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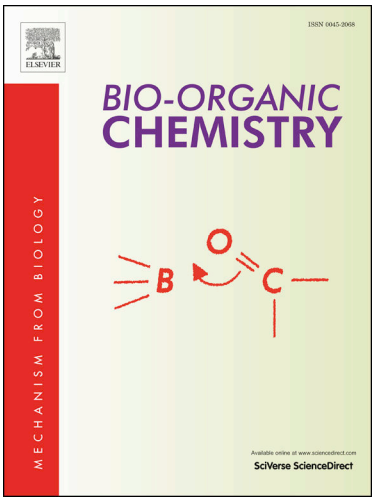

Synthesis of substituted *N*-(2'-nitrophenyl)pyrrolidine-2-carboxamides towards the design of proline-rich antimicrobial peptide mimics to eliminate bacterial resistance to antibiotics

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PII: S0045-2068(20)31638-2
DOI: https://doi.org/10.1016/j.bioorg.2020.104340
Reference: YBIOO 104340

To appear in: *Bioorganic Chemistry*

Received Date: 8 July 2020
Revised Date: 29 September 2020
Accepted Date: 30 September 2020

Please cite this article as: J.A. Odusami, M.I. Ikhile, J.U. Izunobi, I.A. Olasupo, F.O. Osunsanmi, A.R. Opoku, M. C. D. Fotsing, O.T. Asekun, O.B. Familoni, D.T. Ndinteh, Synthesis of substituted *N*-(2'-nitrophenyl)pyrrolidine-2-carboxamides towards the design of proline-rich antimicrobial peptide mimics to eliminate bacterial resistance to antibiotics, *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.104340

Synthesis of substituted *N*-(2'-nitrophenyl)pyrrolidine-2-carboxamides towards the design of proline-rich antimicrobial peptide mimics to eliminate bacterial resistance to antibiotics

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Abstract

The treatment of diseases is under threat due to the increasing resistance of disease-causing bacteria to antibiotics. Likewise, free radical-induced oxidative stress has been implicated in several human disease conditions, such as cancer, stroke and diabetes. In the search for amino acid analogues with antibacterial and antioxidant properties as possible mimics of antimicrobial peptides, substituted *N*-(2'-nitrophenyl)pyrrolidine-2-carboxamides **4a**–**4k** and *N*-(2'-nitrophenyl)piperidine-2-carboxamides **4l**–**4n** have been synthesized via a two-step, one-pot amidation of the corresponding acids, using thionyl chloride with different amines in dichloromethane. The carboxamides were characterized by infrared and nuclear magnetic resonance spectroscopy, mass spectrometry and elemental analysis.

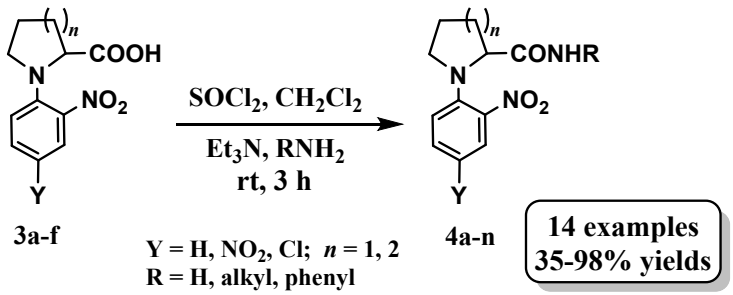
Carboxamides **4a**–**4n** were assayed against five Gram-positive and five Gram-negative bacterial strains using the broth micro-dilution procedure and compared to standard antibiotic drugs (streptomycin and nalidixic acid). **4b** showed the highest antibacterial activity with a minimum inhibitory concentration (MIC) value of 15.6 µg/mL against *Staphylococcus aureus*. Pertinently, **4b** and **4k** are promising candidates for narrow-spectrum (Gram-positive) and broad-spectrum antibiotics, respectively.

The antioxidant properties of the carboxamides were also evaluated using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical and 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation. **4a** and **4k** recorded the lowest IC_{50} values of 1.22 x 10^{-3} mg/mL (with DPPH) and 1.45 x 10^{-4} mg/mL (with ABTS), respectively. Notably, **4k** recorded about 2.5 times better antioxidant capacity than the positive controls – ascorbic acid and butylated hydroxyanisole.

These results bode well for *N*-aryl carboxamides as good mimics and substitutes for antimicrobial peptides towards mitigating bacterial resistance to antibiotics as well as ameliorating oxidative stress-related diseases.

Keywords: antibacterial, antioxidant, piperidine-2-carboxamides, prolinamides, structure–activity relationship.

Graphical Abstract:



Highlights:

- New substituted *N*-(2'-nitrophenyl)pyrrolidine-2-carboxamides **4a**–**4k** and *N*-(2'-nitrophenyl)piperidine-2-carboxamides **4l**–**4n** have been synthesized and characterized.
- An S_N2-type mechanism has been proposed and enumerated.
- *N*-(2',4'-Dinitrophenyl)pyrrolidine-2-carboxamide **4b** gave an MIC of 15.6 μg/mL against *Staphylococcus aureus* relative to streptomycin (256 μg/mL) and nalidixic acid (64 μg/mL) standards.
- *N'*-Phenyl-*N*-(4'-chloro-2'-nitrophenyl)pyrrolidine-2-carboxamide **4k** recorded about 2.5 times better antioxidant capacity than the positive controls, ascorbic acid and BHT (IC_{50}: 3.6 x 10^{-4}; ABTS) with an IC_{50} of 1.45 x 10^{-4} mg/mL (in ABTS) and optimal percentage scavenging activity of 45% (in DPPH).

1.0. Introduction

The world of medicine has not been the same since almost a century ago when Sir Alexander Fleming serendipitously discovered the first antibiotic, penicillin. Widespread use of antibiotics and the permeation of antibiotics into food chains have, however, encouraged the enormous and growing threat of bacterial pathogens with multidrug resistance; leading to many more human infections [1]. World Health Organization (WHO) reports that the escalating resistance of bacteria to antibiotics is a strong threat to global health, food security and development, and a source of serious concern to many people irrespective of country, age or race [2]. Antibiotic resistance is also implicated in longer hospital stays, higher medical costs and increased mortality and morbidity. Current treatments for bacterial infections are therefore increasingly becoming inadequate, making the development of new classes of antimicrobial agents urgent and paramount [3].

An emerging complementary approach to eradicating bacterial resistance is the application of naturally occurring or synthetic host-defense peptides (HDPs) [4–6]. HDPs, also referred to as antimicrobial peptides (AMPs) for their antimicrobial activity, are widely recognized for their multifunctional roles in the innate and adaptive immune responses. Their immunomodulatory capabilities include the modulation of pro- and anti-inflammatory responses, enhancement of extra- and intra-cellular bacterial killing, chemo-attraction, cellular differentiation and activation of the innate and adaptive compartments, wound-healing and the modulation of autophagy as well as apoptosis and pyroptosis [7]. AMPs are produced by most multicellular organisms as a component of the innate immune system and, in comparison to traditional antibiotics, have a different antibacterial mechanism, making it difficult for bacteria to develop resistance to them [8]. In addition, AMPs have broad spectrum activities to directly kill bacteria, yeasts, fungi, viruses and even cancer cells [9].

The ability of AMPs to kill bacteria usually depends on their ability to interact with bacterial cell walls or membranes. AMPs are amphipathic and exhibit a net positive charge and high ratio of hydrophobic amino acids; allowing them to selectively bind to negatively charged bacterial cells to effect non-enzymatic disruptions [10,11]. AMPs also display high diversity in structure (e.g., α-helical, β-stranded, β-hairpin and extended structures), and some peptides are reported to be able to cross the lipid bilayer without causing any damage but kill bacteria by inhibiting intracellular functions, such as blocking enzyme activity or inhibiting protein and nucleic acid synthesis [9]. In addition to direct antimicrobial activities, some AMPs are also able to inhibit biofilm formation and disrupt existing biofilms [5,12]. Other advantages of AMPs include their anti-inflammatory activity, neutralization of virulence factors and slow resistance development [13].

AMPs have been identified at most sites of the human body exposed to microbes, such as the skin, intestinal mucosa, oral mucosa, lung, eye and reproductive tract. It is now clear that most of these peptides are induced during inflammation, injury or infection whereas AMPs are typically constitutively expressed. The specific sites of expression and strict regulation of AMP expressions are key to understanding how they work. This may, therefore, explain why AMPs, as evolutionarily ancient gene products, remain effective antibiotics unlike pharmaceutically derived antibiotics, which can be rapidly negated due to bacterial resistance [14]. It has been shown that AMPs can be

absorbed onto the negative bacterial surface by electrostatic interaction, with the hydrophobic amino acid residues inserting into the lipid bilayer and inducing the formation of pores on the bacterial membrane [15,16].

Most natural AMPs suffer from poor proteolytic stability, bioavailability and cell selectivity due to their peptidic nature thereby limiting their therapeutic applications. They also sometimes cause hemolysis. Consequently, it is often necessary to increase sequence diversity and introduce unnatural D-amino acids or *β*-amino acids, to improve proteolytic stability [17], and conjugate the AMPs to sugars, lipids and proteins as well as employ the use of polyvalent peptide synthesis [18].

It is instructive that microbes produce various AMPs, to limit the growth of other micro-organisms, which are quite distinct from vertebrate antimicrobial peptides as they can be synthesized from non-ribosomal peptide synthase [9,19]. Non-ribosomal peptides often have cyclic or branched structures and can contain non-proteinogenic amino acids, including D-amino acids. They can also be homologated at the ring nitrogen atom (e.g., *N*-methyl and *N*-formyl groups) as well as glycosylated, halogenated or hydroxylated. The glycopeptide, vancomycin; produced by *Amycolatopsis orientalis*, nisin; produced by *Lactococcus lactis* [20], (*cf*. Figure 1) and polymyxin B; produced by *Bacillus polymyxa*, are good examples of antimicrobial peptides, produced by microbes, which are FDA-approved antibiotics [9].

