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Synthesis, characterization of nitro or amino substituted pyridyl ligands bridged by an ester or ether bond, and their antibacterial assessment against drug resistant bacteria

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ABSTRACT

A series of ester and ether bridged compounds bearing the nitro and amino moieties were synthesized and characterized, in order to assess their ability to potentially serve as antibacterial agents against drug resistant bacteria. All the compounds were obtained at considerable yields while characterization techniques confirmed their successful synthesis. Furthermore, their antimicrobial assessments showed that all the compounds exhibited antibacterial activity against the Gram-negative bacterial strains, both *Klebsiella pneumoniae* and its drug resistant counterpart. On the contrary, they were poorly active against *S. aureus* and MRSA. According to the MIC data, all the compounds showed activity of 0.22 to 0.24 mM towards MDR-*K. pneumoniae* which was higher than the 0.13 mM of the control **AgSD**. As for *K. pneumoniae,* the nitro derivatives **L1** and **L4** showed significantly higher MIC of 0.23 and 0.26 mM, respectively; while the amino derivatives **L2** and **L3** were more active, with MIC values of 0.16 mM that was better than the 0.18 mM of the control **AgSD**. Finally, the time kill kinetics study showed that **L2, L3** and the control **AgSD** were bacteriostatic, while **L1** and **L4** initially showed a bactericidal trend towards *K. pneumoniae* but eventually changed to an exponential growth. However, all the compounds were bactericidal towards MDR- *K. pneumoniae.*

Introduction

Antibiotic resistance among *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species (ESKAPE) pathogens such as *Staphylococcus aureus* and *Klebsiella pneumoniae* is a major public health challenge and a key priority area of the World Health Organization (WHO) [1]. The emergence of multi-drug resistant bacteria is caused by misuse of antibiotics and further worsened by the lack of new antibiotics for decades [1]. Antibiotic resistance occurs when bacteria develop mechanisms that help them in the evasion of conventional drugs, thus rendering such drugs useless [2]. Incidentally, more attention is paid to the development of different antimicrobial interventions, including design and synthesis of novel antibacterial agents. Most conventional antimicrobials are small molecules having different heterocyclic nucleus. Examples of these may be found on the drug bank website [3]. The molecules described herein are pyridine derivatives of 4-picolyl bridged with an ester or ether. These were synthesized to test their antibacterial potential against drug resistant bacteria. Functional groups found in bioactive compounds are termed pharmacophores. The pharmacophores found in potential drugs are important choices as they may affect the efficacy, cytotoxicity and genotoxcity of the potential drug. Consequently, we designed a new series of molecules, based on the well-known biological significance of various pharmacophores such as amine, nitro, chloride, ether, and ester as shown in Fig. 1. The chloride halogen has since been used as a substituent in many drugs. Literature has shown that this functional group possesses halogen bonding in biological systems, where halides act as Lewis acids and therefore get

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attracted to any electron donating groups around the chlorine bearing molecule [4-7]. This means that the chloride bearing compound will hypothetically have affinity to electron-rich donor atoms in biomolecules such as oxygen, sulphur, nitrogen and phosphorus. This has previously been demonstrated by Hardegger et al., who showed that the binding affinities of inhibitors of cathepsin L and MEK1 kinase increased with the introduction of halogens (bromine, chlorine and iodine) to the aryl ring as substituents [8]. Chlorinated drugs also improve the chances of drug-membrane permeability because of the polarization of the halogen bond in the main molecule [9].

