±0.15	14.00 ±0.15	19.00 ±0.15	23.00 ±0.15	26.00

[a] No activity in 15 and 25 μg concentrations.
[b] Streptomycin.
[c] Amphotericin (standard antibiotics).

*Antimicrobial activity of spirooxindolopyrrolidine fused acenaphthenone heterocyclic hybrids **4a** and **4b***

The dispiropyrrolidines **4a** and **4b** thus synthesized were evaluated for their *in vitro* antibacterial activity against seven HAMPs such as *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, Enterobacter* sp., *Proteus* sp. by agar well diffusion method. It is pertinent to note that most of the HAIs were caused by these bacterial pathogens. Among the two synthesized SHHs, **4b** possessed a significant antimicrobial activity against all tested pathogens (Table 2 and Fig. 4). The results indicate that compound **4b** was more efficient than compound **4a**. The maximum inhibition zone was observed for compound **4b** at low concentration against four bacterial pathogens such as *S. aureus*, *E. coli*, *K. pneumoniae, Proteus* sp., and *Serratia* sp., whereas the minimum zone of inhibition was observed against two bacterial pathogens *viz*., *P. aeruginosa*, *Enterobacter* sp., and antibacterial activity of SHH **4b** was summarized in Table 2. The promising antibacterial results encouraged us to eval-

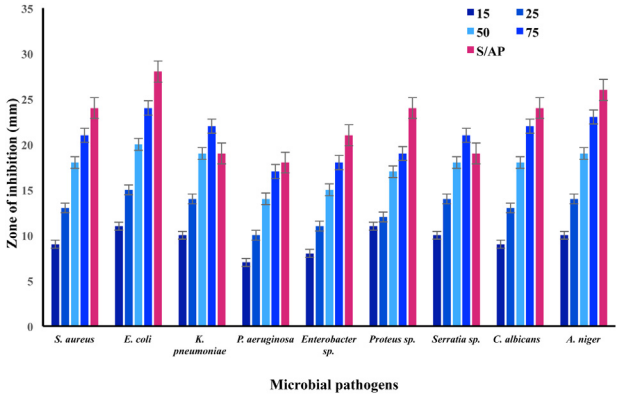


Fig. 4. Antimicrobial activity of hybrid compound **4b** against HAMPs. (*Note*: 15, 25, 50 and 75 – different concentration of compound **4b**; S/AP – standard antibiotics).

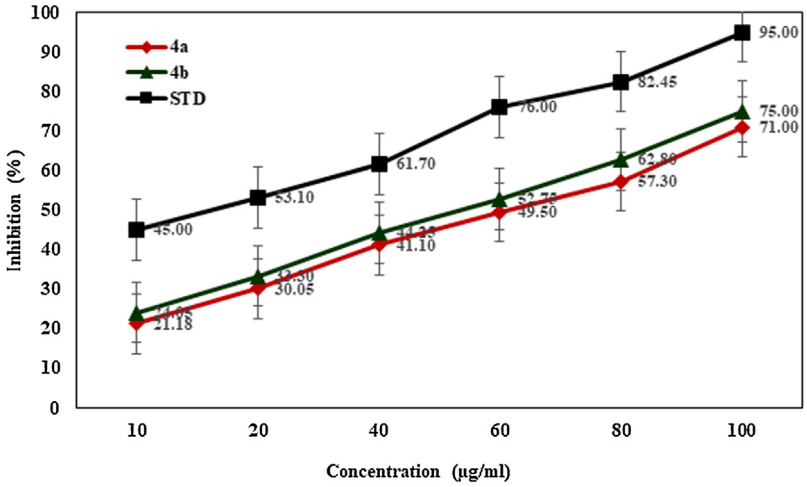


Fig. 5. DPPH radical scavenging activity of heterocyclic hybrids **4a** and **4b** with standard.

Table 3
MIC values of dispiropyrrolidine **4b** against selected HAMPs.

Microbial pathogens	MIC value (μg)	
	Compound **4b**	Streptomycin
Bacterial pathogens		
S. aureus	250.00	15.00
E. coli	31.25	5.00
K. pneumoniae	62.50	10.00
P. aeruginosa	250.00	15.00
Enterobacter sp.	125.00	10.00
Proteus sp.	62.50	5.00
Serratia sp.	62.50	5.00
Fungal pathogens		Amphotericin
C. albicans	250.00	10.00
A. niger	62.50	5.00

Table 4
The DPPH radical scavenging activity of heterocyclic hybrids **4a** and **4b** and standard (ascorbic acid).

Concentration (μg/mL)	% inhibition		% inhibition
		STD	
	4a	4b	
10	21.18 ± 0.05	24.05 ± 0.40	45.00 ± 1.05
20	30.05 ± 0.20	33.30 ± 0.05	53.10 ± 0.08
40	41.10 ± 0.50	44.25 ± 0.15	61.70 ± 0.20
60	49.50 ± 0.40	52.75 ± 0.35	76.00 ± 0.85
80	57.30 ± 0.35	62.80 ± 0.40	82.45 ± 0.60
100	71.00 ± 1.00	75.00 ± 0.85	95.00 ± 0.50

uate their antifungal activity against two fungal pathogens *i.e.*, *C. albicans*, *A. niger* and the results obtained were presented in Table 2 and Fig. 4. Among the tested fungal pathogens, *A. niger* is more susceptible against compound **4b**, whereas the *C. albicans* displayed minimum zone of inhibition.