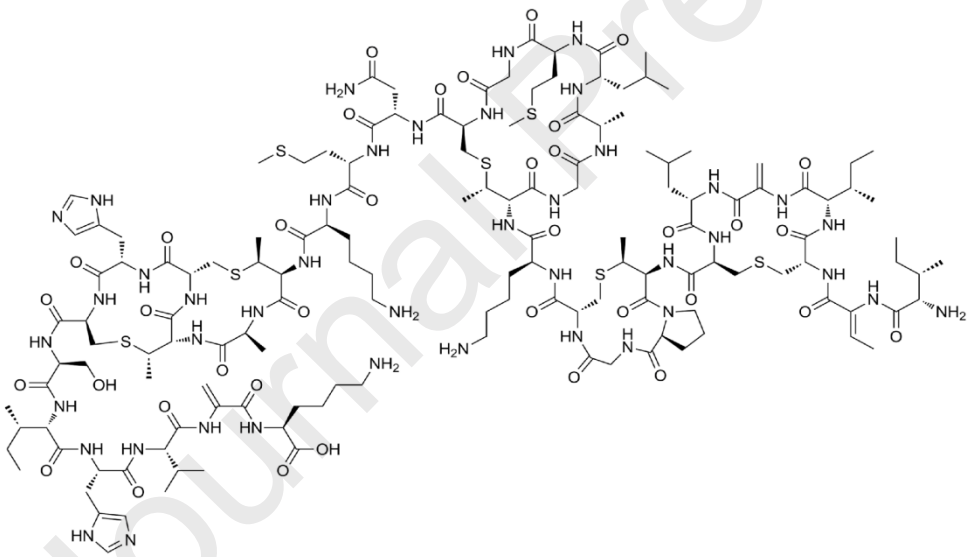


Figure 1: Structure of Nisin [20], an FDA-approved natural AMP, from *Lactococcus lactis* (Gram-positive bacterium)

AMPs with antioxidant properties have also been reported [21]. Antioxidants are important in protecting cells by inhibiting oxidation processes in the body. Oxidation is a vital process in living organisms, which produces free radicals as its side effect. Free radicals initiate reactive species, such as reactive oxygen (ROS) and nitrogen (RNS), with molecules in the body thereby starting a chain reaction (oxidative stress), which lead to many diseases, such as cancer, stroke, arteriosclerosis, diabetes and heart diseases, etc. [22]. Conversely, at low or moderate concentrations, free radicals also play beneficial roles in the wellbeing of organisms. Oxidative

stress has physiological roles, in cellular signaling, for example, and is an important component of the immune system's response to pathogenic microbes [23]. Notably, antioxidants decrease the adverse effects of high reactive species' concentrations on normal physiological functions. They are typically able to delay, retard or prevent auto-oxidation processes [24].

Studies have shown that the antioxidant activities of peptides are dependent on their constituting amino acids, molecular weights and primary structures [25]. They act as metal chelators, active-oxygen quenchers and hydroxy radical scavengers. The antioxidant properties exhibited by amino acids in some systems are also reflective of their different RNH_3^+ moieties. It is also significant to note that the antioxidant activity of amino acids with aromatic and hydrophobic groups have been attributed to their capacity to act as radical scavengers and inhibit lipid peroxidation, respectively.

Synthetic peptides based on naturally occurring AMPs offer potentials for the design of novel drug candidates, where key attributes like positive charge, hydrophobicity and amphipathic structures are linked to the antimicrobial potency of peptide candidates. Synthetic peptides are considered safe because of their pre-determined amino acid sequences, which can be obtained quickly in high purity though they can be susceptible to proteases and rapidly eliminated by the body. Nevertheless, various strategies have been designed to overcome these drawbacks. Thus, in the design of new synthetic peptides, it is imperative to maintain biological activity and address issues pertaining to lability, toxicity and aggregation [6].

We recently reported on the antibacterial evaluation of arylated L-proline and D,L-pipecolinic acid as potential drug candidates for eradicating antibiotic-resistant bacteria [26]; as members of the proline-rich antibacterial peptide family (pyrrhocoricin, apidaecin and drosocin) are reported to kill responsive bacterial species by binding to the multihelical lid region of the bacterial DnaK protein [27]. Piperidine-based broad-spectrum antipathogens [28] and proline-rich natural AMPs are also known [29,30]. In continuation of our quest for small molecules mimicking some properties of AMPs, we have elaborated arylated L-proline and D,L-pipecolinic acid by amidation, in the mode of amide-terminal natural AMPs [31,32], such as indolicidin [33].

Moreover, pyrrolidinyl carboxamide-containing compounds are of immense biological and therapeutic importance [34]. They have also found use as reverse-turn mimetics [35], ligands and asymmetric organocatalysts [36], and in the treatment of cancer [37] and hepatitis C [38]. Reported herein, therefore, is the synthesis of substituted *N*-(2'-nitrophenyl)pyrrolidine-2-carboxamides **4a**–**4k** and *N*-(2'-nitrophenyl)piperidine-2-carboxamides **4l**–**4n**, which are C-terminal amides of L-proline and D,L-pipecolinic acid, respectively.

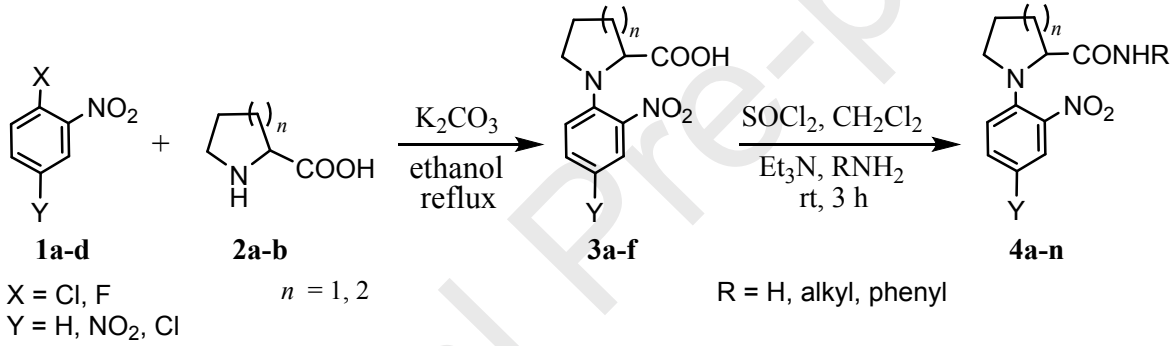
The focus of this study is to synthesize easily accessible amino acid mimics of AMPs and evaluate their antibacterial and antioxidant activities, with a view to producing a new generation of resistance-proof antibiotics as a panacea to the crisis of bacterial resistance and oxidative stress-related diseases.

2.0. Results and discussion

2.1. Chemistry

In conceptualizing the target molecules, substituted *N*-(2'-nitrophenyl)cycloaminno-2-carboxamides **4a**–**4n**, salient structural features of natural AMPs, such as the simultaneous presence of hydrophobic groups and cationic charges, were considered. It was therefore envisaged that the phenyl ring would provide the requisite hydrophobicity while the amino groups, under hydrophilic ambience, are expected to generate cationic quaternary ammonium groups ($-NH_2R^+$). The *N'*-substituents were also varied to assess the effects of conformational flexibility in the compound's antimicrobial activity [39].

The target compounds, substituted *N*-(2'-nitrophenyl)pyrrolidine-2-carboxamides **4a**–**4k** and *N*-(2'-nitrophenyl)piperidine-2-carboxamides **4l**–**4n** were synthesized from their respective *N*-arylated amino acids **3a**–**3f** via a two-step, one-pot amidation reaction using thionyl chloride, to activate the acids to their corresponding acid chloride derivatives before condensing with various amines to afford the desired carboxamides (Scheme 1). The precursor, substituted *N*-(2'-nitrophenyl)cycloamino-2-carboxylic acids **3a**–**3f** were prepared, as previously reported [26], by the condensation of substituted *o*-halogenonitrobenzenes **1a**–**1d** with L-proline **2a** or D,L-pipecolinic acid **2b**, under refluxing ethanol, in the presence of potassium carbonate.



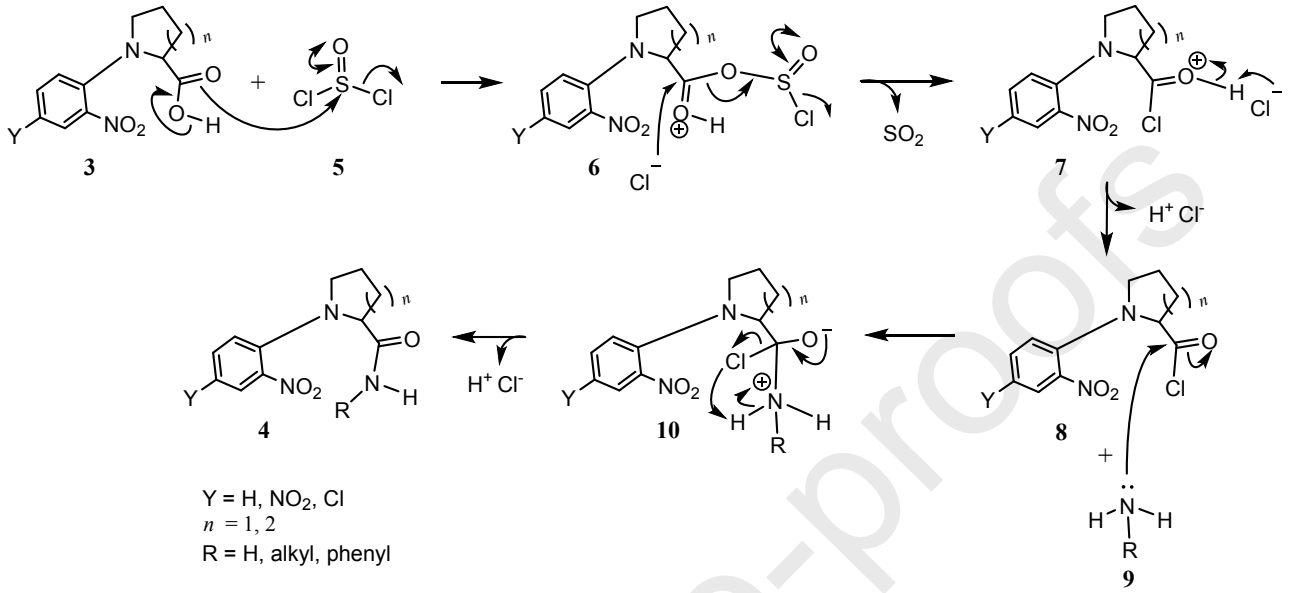
Scheme 1: Synthesis of substituted *N*-aryl cycloamino-carboxamides

The decrease in the strong carbonyl (>*C*=*O*) stretching frequency from 1718–1700 cm^{-1} (in acids **3**) to 1690–1650 cm^{-1} in the IR spectrum of **4** as well as the disappearance of the carboxylic acid proton and appearance of the protons attributable to the amines in the ^{1}H-NMR spectra were good indicators of a successful reaction. Upfield shifts in the resonance peaks of the carbonyl carbon (>***C***=*O*) atoms and the appearance of new carbon atom peaks were also observed in their ^{13}C-NMR spectra. The spectroscopic data collected are in agreement with the proposed structures. The stereo-configurations of the products **4** were, however, assumed based on the configurations of their respective precursors **3**.

A mechanism [40,41] for the amidation reaction is proposed in Scheme 2, involving a two-step nucleophilic substitution of the hydroxide ion ($^-$OH) at the carbonyl carbon atom of the cycloamino acid **3**. Firstly, $^-$OH is substituted by a chloride anion (Cl^-), to form the acyl chloride **8**, in a 1,2-addition–elimination reaction then, secondly, by the amine **9**, to furnish the carboxamide **4**, with the evolution of sulfur dioxide (SO_2) and two molecules of hydrogen chloride (HCl).

The formation of **8** begins with the attack of the electrophilic sulfur atom of thionyl chloride **5** by the π electrons of the carbonyl carbon atom of **3**, propelled by the formation of a π bond between

the hydroxyl oxygen and carbonyl carbon atoms, to give the chlorosulfite **6**. This is followed by the nucleophilic attack of the chlorosulfite-containing carbon atom by Cl^- (from $SOCl_2$ **5**) to afford **7**, with the evolution of SO_2 gas. Subsequently, the *O–H* σ bond in the intermediate **7** breaks, under the influence of the Cl^- on the proton, to give the acyl chloride **8**, with the elimination of HCl.