The ester functional group is known to improve biological activity of drugs and this has been linked with various chemical properties of this functional group, such as its ability to be hydrolysed by esterase enzymes. This property makes it possible for ester group-containing drugs to act as pro-drugs and thus deliver the active molecule to the target biomolecule [10,11]. Furthermore, ester groups are used to mask highly polar functional groups such as alcohols, phenols and carboxylic acids to increase the ability of the drugs to cross cell membranes [11,12]. The amine group is another important pharmacophore that may increase antibacterial activity of compounds by targeting the folic acid biosynthetic pathway. Folic acid is an important biomolecule in bacteria, which is critical for synthesis of nucleic acid and proteins [13]. Subsequently, compounds containing an accessible amine group are hypothesized to compete with Para-aminobenzoic acid (PABA) as competitive antagonists to dihydropteroate synthase (DHPS) enzyme on the folic acid biosynthesis pathway. This means that the production of proteins, nucleic acids and the growth of the bacterial cell will be affected. Compounds bearing the nitro group have mixed reactions, in literature, because they sometimes bear toxicity issues, but also have some important antibacterial properties. One important mode of action of this pharmacophore is the reduction of the nitro moiety by the nitroreductase enzymes, where reactive oxygen species are released and affect the integrity of the cell wall or membrane. These reducing agents include the flavin nucleotides, and nicotinamide adenine dinucleotide [14,15]. The Drugbank website has an array of different nitro-containing antibacterials with various modes of actions. These include cell wall or membrane disruption, enzyme binding and inactivation, genomic material binding compounds and so many more [16]. This pharmacophore is ideal in comparison to the amino group, and this study is expected to show the efficacy of the amino and the nitro groups. Aromatic compounds bearing these functional groups have bioactive properties which include lipophilicity and genomic material intercalation [17-20]. Thus, we hereby describe the synthesis, characterization and preliminary antibacterial activity of the compounds coined **L1, L2, L3** and **L4** as potential lead compounds towards the fight against drug resistant bacteria.

Materials and methods

Materials and Instrumentation

All chemicals for synthesis and biological reagents were purchased from Merck Sigma Aldrich (USA) and used without further purification. The drug resistant sample organisms Methicillin-resistant *Staphylococcus aureus* (MRSA), Multi-Drug resistant *K. pneumoniae* (MDRKP) and *K. pneumoniae* were clinical isolates while *S. aureus* ATCC 25931 was a standard reference organism that were all donated by the National Health Laboratory, Botswana, Gaborone. The neat film infrared spectra of the compounds were recorded in the 4000–500 nm region using a PerkinElmer System 2000 FTIR spectrometer (Perkin Elmer USA). ^{1}H NMR spectra were acquired in 5 mm NMR tubes at 298–310 K using a Bruker 400 MHz spectrometer in the range 0–10 ppm. Solvents used were deuterated chloroform with the reference material as TMS (Bruker Daltonics, Germany). The data were processed on MestReNova version 9.0.1–13254. Elemental analyses (CHN) were performed with a model 240 Perkin Elmer elemental analyzer (PerkinElmer, USA).

Synthesis

The compound labelled **L1** was synthesized following our previous studies on the crystal structure report [21] while **4PY** was a commercial compound. The synthesis of the other compounds is described in sections 2.2.1–2.2.3, and their synthetic scheme is shown in Fig. 2.