*Minimal inhibitory concentration (MIC) determination of compound **4b***

The effectiveness of compound **4b** was determined by MIC, by following the methods described by Andrews [41]. MIC was performed only for SHH **4b** as it exhibited maximum zone of inhibition against tested pathogens in the antimicrobial activity assay (Table 2). The SHH **4b** displayed significant MIC values against *S. aureus* (250.00 μg/mL), *E. coli* (31.25 μg/mL), *K. pneumoniae* (62.50 μg/mL), *P. aeruginosa* (250.00 μg/mL), *Enterobacter* sp., (125.00 μg/mL), *Proteus* sp., (62.50 μg/mL), and *Serratia* sp., (62.50 μg/mL) respectively. However, the MIC values against fungal pathogens were *C. albicans* (250.00 μg/mL) and *A. niger* (62.50 μg/mL). The comprehensive MIC values of compound **4b** against tested microbial pathogens are presented in Table 3. The overall antimicrobial activity of the synthesized SHHs might be due to the presence of biologically active spirooxindole, acenaphthenone and pyrrolidine substituents in the compound.

*DPPH free radical scavenging assay of hybrid compounds **4a** and **4b***

The synthesized dispiropyrrolidines **4a** and **4b** were also evaluated for their DPPH free radical scavenging assays and observed that both the heterocyclic hybrids exhibited almost equivalent antioxidant activity which is comparable to the standard, ascorbic acid at different concentrations as shown in Table 4 and Fig. 5. The antioxidant activity is concentration dependent and not for time dependent. The standard ascorbic acid exhibited percentage of 45.00 + 1.05 and 95.00 + 0.50 towards 10 and 100 μg/mL respectively. While, the heterocyclic compounds showed 21.18 + 0.05 ad 24.05 + 0.40 percentage of inhibition with respect to compounds **4a** and **4b**. It was observed that the compound **4b** showed significant DPPH free radical scavenging property than compound **4a**.

Molecular docking study

The ligand **4b** was docked in to the glide ligand based binding site of 1IWN. The glide SP and XP flexible docking studies frequently applied. The XP docking is provided extra precision and write descriptor information generates favorable ligand poses which were further screened through filters to examine the spatial fit of the ligands in the active site. The best interactive pose of the **4b** ligand pose was confirmed based on the Gscore, energy score and biochemical bonding. The ligand interactive confirmation pose was shown in Fig. 6. The computational molecular docking Gscore was predicted as −7.856 kcal/mol. The binding affinity and the amino acid residues of 1IWN complex with energy score of −39.483. The molecular docking participated in the interactions with **4b** given in Table 5.

Conclusion

In the current study, antimicrobial and antioxidant activities of SHHs **4a** and **4b** were determined and the outcomes displayed

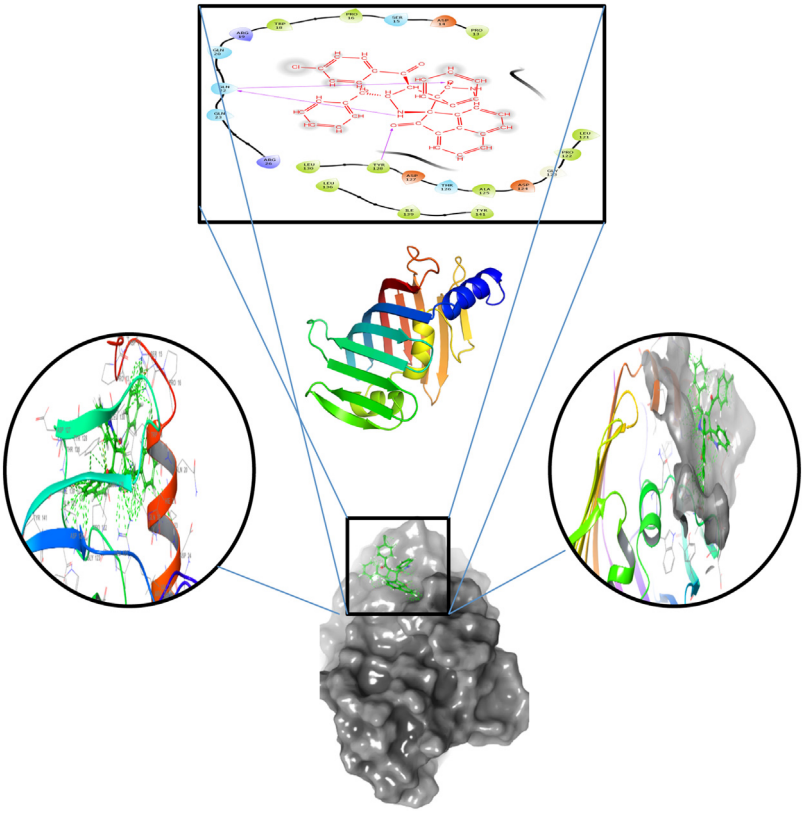


Fig. 6. Binding interactions of compound represented by ligand interaction plot 3D protein molecular cartoon structure with secondary structural feature. The residues shown in 2D plot represent the name of amino acid, positional number, respectively. Pink color dashed lines connectivity indicates hydrogen bonds.

Table 5
Molecular docking data of compound 4b with 1IWN antimicrobial protein receptors.

Complex	Binding affinity (kcal mol^{-1})	Residual interactions
1IWN (4b)	−7.856	GLN 22 (2) TYR 128

prospective antimicrobial efficiency against tested selected HAMPS and also significant antioxidant activities more specifically with compound **4b**. Further, the compound **4b** exhibited maximum competent activity against the bacterial pathogens *E. coli*, *Serratia* sp., *K. pneumoniae* and *S. aureus*, and more efficient antifungal activity against *C. albicans* and *A. niger*. The MIC value was observed from 31.25 to 250.00 μg/mL. Further, these investigations inferred that physiochemical and pharmacological properties are necessary to move these molecules to the next stage in the drug development pipeline.

Competing interests

None declared.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jiph.2020.09.016.

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