Scheme 2: Proposed mechanism of reaction for the one-pot formation of carboxamides

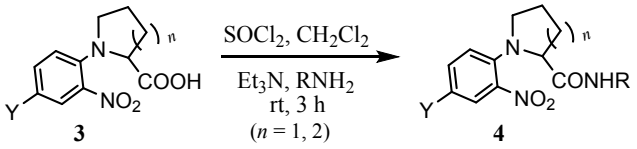
The second step, in the proposed mechanism, involves the nucleophilic attack of the carbonyl carbon atom of the acyl chloride **8** by the lone pair of electrons on the nitrogen atom of the amine (**9**) to form the new *C–N* σ bond of the intermediate, **10**. In a concerted movement of electrons in **10**, the excess lone pair of electrons on the oxygen atom reforms a π bond with the carbonyl carbon atom, to push out the Cl^- leaving group, which picks up the proton, generated from the fission of the *N–H* σ bond, to give the carboxamide **4**, with the removal of a second molecule of HCl.

A variety of primary amines were employed in the amidation of the *N*-aryl cycloamino acids **3** (Table 1) to ensure that the resulting carboxamides **4** were equipped with a secondary nitrogen atom. This was geared towards making the target molecules less hydrophobic and facilitate hydrogen bonding. The occurrence of *CH*/π interactions [42] as well as π–π stacking interactions between the *N*- and *N'*-phenyl groups of the prolyl and amide moieties, respectively, of carboxamides **4i**–**4k** and **4n** were also envisaged.

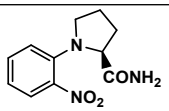
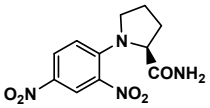
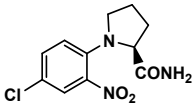
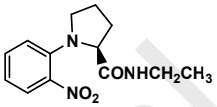
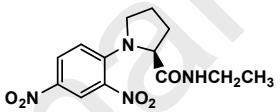
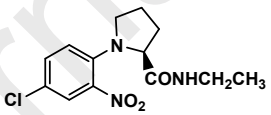
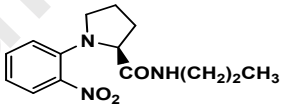
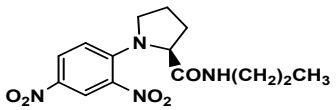
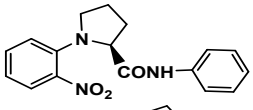
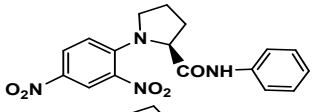
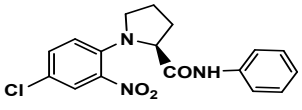
Generally, the amidation reactions of substituted *N*-(2'-nitrophenyl)pyrrolidine-2-carboxylic acids **3a**–**3c** were higher yielding than those of the *N*-(2'-nitrophenyl)piperidine-2-carboxylic acids **3d**–**3f** (*cf*. **4a** v **4l** & **4i** v **4n**; Table 1), as previously observed [26,43], except for the dinitro-compounds, **4b** and **4m**, where the reverse was the case (entry 2 v 13). It is plausible that the electron-withdrawing effect of the additional *para*-NO_2 group combined synergistically with the structural flexibility of the pipecolinyl ring to present a more nucleophilic carbonyl carbon atom to furnish **4m** in 47% yield; relative to the lower 35% yield of **4b**.

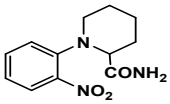
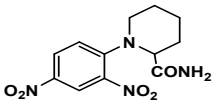
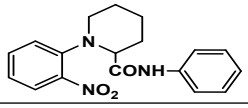
The *para*-substituents on the *N*-phenyl ring appeared to have minimal effect on product yield as the yields of 4'-unsubstituted and 4'-nitro-substituted compounds were comparable (**4d** v **4e**; **4g** v **4h**; **4i** v **4j** & **4l** v **4m**) with the exception of **4a** and **4b**. It is noteworthy that though the nitro-substituted compounds were marginally better yielding, the 4'-chloro-containing substrates were lower-yielding in all the carboxamides formed except for **4c**, where a 91.7% yield was recorded. Furthermore, the yield of the carboxamides appeared to decrease with increasing alkyl chain length, with the *N'*-phenyl carboxamides (**4i** & **4j**) been marginally better-yielding than the *N'*-propyl (**4g** & **4h**) analogues. Other authors have similarly observed this [44,45].

Table 1: Synthesis of substituted *N*-aryl cycloamino-carboxamides (4a–n)



3 4

Entry	Substituents Y	R	Carboxamide			m.p. (°C)	Yield (%)
1.	H	H		L-	**4a**	188–190	98.2
2.	NO_2	H		L-	**4b**	90–92	35.0
3.	Cl	H		L-	**4c**	168–170	91.7
4.	H	ethyl		L-	**4d**	90–92	76.3
5.	NO_2	ethyl		L-	**4e**	149–151	78.3
6.	Cl	ethyl		L-	**4f**	152–154	60.0
7.	H	propyl		L-	**4g**	(oil)	68.9
8.	NO_2	propyl		L-	**4h**	(oil)	70.0
9.	H	phenyl		L-	**4i**	160–162	71.3
10.	NO_2	phenyl		L-	**4j**	232–234	73.0
11.	Cl	phenyl		L-	**4k**	230–232	58.3

12.	H	H		**D**,**L**-	**4l**	189–191	45.0
13.	NO_2	H		**D**,**L**-	**4m**	190–192	47.4
14.	H	phenyl		**D**,**L**-	**4n**	167–169	61.0

2.2. Biology

2.2.1. Antibacterial activity

To investigate the antibacterial potencies of the *N*-(2'-nitrophenyl)cycloaminno-2-carboxamides **4a**–**4n** synthesized in this protocol, well-known, commonplace opportunistic infection-causing bacteria (some with antibiotic resistance) were selected [18]. The carboxamides **4a**–**4n** were assayed against ten pharmaceutical strains of bacteria to evaluate their antibacterial activities. Five Gram-positive bacterial strains, namely: *Bacillus subtilis* (ATCC 19659), *Enterococcus faecalis* (ATCC 14506), *Mycobacterium smegmatis* (ATCC 14468), *Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus aureus* (ATCC 25923) as well as five Gram-negative bacterial strains: *Enterobacter cloacae* (ATCC 13047), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 33420), *Klebsiella oxytoca* (ATCC 8724) and *Proteus mirabilis* (ATCC 7002) were employed.

Amidst the assayed bacteria, *E. coli*, *E. cloacae*, *E. faecalis* and *S. aureus* are associated with opportunistic infections [18,46]. Most *E. coli* bacteria are benign but many strains are linked to persistent diarrhea and food-borne illnesses. They are Gram-negative bacteria and cause enteric diseases, urinary tract infections and sepsis or meningitis [47]. *E. cloacae*, on the other hand, has emerged as a nosocomial pathogen from intensive care patients pathogenic, especially to those on mechanical ventilation [46]. They are Gram-negative and capable of producing a wide variety of infections, such as pneumonia, urinary tract infections, and septicaemia. *E. faecalis* and *S. aureus* are both Gram-positive bacteria and are implicated in endodontic infections and periapical inflammations [48] as well as nosocomial and community-acquired infections [49], respectively. *E. faecalis* infects the urinary tract, abdomen, biliary tract and burn wounds, etc. and forms a biofilm in external medical devices whereas *S. aureus* causes bacteremia, infective endocarditis, skin and soft tissue infections, gastroenteritis, meningitis, toxic shock syndrome, lung and urinary tract infections, amongst others.

The *in-vitro* antibacterial assays of **4a**–**4n** were carried out using the broth micro-dilution method [50], in the presence of resazurin dye, with standard antibiotic drugs, streptomycin and nalidixic acid as controls. Streptomycin is a first-generation aminoglycosidic, broad-spectrum antibiotic used in the treatment of tuberculosis. It is effective against Gram-negative and some Gram-positive bacteria [51]. Nalidixic acid (a 1,8-naphthyridone), on the other hand, is also a broad-spectrum bactericidal, commonly used against the bacteria responsible for urinary tract infections. Typically, it works by blocking bacterial deoxyribonucleic acid (DNA) replication [52]. The minimum inhibitory concentrations **(**MIC) of the carboxamides **4a**–**4n** were evaluated in accordance with

standard procedures [53] and showed moderate to excellent antibacterial activities in different concentrations (15.6–250 μg/mL; Table 2).

Table 2: Minimum Inhibitory Concentrations (MIC) of carboxamides 4a–4n (µg/mL)

Bacterial strains	Carboxamides														Standards	
	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	4m	4n	STM	NLD
(a) Gram-positive																
BS	250	125	125	125	125	125	250	125	250	125	62.5	125	125	125	16	16
EF	250	62.5	125	250	250	250	250	125	125	125	250	125	125	125	128	> 512
MS	125	125	125	250	125	125	125	125	125	125	62.5	125	62.5	125	4	> 512
SA	125	15.6	62.5	250	125	125	125	125	125	125	62.5	250	125	125	256	64
SE	125	125	125	250	125	125	125	125	125	125	250	125	125	250	8	64
(b) Gram-negative																
ECL	125	125	62.5	250	125	250	125	250	125	31.3	125	250	125	125	> 512	16
EC	125	125	125	250	125	125	125	125	125	125	125	125	125	125	64	> 512
PV	250	125	125	250	125	125	125	125	125	125	125	125	125	125	16	500
PM	125	62.5	125	125	125	125	125	125	125	125	62.5	62.5	62.5	250	128	32
KO	125	125	125	125	125	125	125	125	125	125	31.3	125	125	125	16	8

STM: Streptomycin, NLD: Nalidixic acid

BS: *Bacillus subtilis*, **EF***: Enterococcus faecalis*, **MS***: Mycobacterium smegmatis*, **SA***: Staphylococcus aureus*, **SE***: Staphylococcus epidermidis*, **ECL***: Enterobacter cloacae*, **EC***: Escherichia coli*, **PV***: Proteus vulgaris*, **PM***: Proteus mirabilis*, **KO***: Klebsiella oxytoca*.