*Synthesis of 4-(pyridine-4-ylmethoxy)aniline **L2***

p-Acetaminophen (2.0 g, 13.2 mmol), potassium hydroxide (1.86 g,

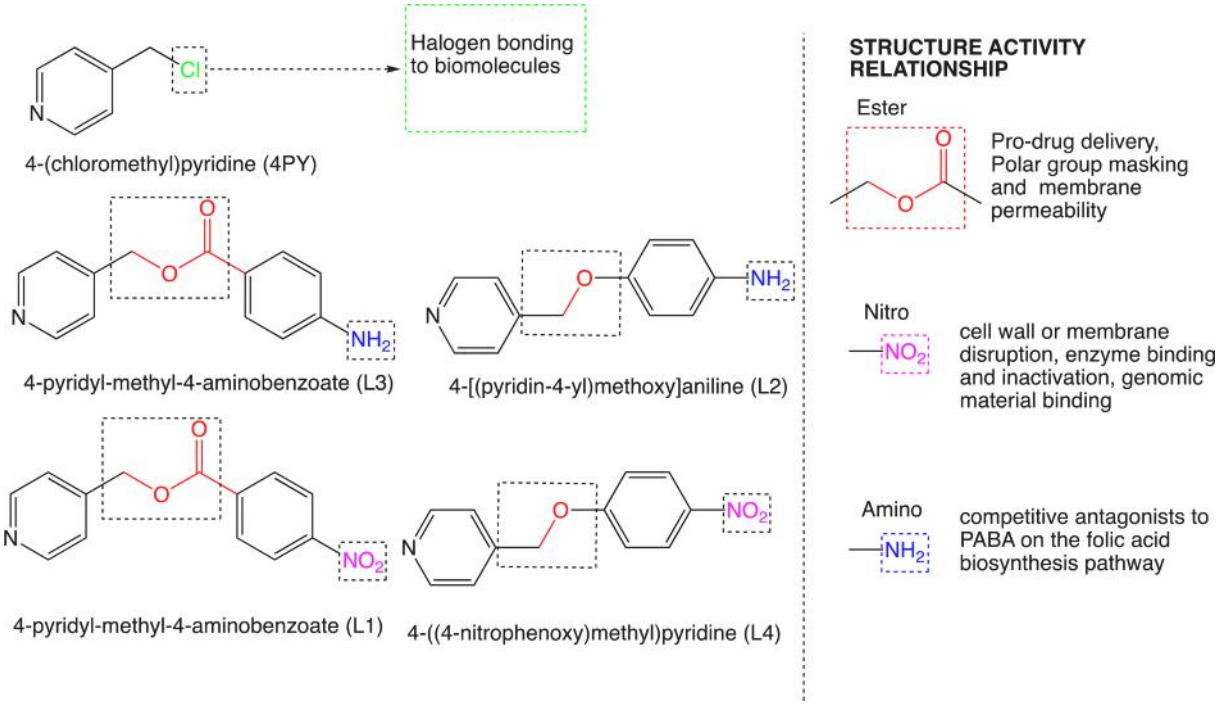

Fig. 1. The synthesized compounds and their important pharmacophores for enhanced antibacterial activity.