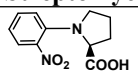
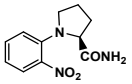
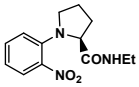
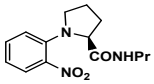
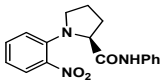
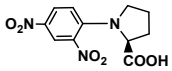
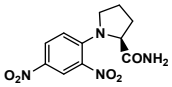
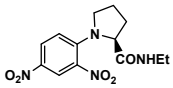
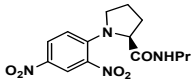
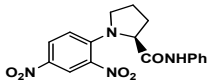
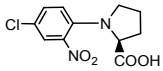
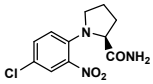
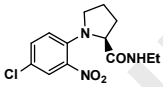
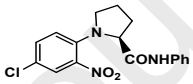
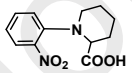
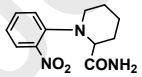
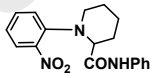
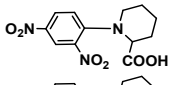
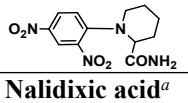
Table 2 shows that the minimum inhibitory concentrations (MIC) of carboxamides **4a**–**4n** were lower than those of the standard, streptomycin, against *S. aureus* (256 μg/mL) and *E. cloacae* (> 512 μg/mL); meaning that the carboxamides were more potent antibacterial agents. *S. aureus* and *E. cloacae* are reported to be aggressive antibiotics-resistant Gram-positive and Gram-negative bacteria, respectively. They are notorious for opportunistic infections [49,54]. Similarly, the MIC value for nalidixic acid (≥ 500 μg/mL) was higher than those obtained for compounds **4a**–**4n** against *E. faecalis*, *M. smegmatis*, *E. coli* and *P. vulgaris*, indicating their inhibitory potencies against these bacterial strains. Conversely, carboxamides **4a**–**4n** recorded higher MIC values than the standards, streptomycin and nalidixic acid, against *B. subtilis*, *S. epidermidis* and *K. oxytoca*. However, **4k** showed some inhibitory effects against *B. subtilis* (62.5 μg/mL) and *K. oxytoca* (31.3 μg/mL) in comparison to nalidixic acid (16 μg/mL; 8 μg/mL) and streptomycin (16 μg/mL), respectively.

It is pertinent to note that *N'*-phenyl-*N*-(4'-chloro-2'-nitrophenyl)pyrrolidine-2-carboxamide **4k** recorded lower MIC values, across the bacterial strains, than the other carboxamides assayed, alluding to its broad-spectrum antibacterial potential. This potency may be due to the presence of a ring chlorine atom and two aromatic (phenyl) systems. *N*-(2',4'-Dinitrophenyl)pyrrolidine-2-carboxamide **4b** also inhibited *E. faecalis* (62.5 μg/mL), *S. aureus* (15.6 μg/mL) and *P. mirabilis* (62.5 μg/mL) better than the standards. Additionally, carboxamide **4c** was more active against *E. faecalis* (125 μg/mL) and *S. aureus* (62.5 μg/mL) than streptomycin and nalidixic acid whereas **4m** was more potent against *E. faecalis* (125 μg/mL). It is vital to point out that many of the synthesized compounds assayed gave MIC values lower than, at least, one of the two standards. The best overall antibacterial activity was exhibited by **4b**; followed by **4k**.

Comparing the carboxamides with the pyrrolidine (**4a**–**4k**) and piperidine (**4l**–**4n**) ring systems, **4l** showed inhibitory activities over more bacterial strains than **4a**, in comparison to both standards, whereas the converse was the case for **4b** and **4m** as well as for **4i** and **4n** (Table 2). Interestingly, all the pipecolyl amides (**4l**–**4n**; 125 μg/mL) assayed inhibited *E. faecalis* more than streptomycin (128 μg/mL) and nalidixic acid (> 512 μg/mL) but against *P. mirabilis*, carboxamides **4a**–**4m** recorded MIC values lower than that of streptomycin (128 μg/mL) while only **4n** recorded higher (250 μg/mL). With the *N'*-substituents, the antibacterial activities increased in the order: *N'-Et* (**4d**–**4f**) < *N'-H* (**4a**–**4c**) < *N'-Pr* (**4g**–**4h**) < *N'-Ph* (**4i**–**4k**) whereas with the 4'-phenyl substituents (*H*, NO_2 and *Cl*), activity increased in the order: 4'-*PhH* (**4a**, **4d**, **4g** & **4l**) < 4'-*PhCl* (**4c** & **4f**) < 4'-$PhNO_2$ (**4b**, **4e**, **4h** & **4m**) except in the *N'*-phenyl carboxamides, **4i**–**4k**, where **4k** (4'-*Cl*) > **4j** (4'-NO_2) > **4i** (4'-*H*).

In general, the compounds evaluated (**4a**–**4n**) exhibited more inhibitory effects on the Gram-negative bacterial strains than the Gram-positive strains. This has been ascribed to the vulnerability of the thinner membrane cell walls of Gram-negative bacteria. Bacterial cell walls contain < 10% peptidoglycan, which, if changed, can disrupt the integrity of the cell membrane, leading to cell death by osmosis [55]. The same trend was previously [26] observed with their carboxylic acid equivalents (**3**; Scheme 1). The inhibitory activities of some of the carboxamides synthesized herein are compared with their carboxylic acid analogues in Table 3.

Table 3: Minimum Inhibitory Concentrations (MIC) of some *N*-aryl amino acids [26] **and carboxamides (µg/mL)**

Entry	Substrate			Bacterial strains									
				Gram-positive					Gram-negative				
				BS	**EF**	**MS**	**SA**	**SE**	**ECL**	**EC**	**PV**	**PM**	**KO**
1.	**Streptomycin**[a]		**STM**	16	128	4	256	8	> 512	64	16	128	16
2.		**L-**	**3a**[b]	–[c]	62.5	62.5	31.2	–[b]	15.6	62.5	250	125	–[b]
3.		**L-**	**4a**	–[c]	250	125	125	–[c]	125	125	250	125	–[c]
4.		**L-**	**4d**	–[c]	250	250	250	–[c]	250	250	250	125	–[c]
5.		**L-**	**4g**	–[c]	250	125	125	–[c]	125	125	125	125	–[c]
6.		**L-**	**4i**	–[c]	125	125	125	–[c]	125	125	125	125	–[c]
7.		**L-**	**3b**[b]	–[c]	125	125	125	–[c]	250	125	125	125	–[c]
8.		**L-**	**4b**	–[c]	62.5	125	15.6	–[c]	125	125	125	62.5	–[c]
9.		**L-**	**4e**	–[c]	250	125	125	–[c]	125	125	125	125	–[c]
10.		**L-**	**4h**	–[c]	125	125	125	–[c]	250	125	125	125	–[c]
11.		**L-**	**4j**	–[c]	125	125	125	–[c]	31.3	125	125	125	–[c]
12.		**L-**	**3c**[b]	–[c]	15.6	31.3	62.5	–[c]	62.5	31.3	31.3	31.3	–[c]
13.		**L-**	**4c**	–[c]	125	125	62.5	–[c]	62.5	125	125	125	–[c]
14.		**L-**	**4f**	–[c]	250	125	125	–[c]	250	125	125	125	–[c]
15.		**L-**	**4k**	–[c]	250	62.5	62.5	–[c]	125	125	125	62.5	–[c]
16.		**D,L-**	**3e**[b]	–[c]	250	250	125	–[c]	125	250	125	250	–[c]
17.		**D,L-**	**4l**	–[c]	125	125	250	–[c]	250	125	125	62.5	–[c]
18.		**D,L-**	**4n**	–[c]	125	125	125	–[c]	125	125	125	250	–[c]
19.		**D,L-**	**3f**[b]	–[c]	125	125	250	–[c]	250	125	125	250	–[c]
20.		**D,L-**	**4m**	–[c]	125	62.5	125	–[c]	125	125	125	62.5	–[c]
21.	**Nalidixic acid**[a]		**NLD**	16	> 512	> 512	64	64	16	> 512	500	32	8

[a]Standard, [b]Data from Ref. [26], [c]$MIC_{Standards} < MIC_{Substrate}$; **BS**: *Bacillus subtilis*, **EF***: Enterococcus faecalis*, **MS***: Mycobacterium smegmatis*, **SA***: Staphylococcus aureus*, **SE***: Staphylococcus epidermidis*, **ECL***: Enterobacter cloacae*, **EC***: Escherichia coli*, **PV***: Proteus vulgaris*, **PM***: Proteus mirabilis*, **KO***: Klebsiella oxytoca*; Et: ethyl, Pr: propyl, Ph: phenyl.

Table 3 revealed that the amide derivatives (**4a**, **4d**, **4g** and **4i**) of acid **3a** (entry 2) had lower inhibitory effects on the bacterial strains assayed than the parent acid (**3a**) whereas the scenario was the reverse in the case of **3b** (entry 7) where its concomitant derivatives (**4b**, **4e**, **4h** and **4j**) showed marginally higher inhibitory effects, with carboxamide **4b** giving the lowest MIC value of 15.6 µg/mL against *S. aureus*. In the same vein, the derivatization of *N*-(2'-nitrophenyl)piperidine-2-carboxylic acid **3e** and *N*-(2',4'-dinitrophenyl)piperidine-2-carboxylic acid **3f** to carboxamides **4l** and **4n**, and **4m**, respectively, in the piperidine-containing compounds (entries 16–20), resulted in increases in their antibacterial activities.

Notably, the acid and amides with the *para*-chlorophenyl moiety (entries 12–15) gave the best overall assay results, with *N*-(4'-chloro-2'-nitrophenyl)pyrrolidine-2-carboxylic acid **3c** showing the highest antibacterial activity (entry 12) against three Gram-positive and four Gram-negative strains, followed by *N'*-phenyl-*N*-(4'-chloro-2'-nitrophenyl)pyrrolidine-2-carboxamide **4k** (entry 15), which recorded an MIC value of 62.5 µg/mL against *M. smegmatis*, *S. aureus* and *P. mirabilis*. These results corroborate the acclaimed antimicrobial activities of free and bonded chlorine [56]. Active chlorine compounds are reported to kill bacteria by chlorination. They produce a persisting oxidation capacity [c(Ox)]; designated as "chlorine cover", which is responsible for the observed attenuation of bacterial virulence and post-antibiotic effect [56].

2.2.2. Antioxidant activity

The carboxamides were screened for possible antioxidant activities with 1,1-diphenyl-2-picryl hydrazyl (DPPH) and 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) [57]. Delineated in Figures 2 and 3, are plots of the percentage scavenging activities of carboxamides **4a**, **4e**, **4k** and **4l** with DPPH radicals and ABTS radical cations, respectively. The four compounds evaluated represent four different series of the carboxamides synthesized herein with varying substituents. Ascorbic acid (AA) and butylated hydroxyanisole (BHA) were used as positive controls while Tween 20 served as the negative control.

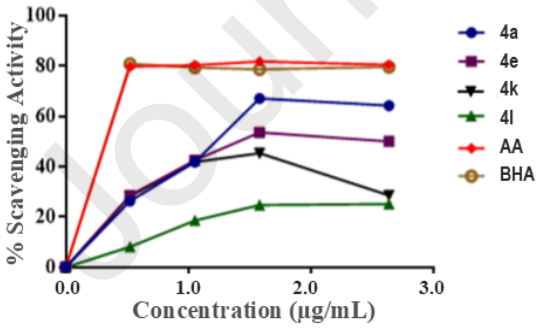


Figure 2: Percentage Scavenging Activity (DPPH)

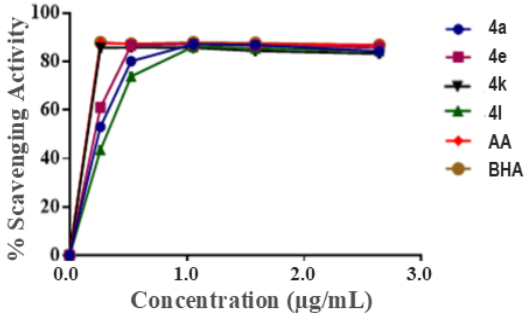


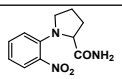
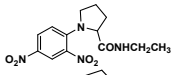
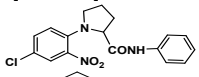
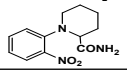
Figure 3: Percentage Scavenging Activity (ABTS)

In the DPPH assay, all the screened carboxamides scavenged the DPPH radicals in a dose-dependent manner (Figure 2). The highest (70%) and lowest (25%) scavenging activities were exhibited by *N*-(2'-nitrophenyl)pyrrolidine-2-carboxamide **4a** and *N*-(2'-nitrophenyl)piperidine-2-carboxamide **4l**, respectively. The carboxamides showed optimal scavenging activities at about 1.5

μg/mL whereupon further increments in concentration led to decreases in activity, except with **4l**, which scavenging activity remained almost constant.

Gratifyingly, the compounds showed better scavenging activity with the ABTS radical cations (Figure 3) in comparison to their activities with the DPPH radicals. The carboxamides, **4a**, **4e**, **4k** and **4l**, recorded a 90% scavenging activity; comparable to the percentage scavenging activities of the positive controls, AA and BHA. Figure 3 also shows that their activities were dose dependent. In contrast to the DPPH assays, the scavenging activities of the carboxamides did not decrease with increasing concentration.

Table 4: The IC_{50} values from the DPPH and ABTS assays of some carboxamides

Entry	Substrate		IC_{50} (mg/mL)[a]	
			DPPH	ABTS
1.	Ascorbic acid	**AA**	6.6×10^{-4}	3.6×10^{-4}
2.		**4a**	1.22×10^{-3}	2.55×10^{-4}
3.		**4e**	2.15×10^{-4}	2.15×10^{-4}
4.		**4k**	ND	1.45×10^{-4}
5.		**4l**	ND	3.25×10^{-4}
6.	Butylated hydroxyanisole	**BHA**	6.6×10^{-4}	3.6×10^{-4}

[a]All IC_{50} values are significant ($P < 0.05$); DPPH: 1,1-diphenyl-2-picryl hydrazyl, ABTS: 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid); ND: Not Determined.

The half maximal inhibitory concentration (IC_{50}) values for the antioxidant activities of the four carboxamides and two positive controls, with both DPPH and ABTS, were also calculated (Table 4). Interestingly, *N'*-ethyl-*N*-(2',4'-dinitrophenyl)pyrrolidine-2-carboxamide **4e** gave the same IC_{50} of 2.15×10^{-4} mg/mL in both assays. This was lower than the IC_{50} values of AA and BHA in DPPH (6.6×10^{-4} mg/mL) and ABTS (3.6×10^{-4} mg/mL), respectively. Remarkably, the four carboxamides **4a**, **4e**, **4k** and **4l** gave IC_{50} values lower than those of the controls, in the ABTS assay, with **4k** recording the lowest IC_{50} of 1.45×10^{-4} mg/mL. The IC_{50} value of 3.25×10^{-4} mg/mL of compound **4l** was also comparable to those of AA and BHA.

A corollary of the antioxidant assays is that all the carboxamides screened exhibited good antioxidant activities, with maximum percentage scavenging activities of 70–90% and IC_{50} values in the range of $1.45–3.25 \times 10^{-4}$ mg/mL; lower than those of the positive controls, ascorbic acid and butylated hydroxyanisole. The carboxamides, **4a**, **4e**, **4k** and **4l**, performed better in the assays with the ABTS radical cations than the DPPH radicals. It is unclear whether this is connected to the fact that the DPPH assay is a biphasic (alcohol/water) medium-based assay in contrast to the ABTS assay, which is a monophasic (aqueous) medium-based assay [58]. It is pertinent to point out that whilst the former was conducted to determine the antioxidant's ability to donate electrons to neutralize the DPPH radicals, the latter was carried out to determine antioxidant capacity, i.e., the ability of the antioxidant to scavenge ABTS radical cations [59].

3.0. Conclusion

N-(4'-substituted-2'-nitrophenyl)cycloamino acids (**3a**–**3f**) have been successfully subjected to a one-pot amidation reaction involving the *in situ* formation of their acyl chloride derivatives, using thionyl chloride, and subsequent condensation with different primary amines, at ambient temperature to furnish various carboxamides (**4a**–**4n**), via an S_N2-type mechanism. The chemical structures, composition and molecular weights of the resulting products were identified and confirmed using IR, ^1H, ^{13}C and 2-D NMR spectroscopy, mass spectrometry and elemental analysis. The concomitant data were all in agreement with the proposed structures.

The antibacterial activity assays of the compounds, using the broth micro-dilution method, showed that all the compounds screened possessed moderate to excellent inhibitory potencies against the ten bacterial strains tested. *N*-(2',4'-Dinitrophenyl)pyrrolidine-2-carboxamide **4b** exhibited the highest antibacterial activity (MIC = 15.6 μg/mL) against *Staphylococcus aureus*; a Gram-positive, antibiotic-resistant, opportunistic infection-causing bacteria of global concern as well as *Enterococcus faecalis* (MIC = 62.5 μg/mL). Carboxamide **4b** was also more potent than nalidixic acid (≥ 500 μg/mL) against *M. smegmatis, E. coli* and *P. vulgaris* (125 μg/mL), and inhibited *E. cloacae* (125 μg/mL) and *P. mirabilis* (62.5 μg/mL) better than streptomycin. Similarly, *N'*-phenyl-*N*-(4'-chloro-2'-nitrophenyl)pyrrolidine-2-carboxamide **4k** gave a lower MIC reading against *S. aureus* (62.5 μg/mL), in comparison to streptomycin (256 μg/mL) and nalidixic acid (64 μg/mL).

In addition, **4k** showed higher antibacterial activity than nalidixic acid against two Gram-positive (*E. faecalis* and *M. smegmatis*) and Gram-negative (*E. coli* and *P. vulgaris*) bacterial strains as well as against two Gram-negative (*E. cloacae* and *P. mirabilis*) strains, where its MIC values were lower than that of streptomycin. Consequently, it can be inferred that compounds **4b** and **4k** are good candidates for narrow-spectrum (Gram-positive) and broad-spectrum antibiotics, respectively. The carboxamides herein are auspicious, inexpensive, easy-to-access, potential synthetic mimics for antimicrobial peptides and are probable candidates for the development of antibiotics that are not disposed to bacterial resistance.

It is also notable that of the four carboxamides submitted for antioxidant activity assays, all were inhibitory, with **4l** giving the lowest percentage scavenging activity of 25% and IC_{50} of 3.25 x 10^{-4} mg/mL and **4k**, with an optimal percentage scavenging activity of 45% (in DPPH), having the lowest IC_{50} value of 1.45 x 10^{-4} mg/mL (in ABTS). It is equally significant to point out that the carboxamides screened for radical scavenging abilities also possessed bactericidal capabilities. In the ABTS assay, the antioxidant properties increased in the order: **4l** < **4a** < **4e** < **4k** whereas in the antibacterial assays, broadly, **4a** < **4e** < **4l** < **4k**. Consequently, it is plausible to surmise that these carboxamides follow analogous trends in their antibacterial and antioxidant activities, and are, therefore, good candidates for further drug development.

4.0. Experimental

4.1. Chemistry

All reagents and solvents were obtained from commercial sources and used without further purification. Melting points were determined on an Electrothermal digital apparatus and are uncorrected. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} precoated plates using an ethyl acetate/*n*-hexane (1:2) solvent system and visualized under a

UV lamp (254 nm). Infrared spectra were recorded on a Perkin Elmer Universal (ATR Spectrum 100) FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded, in deuterated $CDCl_3$ or DMSO-d_6, on a Bruker Ultrashield (400 MHz or 500 MHz) spectrometer. Chemical shifts (δ) values were reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and signals were expressed as a s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), m (multiplet). Mass spectra (*m/z*) were obtained from a Nexera UHPLC coupled to an LC-MS 8040 using electrospray ionization (ESI) in positive mode and elemental analyses were performed on a 2400 series CHNS PerkinElmer analyzer.

4.1.1. General procedure for the preparation of 4a–4n

Into a clean round-bottomed flask containing **3** (1 mmol) dissolved in CH_2Cl_2 (10 mL) was added triethylamine (3 mmol), thionyl chloride (1 mmol) and amine (1 mmol), in succession, with stirring. The reaction mixture was then stirred at ambient temperature for 3 h, monitored by TLC, and concentrated under reduced pressure. The resulting residue was extracted into CH_2Cl_2 (20 mL), diluted with 1*M* HCl (10 mL), neutralized with 1*M* NaOH (10 mL) and washed with saturated brine solution (25 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford the carboxamides **4**. Some of the products were purified via column chromatography (alumina/*n*-hexane:ethyl acetate; 2:1).

4.1.2. Spectroscopic data

4.1.2.1. *N*-(2'-Nitrophenyl)pyrrolidine-2-carboxamide (4a)

Yellow solid (0.982 g, 98.2%); m.p. 188–190 ^{o}C; R_f = 0.47 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3440, 3328, 3187, 3091, 2926, 2877, 1651, 1526, 1351, 1266, 1127, 1052; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 7.75 (1H, dd, *J* = 8.2, 1.5 Hz, Ar**H**), 7.39 (1H, m, Ar**H**), 7.01 (1H, d, *J* = 8.4 Hz, Ar**H**), 6.90 (1H, t, *J* = 7.7 Hz, Ar**H**), 6.67; 5.78 (2H, 2 x s, -CO*N*'**H**$_2$), 4.36 (1H, t, *J* = 7.8 Hz -*N*C**H**CO*N*'-), 3.65 (1H, t, *J* = 4.9 Hz, -C**H**$_a$H$_b$*N*-), 2.80 (1H, dd, *J* = 13.2, 5.4 Hz, -CH$_a$**H**$_b$*N*-), 2.57 (1H, t, *J* = 5.0 Hz, -CH$_2$C**H**$_a$H$_b$CH-), 2.04 (2H, d, *J* = 3.7 Hz, -C**H**$_2$CH$_a$H$_b$CH-), 1.87 (1H, m -CH$_2$CH$_a$**H**$_b$CH-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 175.8 (-**C**O*N*'H$_2$), 142.0; 133.5; 126.5; 126.3; 119.3; 117.0 (**Ar**H), 63.3 (-*N***C**HCO*N*'-), 53.1 (-**C**H$_2$*N*-), 31.7 (-CH$_2$**C**H$_2$CH-), 25.6 (-**C**H$_2$CH$_2$CH-); ESI-MS *m/z*: 236.05 $[M+H]^+$; Anal. calcd for $C_{11}H_{13}N_3O_3$ (%): C, 56.17; H, 5.53; N, 17.87; O, 20.43. Found: C, 55.89; H, 5.86; N, 17.78; O, 20.47.