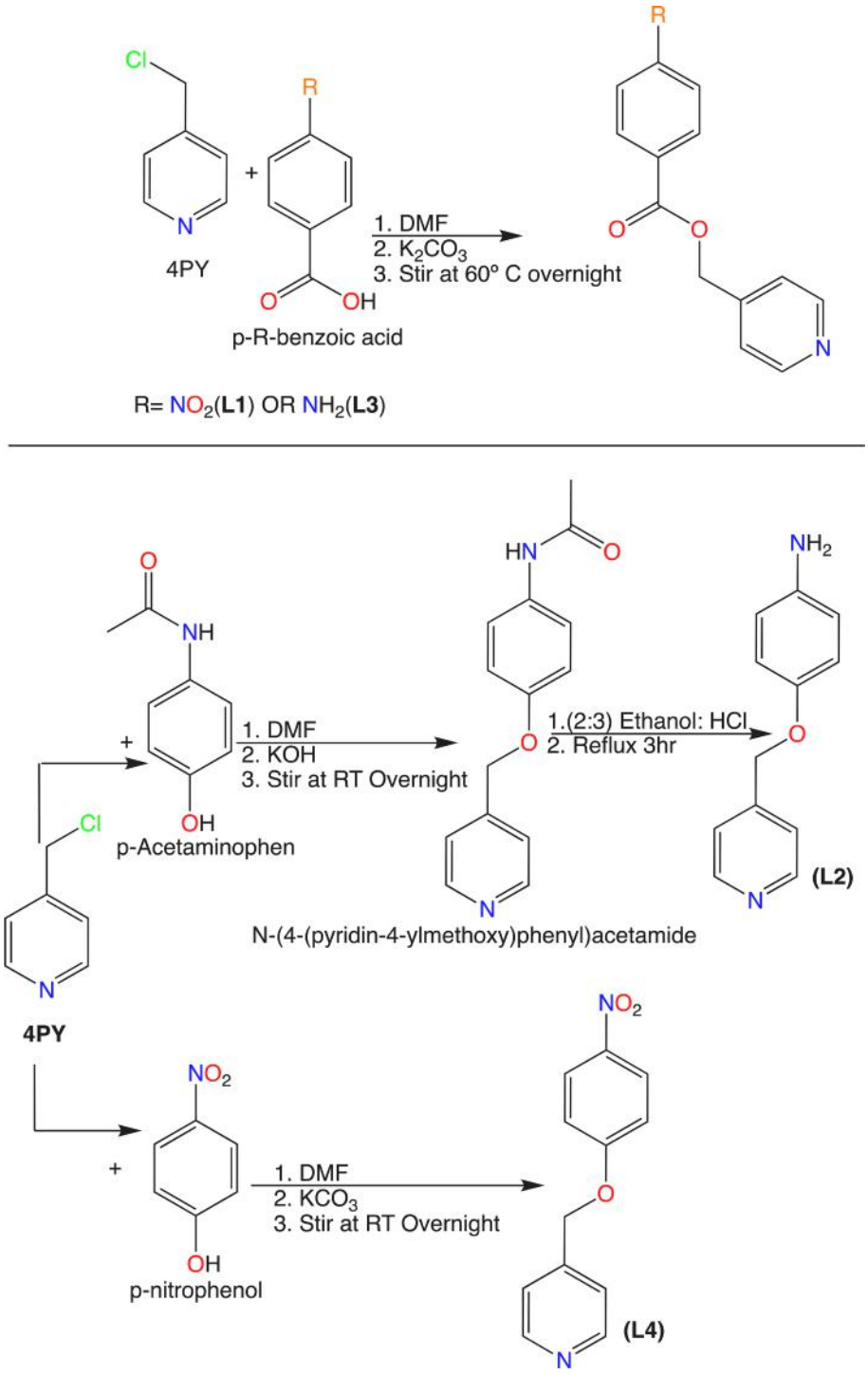

Fig. 2. The stepwise synthetic scheme of the compounds **L1, L2, L3 and L4.**

33.1 mmol) and dimethylformamide, DMF (20 ml) were charged into a 250 ml round bottom flask, thereafter 4-chloromethylpyridine hydrochloride (2.17 g, 13.2 mmol) dissolved in 10 ml DMF was added. The reaction proceeded at room temperature for 4 hr. After this, water was added slowly to the mixture and the white precipitated crystals were then filtered. To acquire the amine group, the acetamide moiety was hydrolyzed. The hydrolysis was done in 20 ml ethanol and 30 ml concentrated hydrochloric acid. The refluxing was maintained for 3 h after which the reaction mixture was allowed to cool and neutralized to pH 7 using Sodium hydrogen carbonate and the reaction mass was allowed to stand overnight to give the title compound 2.00 g, yield 76.4 %. IR (υ_{max} /cm^{-1}): (C═C) 1507.1, (C-O) 1232.1, (Ar-N) 1463.2, (O-C-O) 1094.6, (1° N–H) 3297.6–3363.2. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (dd, J = 5.1, 1.3 Hz, 2H), 7.35 (s, 2H), 6.79 (m, 2H), 6.64 (m, 2H), 5.02 (s, 2H), 3.74 (s, 2H). Anal. Found (%) for $C_{13}H_{14}N_2O$·0.55p-Acetaminophen: C, 69.342%; H, 5.9725 %; N, 12.8085 %. Calcd (%) C, 69.51 %; H, 6.03 %; N, 12.6 %.

Synthesis of pyridin-4-ylmethyl 4-aminobenzoate ***L3.*** 4-Aminobenzoic acid (2.8 g, 20.4 mmol) was dissolved in 25 ml of DMF. Potassium carbonate (2.8 g, 20.4 mmol) was then added to the resulting mixture before being stirred for 30 min with an anhydrous calcium chloride

drying tube. Then to the stirred white precipitate, 4-chloromethylpyridine hydrochloride (3.3 g, 20.4 mmol) dissolved in 25 ml DMF was added quickly before covering with a dry calcium chloride drying tube, the contents were then stirred overnight at 70 ℃. Afterwards, the maroon precipitate was cooled and neutralized with 10 % sodium hydrogen carbonate. The mixture was extrated three times with ethyl acetate. The brown product was then air dried, before characterization. 4.45 g, yield 96%. IR (υ_{max} /cm^{-1}): (C═C) 1520.8, (C-O) 1265.2, (Ar-N) 1439.8, (O-C═O) 1682.3, (1° N–H) 3308.7–3402.5. ^{1}H NMR (400 MHz, Acetone): δ 8.62 (m, 2H), 7.95 – 7.88 (m, 2H), 7.34 (m, 2H), 6.71 – 6.63 (m, 2H), 5.33 (s, 2H), 4.12 (s, 2H). Anal. Found (%) for $C_{13}H_{12}N_2O_2$, ·1.6 DCM: C, 47.52 %; H, 3.73 %; N, 8.54 %. Calcd (%) C, 48.16 %; H, 4.21 %; N, 7.69 %.

*Synthesis of 4-((4-nitrophenoxy) methyl)pyridine **L4**.* 4-nitrophenol (1.5 g, 10.7 mmol), potassium carbonate (5.6 g, 40.5 mmol) and dimethylformamide (20 ml) were charged into a 250 ml round bottom flask, thereafter 4-chloromethylpyridine hydrochloride dissolved (1.77 g, 10.7 mmol) in 10 ml DMF was added. The reaction proceeded at room temperature for 4 hr. After this, water was added to the mixture and white precipitate crystals formed. These crystals were then filtered. To acquire the compound 2.00 g, 81 %. IR (υ_{max} /cm^{-1}): (C═C) 1502.9, (C-O) 1265.9, (Ar-N) 1459.5, (O-C-O) 1179.2. ^{1}H NMR (400 MHz, Acetone): δ 8.55 (m, 2H), 8.19 (m, 2H), 7.43 (m, 2H), 7.21 (m, 2H), 5.34 (s, 2H). Anal. Found (%) for $C_{13}H_{12}N_2O_3$ ·0.8 K_2CO_3: C, 45.0685 %; H, 3.1425 %; N, 8.784 %. Calcd (%) C, 45.11 %; H, 2.96 %; N, 8.22 %.

Minimum Inhibitory Concentration Screening (MIC). The *in vitro* antibacterial activity of the compounds was tested by broth microdilution method against the bacterial strains *K. pneumoneiae*, MDRKP, *S. aureus,* and MRSA. Eight concentrations of each of the test compounds were serially diluted: (0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.00390625) mM. Bacterial inocula, with an incubation time not over 6 h, was adjusted to the 0.5 McFarland standard and further diluted down to 5 × 10^5 CFU/ml using Mueller Hinton Broth (MHB). For the determination of the MIC, MHB was used for serial dilutions to make up a final volume of 100 µl in 96-well plates, and 100 µl of bacterial solution was added to each solution. The bioassays experiment was analyzed in triplicate using a 96 well microplate reader. Then finally the assay was analyzed, graphed, and statistically evaluated using GraphPad prism.

Time kill kinetics assay

The *in vitro* growth analysis of the bacteria with the best performing compounds (those with MIC < 0.3 mM) was tested against the bacterial species *K. pneumoniae*, MDRKP, *S. aureus,* and MRSA following a previously used method with some ammendments [22]. In brief, the time kill assay was done using the compounds at their MIC using optical density (OD) measurements to assess whether the compounds were bacteriostatic or bactericidal. The bacterial isolates were grown to McFarlan standard. Thereafter the cultures were diluted to 5 × 10^5 CFU/ml using nutrient broth (NA). The compounds were dissolved and loaded in 96 well plates that were pre-loaded with nutrient broth, with some wells left without treatment for vehicle control. The diluted cultures were added to the wells and incubated at 37 °C and thereafter the plates were run on a plate reader at time intervals of 0, 1, 2, 3, 4, 5, 6 and 24 hr. The procedure was performed in triplicate and a graph of the $\log_{10}$ % viability at the active MIC was plotted against time using Graphpad Prism.

Results and discussions

Herein, the focus is on four potential compounds as leads towards development of novel products against multi-drug resistant bacteria. The compound **L3** has not been described before, while **L2** and **L4** have been synthesized before in various patents as a precursor to other compounds [23,24]. Nonetheless, all these compounds antibacterial activity towards *K. pneumoniae*, MDRKP, *S. aureus*, and MRSA has not been described before.