4.1.2.2. *N*-(2',4'-Dinitrophenyl)pyrrolidine-2-carboxamide (4b)

Yellow powder (0.328 g, 35.0%); m.p. 90–92 ^{o}C; R_f = 0.45 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3442, 3295, 3162, 3115, 2926, 2860, 1689, 1597, 1515, 1448, 1385, 1326, 1137, 1061; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 8.67 (1H, d, *J* = 2.7 Hz, Ar**H**), 8.25 (1H, dd, *J* = 9.4, 2.7 Hz, Ar**H**), 7.00 (1H, d, *J* = 9.4 Hz, Ar**H**), 6.15; 5.30 (2H, 2 x s, -CO*N*'**H**$_2$), 4.42 (1H, t, *J* = 8.1 Hz, -*N*C**H**CO*N*'-), 3.70 (1H, d, *J* = 6.1 Hz, -C**H**$_a$H$_b$*N*-), 2.02 (3H, s, -CH$_a$**H**$_b$*N*-; -CH$_2$C**H**$_2$CH-), 1.23 (2H, s, -C**H**$_2$CH$_2$CH-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 175.1 (-**C**O*N*'H$_2$), 144.9; 136.7; 136.1; 127.5; 123.4; 116.2 (**Ar**H), 62.2 (-*N***C**HCO*N*'-), 51.9 (-**C**H$_2$*N*-), 30.7 (-CH$_2$**C**H$_2$CH-), 24.5 (-**C**H$_2$CH$_2$CH-); ESI-MS *m/z*: 281.10 $[M+H]^+$; Anal. calcd for $C_{11}H_{12}N_4O_5$ (%): C, 47.14; H, 4.25; N, 20.00; O, 28.61. Found: C, 47.32; H, 4.27; N, 19.95; O, 28.46.

4.1.2.3. *N*-(4'-Chloro-2'-nitrophenyl)pyrrolidine-2-carboxamide (4c)

Yellow powder (1.827 g, 91.7%); m.p. 168–170 °C; R_f = 0.49 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3369, 3087, 2971, 2933, 2875, 2492, 2608, 1680, 1611, 1546, 1504, 1413, 1346, 1254, 1176, 1154, 1103, 1073, 730; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 7.68 (1H, s, Ar**H**), 7.46 (1H, d, *J* = 1.5 Hz, Ar**H**), 6.90 (1H, d, *J* = 9.1 Hz, Ar**H**), 6.50; 6.27 (2H, 2 x s, -CO*N*'**H**$_2$), 4.26 (1H, t, *J* = 7.9 Hz, -*N*C**H**CO*N*'-), 3.57 (1H, m, -C**H**$_a$H$_b$*N*-), 3.06 (1H, q, *J* = 12.2 Hz, -CH$_a$**H**$_b$*N*-), 2.73 (1H, t, *J* = 6.2 Hz, -CH$_2$C**H**$_a$H$_b$CH-), 2.50 (1H, dd, *J* = 14.2, 11.6 Hz, -CH$_2$CH$_a$**H**$_b$CH-), 1.95 (1H, m, -C**H**$_a$H$_b$CH$_2$CH-), 1.26 (1H t, *J* = 7.9 Hz, -CH$_a$**H**$_b$CH$_2$CH-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 175.2 (-**C**O*N*'H$_2$), 140.7; 139.2; 133.4; 125.8; 123.6; 118.2 (**Ar**H), 63.6 (-*N***C**HCO*N*'-), 53.1 (-**C**H$_2$*N*-), 31.7 (-CH$_2$**C**H$_2$CH-), 25.5 (-**C**H$_2$CH$_2$CH-); ESI-MS *m/z*: 270.55 $[M+H]^+$; Anal. calcd for $C_{11}H_{12}ClN_3O_3$ (%): C, 48.98; H, 4.45; N, 17.81; O, 28.76. Found: C, 49.10; H, 4.48; N, 17.92; O, 28.50.

4.1.2.4. *N'*-Ethyl-*N*-(2'-nitrophenyl)pyrrolidine-2-carboxamide (4d)

Yellow powder (0.425 g, 76.3%); m.p. 90–92 °C; R_f = 0.46 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3296, , 3091, 2983, 2944, 2875, 1652, 1605, 1555, 1446, 1350, 1268, 1183, 1142; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H ppm: 7.77 (1H, d, *J* = 15.4 Hz, Ar**H**), 7.41 (1H, t, *J* = 10.9 Hz Ar**H**), 6.96 (2H, dt, *J* = 10.6, 9.7 Hz Ar**H**), 6.68 (1H, s, -CO*N*'**H**), 4.36 (1H, t, *J* = 7.7 Hz, -*N*C**H**CO*N*'-), 3.64 (1H, q, *J* = 8.4 Hz, -C**H**$_a$H$_b$*N*-), 3.21 (2H, m, -*N*'C**H**$_2$CH$_3$), 2.80 (1H, t, *J* = 6.6 Hz, -CH$_a$**H**$_b$*N*-), 2.57 (1H, m -CH$_2$C**H**$_a$H$_b$CH-), 2.01–1.79 (3H, m, -C**H**$_2$CH$_a$**H**$_b$CH-), 0.99 (3H, t, *J* = 7.3 Hz, -*N*'CH$_2$C**H**$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C ppm: 172.2 (-**C**O*N*'H), 142.2; 139.9; 133.5; 126.3; 119.1; 117.1 (**Ar**H), 63.6 (-*N***C**HCO*N*'-), 53.2 (-**C**H$_2$*N*-), 34.1 (-*N*'**C**H$_2$CH$_3$), 31.7 (-CH$_2$**C**H$_2$CH-), 25.6 (-**C**H$_2$CH$_2$CH-), 14.5 (-*N*'CH$_2$**C**H$_3$); ESI-MS *m/z*: 264.10 $[M+H]^+$; Anal. calcd for $C_{13}H_{17}N_3O_3$ (%): C, 59.31; H, 6.46; N, 15.96; O, 18.27. Found: C, 59.24; H, 6.51; N, 15.96; O, 18.29.

4.1.2.5. *N'*-Ethyl-*N*-(2',4'-dinitrophenyl)pyrrolidine-2-carboxamide (4e)

Yellow solid (0.858 g, 78.3%); m.p. 149–151 °C; R_f = 0.62 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3292, 3090, 2973, 2938, 1666, 1604, 1574, 1551, 1496, 1374, 1321, 1246, 1144, 1177; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 8.69 (1H, d, *J* = 5.6 Hz, Ar**H**), 8.23 (1H, dd, *J* = 9.4, 2.7 Hz, Ar**H**), 7.01 (1H, d, *J* = 9.4 Hz, Ar**H**), 6.13 (1H, s, -CO*N*'**H**), 4.43 (1H, t, *J* = 13.7 Hz, -*N*C**H**CO*N*'-), 3.71 (1H, m, -C**H**$_a$H$_b$*N*-), 3.23 (2H, m, -*N*'C**H**$_2$CH$_3$), 3.00 (1H, t, *J* = 7.5 Hz, -CH$_a$**H**$_b$*N*-), 2.63 (1H, d, *J* = 12.4 Hz, -CH$_2$C**H**$_a$H$_b$CH-), 1.99 (3H, m, -C**H**$_2$CH$_a$**H**$_b$CH-), 1.03 (3H, t, *J* = 7.3 Hz, -*N*'CH$_2$C**H**$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 170.4 (-**C**O*N*'H), 145.9; 138.0; 137.0; 128.0; 123.3; 116.5 (**Ar**H), 63.5 (-*N***C**HCO*N*'-), 53.6 (-**C**H$_2$*N*-), 34.4 (-*N*'**C**H$_2$CH$_3$), 31.7 (-CH$_2$**C**H$_2$CH-), 25.5 (-**C**H$_2$CH$_2$CH-), 14.5 (-*N*'CH$_2$**C**H$_3$); ESI-MS *m/z*: 309.10 $[M+H]^+$; Anal. calcd for $C_{13}H_{16}N_4O_5$ (%): C, 50.46; H, 5.19; N, 18.18; O, 25.99. Found: C, 50.42; H, 5.25; N, 18.25; O, 26.08.

4.1.2.6. *N'*-Ethyl-*N*-(4'-chloro-2'-nitrophenyl)pyrrolidine-2-carboxamide (4f)

Orange solid (0.857 g, 60.0%); m.p. 152–154 °C R_f = 0.48 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3286, 3085, 2963, 2927, 1671, 1605, 1553, 1497, 1376, 1331 1236, 1172, 1128; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 7.73 (1H, d, *J* = 2.6 Hz, Ar**H**), 7.32 (1H, dd, *J* = 9.0, 2.6 Hz, Ar**H**), 6.91 (1H, d, *J* = 9.0 Hz, Ar**H**), 6.51 (1H, s, -CO*N*'**H**), 4.29 (1H, t, *J* = 7.9 Hz, -*N*C**H**CO*N*'-), 3.58 (1H, m, -C**H**$_a$H$_b$*N*-), 3.17 (2H, m, -*N*'C**H**$_2$CH$_3$), 2.76 (1H, t, *J* = 8.5 Hz, -CH$_a$**H**$_b$*N*-), 2.54 (1H, m, -CH$_2$C**H**$_a$H$_b$CH-), 1.90 (3H, m, -C**H**$_2$CH$_a$**H**$_b$CH-), 0.99 (3H, t, *J* = 7.3 Hz, -*N*'CH$_2$C**H**$_3$); ^{13}C-

NMR (125 MHz, $CDCl_3$) δ_C ppm: 171.6 (-**C**O*N*'H), 140.8; 139.8; 133.4; 125.7; 123.8; 118.3 (**Ar**H), 63.8 (-*N***C**HCO*N*'-), 53.2 (-**C**H_2*N*-), 34.0 (-*N*'**C**H_2CH_3), 31.7 (-CH_2**C**H_2CH-), 25.4 (-**C**H_2CH_2CH-), 14.4 (-*N*'CH_2**C**H_3); ESI-MS *m/z*: 297.95 $[M+H]^+$; Anal. calcd for $C_{13}H_{16}ClN_3O_3$ (%): C, 52.44; H, 5.38; N, 14.12; O, 28.04. Found: C, 52.48; H, 5.40; N, 14.25; O, 27.87.

4.1.2.7. *N*'-Propyl-*N*-(2'-nitrophenyl)pyrrolidine-2-carboxamide (4g)

Yellow oil (0.372 g, 68.9%); R_f = 0.40 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3286, 3086, 2957, 2932, 2876, 1657, 1598, 1499, 1444, 1313, 1282, 1174, 1141, 1037; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 7.69 (1H, d, *J* = 8.2 Hz, Ar**H**), 7.33 (1H, t, *J* =7.8 Hz, Ar**H**), 6.96 (1H, d, *J* = 8.5 Hz, Ar**H**), 6.84 (1H, t, *J* = 7.7 Hz, Ar**H**), 6.72 (1H, s, -CO*N*'**H**), 4.33 (1H, t, *J* = 7.8 Hz, -*N*C**H**CO*N*'-), 3.58 (1H, q, *J* = 12.8 Hz, -C**H**$_a$H$_b$*N*-), 3.07 (2H, m, -*N*'C**H**$_2CH_2CH_3$), 2.75 (1H, t, *J* = 8.9 Hz, -CH$_a$**H**$_b$*N*-), 2.50 (1H, d, *J* = 5.9 Hz -CH_2C**H**$_a$H$_b$CH-), 1.95 (2H, m, -C**H**$_a$H$_b$CH$_a$**H**$_b$CH-), 1.30 (3H, m, -CH$_a$**H**$_b$$CH_2$CH-; -*N*'$CH_2$C**H**$_2CH_3$), 0.66 (3H, t, *J* = 7.4 Hz, -*N*'CH_2CH_2C**H**$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 172.2 (-**C**O*N*'H), 142.0; 139.9; 133.4; 126.2; 119.1; 117.2 (**Ar**H), 63.6 (-*N***C**HCO*N*'-), 53.0 (-**C**H_2*N*-), 40.7 (-*N*'**C**$H_2CH_2CH_3$), 31.7 (-CH_2**C**H_2CH-), 25.6 (-**C**H_2CH_2CH-), 22.5 (-*N*'CH_2**C**H_2CH_3), 10.9 (-*N*'CH_2CH_2**C**H_3); ESI-MS *m/z*: 278.05 $[M+H]^+$; Anal. calcd for $C_{14}H_{19}N_3O_3$ (%): C, 60.64; H, 6.85; N, 15.16; O, 17.35. Found: C, 60.88; H, 6.95; N, 15.20; O, 16.97.