Synthesis

The two ester compounds were prepared through the o-alkylation of the carboxylic oxygen in both *p*-nitrobenzoic acid and *p*-aminobenzoic acid. Since *p*-chloromethyl pyridine possesses a primary alkyl halide, the reaction was designed to favour S_N2 mechanism which was achieved by using a polar aprotic solvent dimethylformamide. Since potassium carbonate is a weak base, the *p*-nitrobenzoic acid or aminobenzoic acid were stirred with the base to deprotonate the acid prior to adding the *p*-chloromethyl pyridine. To ensure complete alkylation, the reaction was stirred at a 70 °C overnight. The compound **L1** showed an impurity of the *p*-nitrobenzoic acid through TLC which was alleviated by limiting *p*-nitrobenzoic acid and adding the base in a little excess to accommodate the acidic *p*-chloromethyl pyridine hydrochloride. This amendment proved to reduce the impurity of the compound where there was an observation of golden crystals of **L1**. On the other hand, **L3** was obtained as a pure yellow oily substance that easily crystalized when mixed with methanol when left to dry. The compounds **L1** and **L3** both formed at high yields of 94.1 and 96% respectively. Furthermore, **L2** was synthesized through the o-alkylation of the *p*-Acetaminophen by *p*-chloromethyl pyridine. The use of *p*-Acetaminophen was due to the competitive nucleophilicity of the oxygen and nitrogen moieties in *p*-aminophenol. Thus, the amine group in *p*-aminophenol was protected by the acetamide moiety by forming *p*-Acetaminophen. The alkylation was proposed to follow an S_N2 reaction mechanism, this was because of the use potassium hydroxide which is a strong base, a primary alkyl-halide *p*-chloromethyl pyridine, and the use of a deprotonated *p*-Acetaminophen ion which is a strong nucleophile. Slight excess of potassium hydroxide was used to deprotonate the hydroxyl group in *p*-Acetaminophen. Thereafter, the nucleophilic oxygen attacks the carbocation on the *p*-chloromethyl pyridine. After the alkylation, the deprotection was archived by the acid hydrolysis of the amide group to amine group. As for **L4**, the same procedure for the preparation of **L2** was followed, but because **L4** is a nitro analogue of **L2**, there was no need for the protection of this group and the reaction was done using *p*-nitrophenol. **L2** was recovered as a brown powder with a yield of 76.4% while **L4** was recovered as a grey solid at a good yield of 81%.

Characterization

The following expected functional groups were observed in these compounds FTIR data: the aromatic (C–H) vibration at frequencies 3082 cm^{-1}; the aromatic (C═C) vibrations at frequencies 1507.1 cm-1, the (Ar-N) vibrations at frequencies 1463.2 cm^{-1}, and the (C-O) vibration, which was a sharp and strong peak at 1232.1 cm^{-1}. The primary amine (N–H) vibration stretches at 3297.6–3363.2 cm^{-1} exhibited that the hydrolysis did take place. These functional groups indicated the formation of **L2** compound. The **L4** compound also showed its important functional groups from its spectra, the aromatic (C–H) vibration was observed at frequencies 3083.5 cm^{-1}; the aromatic (C═C) vibrations at frequencies 1502.9 cm-1, the (A-N) vibrations at frequencies 1459.5 cm^{-1}, and the (C-O) vibration, which was a sharp and strong peak at 1265.9 cm^{-1}. The nitro vibration band was also observed at 1341–1445 cm^{-1}. As for the amine analogue **L3**, all the necessary functional groups were also observed. The aromatic (C–H) was assigned to 3182.6 cm^{-1} while the (C═C) aromatic stretch was assigned to the 1646.4 cm^{-1} peak. The ester peak was observed at the 1682.3 cm^{-1} peak. Furthermore, the aromatic amine group was observed at 3308.7–3402.5 cm^{-1}. Finally, the (Ar-N) pyridinyl peak at 1439.8 cm^{-1}.