4.1.2.8. *N*'-Propyl-*N*-(2',4'-dinitrophenyl)pyrrolidine-2-carboxamide (4h)

Brown oil (0.40 g, 70%); R_f = 0.37 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3288, 3094, 2967, 2928, 2875, 1743, 1651, 1601, 1578, 1495, 1290, 1321, 1241, 1142, 1116, 1063; ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H ppm: 8.54 (1H, d, *J* = 2.7 Hz, Ar**H**), 8.21 (1H, d, *J* = 2.8 Hz, Ar**H**), 8.20 (1H, s, -CO*N*'**H**), 6.95 (1H, d, *J* = 9.6 Hz, Ar**H**), 4.45 (1H, t, *J* = 7.0 Hz, -*N*C**H**CO*N*'-), 3.20 (1H, m, -C**H**$_a$H$_b$*N*-), 2.98 (2H, m, -*N*'C**H**$_2CH_2CH_3$), 2.50 (1H, t, *J* = 3.7 Hz, -CH$_a$**H**$_b$*N*-), 2.36 (1H, m, -CH_2C**H**$_a$H$_b$CH-), 2.01 (1H, s, -CH_2CH_a**H**$_b$CH-), 1.88 (2H, m, -C**H**$_2CH_a$H$_b$CH-), 1.39 (2H, q, *J* = 7.2 Hz -*N*'CH_2C**H**$_2CH_3$), 0.81 (3H, t, *J* = 7.4 Hz, -*N*'CH_2CH_2C**H**$_3$); ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C ppm: 169.7 (-**C**O*N*'H), 145.2; 135.1; 135.06; 127.2; 123.1; 117.1 (**Ar**H), 63.8 (-*N***C**HCO*N*'-), 52.1 (-**C**H_2*N*-), 41.0 (-*N*'**C**$H_2CH_2CH_3$), 31.8 (-CH_2**C**H_2CH-), 24.3 (-**C**H_2CH_2CH-), 21.7 (-*N*'CH_2**C**H_2CH_3), 11.2 (-*N*'CH_2CH_2**C**H_3); ESI-MS *m/z*: 323.15 $[M+H]^+$; Anal. calcd for $C_{14}H_{18}N_4O_5$ (%): C, 52.17; H, 5.43; N, 17.38; O, 24.82. Found: C, 52.47; H, 5.72; N, 17.42; O, 24.39.

4.1.2.9. *N*'-Phenyl-*N*-(2'-nitrophenyl)pyrrolidine-2-carboxamide (4i)

Yellow powder (0.942 g, 71.3%); m.p. 160–162 ^oC; R_f = 0.52 (*n*-hexane/ethyl acetate, 1:1); IR ν_{max} (ATR, cm^{-1}): 3301, 3085, 2953, 2881, 1673, 1606, 1507, 1346, 1256, 1169, 1108, 1046; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 8.78 (1H, s, -CO*N*'**H),** 7.76 (1H, d, *J* = 8.2 Hz, Ar**H**), 7.54 (2H, d, *J* = 8.1 Hz, Ar**H**), 7.43 (1H, t, *J* = 7.6 Hz, Ar**H**), 7.28 (2H, t, *J* = 7.0 Hz, Ar**H**), 7.15 (1H, t, *J* = 7.4 Hz, Ar**H**), 7.07 (1H, t, *J* = 7.2 Hz, Ar**H**), 6.97 (1H, d, *J* = 9.7 Hz, Ar**H**), 4.56 (1H, t, *J* = 7.7 Hz, -*N*C**H**CO*N*'-), 3.73–3.72 (1H, m -C**H**$_a$H$_b$*N*-), 3.11–3.10 (1H, m, -CH$_a$**H**$_b$*N*-), 2.89–2.88 (1H, m, -CH_2C**H**$_a$H$_b$CH-), 2.63 (1H, dd, *J* = 12.8, 6.0 Hz, -CH_2CH_a**H**$_b$CH-), 2.10–2.08 (1H, m, -C**H**$_a$H$_b$$CH_2$CH), 1.95–1.94 (1H, m, -CH$_a$**H**$_b$$CH_2$CH-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 170.8 (-**C**O*N*'H), 137.7; 133.8; 133.1; 131.9; 128.9; 127.5; 126.5; 125.6; 124.3; 120.4; 119.7; 117.7 (**Ar**H), 63.9 (-*N***C**HCO*N*'-), 53.5 (-**C**H_2*N*-), 31.8 (-CH_2**C**H_2CH-), 25.7 (-**C**H_2CH_2CH-); ESI-MS

m/z: 312.05 $[M+H]^+$; Anal. calcd for $C_{17}H_{17}N_3O_3$ (%): C, 65.59; H, 5.46; N, 13.50; O, 15.45. Found: C, 65.76; H, 5.42; N, 13.72; O, 15.10.

4.1.2.10. *N'*-Phenyl-*N*-(2',4'-dinitrophenyl)pyrrolidine-2-carboxamide (4j)

Yellow solid (0.46 g, 73.0%); m.p. 232–234 °C; R_f = 0.63 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3285, 3086, 2929, 2876, 1658, 1597, 1496, 1444, 1376, 1313, 1171, 1143, 1067, 1118; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 9.11 (1H, s, -CO*N*'**H**), 8.75 (1H, s, Ar**H**), 8.32 (1H, d, *J* = 9.3 Hz, Ar**H**), 7.68 (2H, d, *J* = 7.7 Hz, Ar**H**), 7.32–7.31 (3H, m, Ar**H**), 7.12 (1H, t, *J* = 8.4 Hz, Ar**H**), 4.58 (1H, d, *J* = 4.7 Hz, -*N*C**H**CO*N*'), 3.45 (1H, t, *J* = 12.6 Hz, -C$\mathbf{H}_a$H$_b$*N*-), 2.92 (1H, d, *J* = 13.6 Hz, -CH$_a$$\mathbf{H}_b$*N*-), 2.67 (1H, d, *J* = 13.4 Hz, -CH$_2$C$\mathbf{H}_a$H$_b$CH-), 1.97–1.53 (3H, m, C$\mathbf{H}_2$CH$_a$$\mathbf{H}_b$CH-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 167.6 (-**C**O*N*'H), 149.9; 139.9; 139.4; 137.8; 129.6; 129.0; 128.7; 124.6; 123.7; 121.4; 119.5; 118.8 (**Ar**H), 61.4 (-*N***C**HCO*N*'-), 52.4 (-**C**H$_2$*N*-), 25.3 (-CH$_2$**C**H$_2$CH-), 20.2 (-**C**H$_2$CH$_2$CH-); ESI-MS *m/z*: 357.90 $[M+H]^+$; Anal. calcd for $C_{17}H_{16}N_4O_5$ (%): C, 57.30; H, 4.49; N, 15.73; O, 22.48. Found: C, 57.44; H, 4.53; N, 15.72; O, 22.31.

4.1.2.11. *N'*-Phenyl-*N*-(4'-chloro-2'-nitrophenyl)pyrrolidine-2-carboxamide (4k)

Yellow powder (0.356 g, 58.3%); m.p. 230–232 °C; R_f = 0.82 (*n*-hexane/ethyl acetate, 1:1); IR ν_{max} (ATR, cm^{-1}): 3257, 3134, 3068, 2924, 2876, 1665, 1600, 1499, 1447, 1387, 1344, 1248, 1173, 1101, 1071, 743; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 8.80 (1H, s, -CO*N*'**H**), 7.79 (1H, d, *J* = 8.2 Hz, Ar**H**), 7.54 (1H, d, *J* = 4.5 Hz, Ar**H**), 7.43 (1H, t, *J* = 7.9 Hz, Ar**H**), 7.27 (2H, d, *J* = 7.9 Hz, Ar**H**), 7.15 (1H, d, *J* = 7.4 Hz, Ar**H**), 7.07 (1H, t, *J* = 8.6 Hz, Ar**H**), 6.97 (1H, d, *J* = 7.7 Hz, Ar**H**), 4.56 (1H, *J* = 7.7 Hz, -*N*C**H**CO*N*'-), 3.75 (1H, q, *J* = 10.5 Hz, -C$\mathbf{H}_a$H$_b$*N*-), 2.91 (1H, t, *J* = 7.3 Hz, -CH$_a$$\mathbf{H}_b$*N*-), 2.65 (1H, q, *J* = 7.5 Hz, -CH$_2$C$\mathbf{H}_a$H$_b$CH-), 2.11–2.07 (2H, m, -C$\mathbf{H}_a$H$_b$CH$_a$$\mathbf{H}_b$CH-), 1.97–1.96 (1H, m, -CH$_a$$\mathbf{H}_bCH_2$CH-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 170.8 (-**C**O*N*'H), 142.0; 140.4; 137.7; 133.8; 128.9; 128.6; 126.3; 124.3; 120.3; 119.4; 119.0; 117.8 (**Ar**H),), 63.9 (-*N***C**HCO*N*'-), 53.5 (-**C**H$_2$*N*-), 31.8 (-CH$_2$**C**H$_2$CH-), 25.7 (-**C**H$_2$CH$_2$CH-); ESI-MS *m/z*: 346.52 $[M+H]^+$; Anal. calcd for $C_{17}H_{16}ClN_3O_3$ (%): C, 59.04; H, 4.63; N, 12.16; O, 24.17. Found: C, 58.93; H, 4.69; N, 12.19; O, 24.19.

4.1.2.12. *N*-(2'-Nitrophenyl)piperidine-2-carboxamide (4l)

Yellow powder (0.443 g, 45.0%); m.p. 189–191 °C R_f = 0.52 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3440, 3328, 3073, 2924, 2880, 1681, 1599, 1503, 1316, 1206, 1175, 1121; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 7.75 (1H, d, *J* = 6.5 Hz, Ar**H**), 7.40 (1H, t, *J* = 7.9 Hz, Ar**H**), 7.01 (1H, d, *J* = 9.7 Hz, Ar**H**), 6.91 (1H, t, *J* = 8.3 Hz, Ar**H**), 6.63; 5.61 (2H, 2 x s, -CO*N*'**H$_2$**), 4.36 (1H, t, *J* = 7.3 Hz, -*N*C**H**CO*N*'-), 3.68–3.67 (1H, m, -C$\mathbf{H}_a$H$_b$*N*-), 2.82–2.81 (1H, m, -CH$_a$$\mathbf{H}_b$*N*-), 2.59–2.58 (1H, m, -CH$_2$CH$_2$C$\mathbf{H}_a$H$_b$-), 2.08–2.06 (2H, m, -CH$_2$C$\mathbf{H}_a$H$_b$CH$_a$$\mathbf{H}_b$-), 1.91–1.89 (1H, m, -C$\mathbf{H}_aH_bCH_2CH_2$-), 1.34–1.32 (2H, m, -CH$_a$$\mathbf{H}_bCH_a$$\mathbf{H}_bCH_2$-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 175.4 (-**C**O*N*'H$_2$), 142.0; 139.9; 133.5; 126.3; 119.2; 116.9 (**Ar**H), 63.3 (-*N***C**HCO*N*'-), 53.1 (-**C**H$_2$*N*-), 31.7 (-CH$_2$CH$_2$**C**H$_2$-), 29.6 (-CH$_2$**C**H$_2$CH$_2$-), 25.6 (-**C**H$_2$CH$_2$CH$_2$-); ESI-MS *m/z*: 250.10 $[M+H]^+$; Anal. calcd for $C_{12}H_{15}N_3O_3$ (%): C, 57.83; H, 6.02; N, 16.86; O, 19.29. Found: C, 57.70; H, 6.24; N, 16.64; O, 19.42.

4.1.2.13. *N*-(2',4'-Dinitrophenyl)piperidine-2-carboxamide (4m)

Yellow powder (0.473 g, 47.4%); m.p. 190–192 °C; R_f = 0.54 (*n*-hexane/ethyl acetate, 2:1); IR ν_{max} (ATR, cm^{-1}): 3447, 3325, 3182, 3094, 2979, 2890, 1680, 1598, 1500, 1370, 1315, 1272, 1178, 1118, 1063; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 8.66 (1H, s, Ar**H**), 8.22 (1H, d, *J* = 6.7 Hz, Ar**H**), 7.01 (1H, d, *J* = 9.4 Hz, Ar**H**), 6.19; 5.83 (2H, 2 x s, -CO*N*'**H**$_2$), 4.43 (1H, t, *J* = 8.0 Hz, -*N*C**H**CO*N*'-), 3.72–3.71 (1H, m -C**H**$_a$H$_b$*N*-), 3.49–3.47 (1H, m, -CH$_a$**H**$_b$*N*-), 3.00 (1H, t, *J* = 8.7 Hz, -CH$_2$CH$_2$C**H**$_a$H$_b$-), 2.66–2.64 (1H, m, -CH$_2$CH$_2$CH$_a$**H**$_b$-), 2.29–2.26 (3H, m -C**H**$_2$C**H**$_a$H$_b$CH$_2$-), 1.94–1.92 (1H, m -CH$_2$CH$_a$**H**$_b$CH$_2$-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 173.4 (-**C**O*N*'H$_2$), 145.8; 137.9; 137.0; 128.0; 123.2; 116.4 (**Ar**H), 64.1 (-*N***C**HCO*N*'-), 61.8 (-**C**H$_2$*N*-), 53.5 (CH$_2$CH$_2$**C**H$_2$-), 31.6 (-CH$_2$**C**H$_2$CH$_2$-), 25.5 (-**C**H$_2$CH$_2$CH$_2$-); ESI-MS *m/z*: 295.05 $[M+H]^+$; Anal. calcd for $C_{12}H_{14}N_4O_5$ (%): C, 48.97; H, 4.76; N, 19.04; O, 27.23. Found: C, 49.10; H, 4.89; N, 19.20; O, 26.81.

4.1.2.14. *N*-Phenyl-*N*-(2'-nitrophenyl)piperidine-2-carboxamide (4n)

Yellow powder (0.394 g, 61.0%); m.p. 167–169 °C; R_f = 0.93 (*n*-hexane/ethyl acetate, 1:1); IR ν_{max} (ATR, cm^{-1}): 3299, 3071, 2926, 2879, 1676, 1599, 1506, 1441, 1316, 1266, 1207, 1173, 1121, 1067, 1118; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H ppm: 8.80 (1H, s, -CO*N*'**H**), 7.80 (1H, d, *J* = 8.2 Hz, Ar**H**), 7.54 (2H, d, *J* = 6.7 Hz, Ar**H**), 7.43 (1H, t, *J* =7.3 Hz, Ar**H**), 7.29 (2H, d, *J* = 10.1 Hz, Ar**H**), 7.18–7.16 (1H, m, Ar**H**), 7.09–7.07 (1H, m, Ar**H**), 6.96 (1H, d, *J* = 8.2 Hz, Ar**H**), 4.56 (1H, t, *J* = 7.8 Hz, -*N*C**H**CO*N*'-), 3.75 (1H, t, *J* = 9.5 Hz, -C**H**$_a$H$_b$*N*-), 2.91–2.89 (1H, m, -CH$_a$**H**$_b$*N*-), 2.67–2.66 (1H, m, -CH$_2$CH$_2$C**H**$_a$H$_b$-), 2.14–2.11 (2H, m, -CH$_2$C**H**$_a$H$_b$CH$_a$**H**$_b$-), 1.98–1.97 (2H, m, -C**H**$_2$CH$_2$CH$_2$-), 1.58–1.57 (1H, m, -CH$_2$CH$_a$**H**$_b$CH$_2$-); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C ppm: 170.8 (-**C**O*N*'H), 142.0; 140.2; 137.7; 136.6; 133.8; 130.9; 128.9; 126.3; 124.3; 120.3; 119.4; 117.8; (**Ar**H), 63.9 (-*N***C**HCO*N*'-), 53.5 (-**C**H$_2$*N*-), 31.8 (-CH$_2$CH$_2$**C**H$_2$-), 29.9 (-CH$_2$**C**H$_2$CH$_2$-), 25.7 (-**C**H$_2$CH$_2$CH$_2$-); ESI-MS: 326.05 $[M+H]^+$; Anal. calcd for $C_{18}H_{19}N_3O_3$ (%): C, 66.46; H, 5.85; N, 12.92; O, 14.77. Found: C, 66.68; H, 5.97; N, 12.98; O, 14.37.

4.2. Biology

4.2.1 Materials

For the antibacterial assays, 96-well plates were purchased from Sigma-Aldrich and ten bacterial strains were obtained from Davis Diagnostics, viz: *Bacillus subtilis* (ATCC 19659), *Enterococcus faecalis* (ATCC 14506), *Mycobacterium smegmatis* (ATCC 14468), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 13048), *Enterobacter cloacae* (ATCC 13047), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 33420), *Klebsiella oxytoca* (ATCC 8724) and *Proteus mirabilis* (ATCC 7002) and cultured overnight in a Mueller–Hinton broth at 25 °C.

For the antioxidant assays, 1,1-diphenyl-2-picryl hydrazyl (DPPH), butylated hydroxyanisole, Tween 20, ascorbic acid and 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich. All chemicals and reagents were of analytical grade.

4.2.2. Antibacterial Activity Assays

The *in vitro* antibacterial activity assays of the synthesized compounds **4a**–**4n** were evaluated by the broth micro-dilution method against five Gram-positive bacterial strains: *Bacillus subtilis* (ATCC 19659), *Enterococcus faecalis* (ATCC 14506), *Mycobacterium smegmatis* (ATCC 14468), *Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus aureus* (ATCC 25923), and five Gram-negative bacterial strains: *Enterobacter cloacae* (ATCC 13047), *Escherichia coli* (ATCC

25922), *Proteus vulgaris* (ATCC 33420), *Klebsiella oxytoca* (ATCC 8724) and *Proteus mirabilis* (ATCC 7002). The standard M38 procedure [60], was adopted in the preparation of stock solutions for serial dilutions. A stock solution (1 mg/mL) of each compound was prepared in dimethyl sulfoxide (DMSO) and serially diluted according to the micro-dilution method to concentrations of 500, 250, 125, 62.5, 31.25 and 15.6 µg/mL in 100 µL-seeded 96-well plates. Each concentration was tested in duplicate against each bacterial strain. The cultured bacterial strain containing 1.5 x 10^8 cfu bacteria corresponding to the 0.5 McFarland standards in Muller–Hilton broth was also prepared. 100 µL of each prepared concentration of the compounds in the 96-well plates were then inoculated with 100 µL of the bacteria and incubated at 37 ^oC for 24 h. Next, 10 µL of 0.02% tetrazolium sodium solution (resazurin dye) was added to each well after 24 h and the plates were re-incubated for 1 h. Change of the solution from blue to pink indicated the viability of the bacteria, and the smallest concentration that killed the bacteria was considered as the minimum inhibitory concentration (MIC). Their activities were compared with streptomycin (STM) and nalidixic acid (NLD) as standard antibiotic drugs [61].

4.2.3. Antioxidant Activity Assays

The hydrogen atom or electron donation abilities and radical scavenging capabilities of the carboxamides were determined with 1,1-diphenyl-2-picryl hydrazyl (DPPH) radicals and 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cations.

4.2.3.1. DPPH Assay

The $DPPH^{\cdot}$ assay of compounds **4a**, **4e**, **4k** and **4l** was evaluated as described [62] with slight modifications. Briefly, a solution of DPPH in methanol (0.1 mM; 1 mL) was mixed with varying concentrations (0.00, 0.01, 0.015, 0.020, 0.025 and 0.003 mg/mL) of the carboxamides in methanol (1 mL), in triplicates. The mixtures were then incubated (Daihan Labtech Co. Ltd.) for 1 hour at room temperature, and the absorbance was read, against a blank, at 517 nm using a plate reader (Bioteck ELx808 Ul). Ascorbic acid and butylated hydroxyl anisole (BHA) were used as positive controls while Tween 20 served as the negative control. The percentage scavenging activity was determined using the formula:

$$\text{Scavenging Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} x\ 100$$ where A is absorbance.

4.2.3.2. ABTS Assay

Following a modified procedure [63], the ability of the carboxamides: **4a**, **4e**, **4k** and **4l**, to scavenge $ABTS^{\cdot+}$ was investigated. Briefly, different concentrations of the carboxamides (0.00, 0.01. 0.015, 0.020, 0.025 and 0.003 mg/mL) were each thoroughly mixed with the ABTS solution (0.003 g/mL) in 1:1 (v/v) and incubated for 14 min. at 25 °C. The absorbance was read at 734 nm using a plate reader (Bioteck ELx808 Ul). Ascorbic acid and butylated hydroxyl anisole (BHA) were used as positive controls. The negative control was Tween 20, and the tests were carried out in triplicates. The percentage scavenging activity was determined using the formula:

$$\text{Scavenging Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} x\ 100$$ where A is absorbance.

4.2.4. Data Analysis

The experiments in this study were in triplicates. The data obtained are expressed as mean ± standard deviation. The Tukey post-hoc and one-way analysis of variance (ANOVA) was adopted to analyze the data with the aid of Graphpad Prism (version 5.03). The half maximal inhibitory concentration (IC_{50}) values for the compounds were determined from the plotted regression graph.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors gratefully acknowledge the University of Lagos, University of Johannesburg and the National Research Foundation (NRF) of South Africa for their generous support of this project.

Appendix A. Supplementary material

Supplementary data to this article, containing the ^{1}H- & ^{13}C-NMR spectra of the synthesized compounds, can be found online at https://...

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