The compound **L3** showed an impurity of the dichloromethane used for extraction after the reaction. This was corrected by using ethyl

acetate as the alternative solvent. Both **L2** and **L4** showed some negligible quantity of impurities of the starting material, or the base used in the reaction. **L2** showed an impurity of *p*-Acetaminophen, and this was corrected by running the reaction longer while for **L4** the compound was washed with hot distilled water to get rid of the potassium carbonate base. However, correction of the elemental analytic data with these impurities shows the successful synthesis of the two compounds. Nuclear magnetic resonance spectroscopy was employed to elucidate the structure of the compounds. The proton NMR spectra of the compound **L3** showed the chemical shifts of the expected protons in the compound. The amino ester compound **L3**, showed a nudge at 4.12 ppm which was assigned to the amino group protons. There was a diagnostic singlet methyl shift at 5.33 ppm. This signal was also observed at downfield as it was deshielded by the ester moiety. The more deshielded protons, such as those close to the pyridyl nitrogen were observed further downfield on the spectrum. The proton NMR spectra of the nitro and amino ether analogues **L2** and **L4** also showed that the compounds were successfully synthesized. Considering the spectra of the nitro compound **L4**, a diagnostic methyl singlet peak was observed at 5.34 ppm, this deshieded methyl peak was assigned to the picolinic arm that bridges the nitrophenolate to the pyridyl moiety. Aromatic protons from the nitrophenolate moiety were observed more up field than the pyridyl protons. Similarly, the amino ether analogue **L2** diagnostic singlet peak was observed at 5.02 ppm. Also, the aminophenolate amine protons peak was observed at 3.74 ppm. Contrary to **L4,** the aromatic protons showed that **L2** has a different geometry, the ether compounds showed that the nitro analogue did not have the aromatic doublet of a doublet, while it was found in **L2** at 6.64–6.79 ppm. All the characterization are listed under the experimental section for each synthesis protocol of each compound.

Antibacterial studies

The synthesized compounds were tested for their preliminary antibacterial properties, to establish them as potential lead compounds against drug resistant bacteria. MIC assay was done to determine the minimum concentration needed to show bioactivity from the compounds. Silver sulfadiazine (**AgSD**) was used as a control because it is an approved antibiotic [25]. The **AgSD** showed better activity across all the organisms but the compounds **L2** and **L3** were more potent than **AgSD** towards *K. pneumoniea.* This drug is similar to **L2** and **L3** in terms of the amino pharmacophore in sulfadiazine. It is both a competitive inhibitor of bacterial PABA (through sulfadiazine) and affects both cell walls and cell membranes of bacteria (through the silver (I) ion) [25]. The compounds displayed better activity towards the Gram-negative bacteria as compared to the Gram-positives (Table 1). This activity was interesting because Gram-negative bacteria can create resistance via modifying the hydrophobic characteristics of the outer membrane, as well as mutations in porins and other variables. Gram-positive bacteria lack this layer, making Gram-negative bacteria more antibiotic-resistant than Gram-positive bacteria [26]. The difference in the effective concentrations towards the non-drug resistant and resistant *K. pneumoniae* strains were not entirely significant especially for **L1**, **L4** and **4PY**. Conversely, it is important to note that the bioactivity of the nitro compounds **L1** and **L4** showed less bioactivitity, while the amino derivatives **L2** and **L3** were more bioactive against *K. pneumoniae*. The observed bioactivity of the amino derivatives **L2** and **L3** corroborates with previous findings of a combination Trimethoprim–Sulfamethoxazole therapy against *K. pneumoniae*, which established amino based compounds for use against bacteria [27]. This indicates that the nitro and amino moieties were the important pharmacophores instead of the ether or ester in *K. pneumoniae*. The amino group may play an important role as a competitive anatagonist to DHPS than PABA in the production of folic acid, resulting in inhibition of the growth and survival of the bacteria [13]. On the contrary, the nitro moiety has many potential modes of action, such as disruption of cell wall or membrane, binding and inactivation of enzymes and binding genomic material [16]. Interestingly, the combination (Trimethoprim–Sulfamethoxazole) has been studied *in vitro* on various clinical isolates of *S. aureus*, and it was found to be potent as opposed to the ester and ether bridged compounds in [26,27]. Finally, the commercially available **4PY** compound is precipitated by hydrochloric acid (HCl) which reduces the pH of the media during growth. Consequently, this acid may have been responsible for the bioactivity in *K. pneumoniae* and not the compound itself, while the proliferation of *S. aureus* is tolerant to lower pH media as opposed to *K. pneumoniae* [28,29].

Compounds with MIC of less than 0.3 mM were further characterised by determining their time kill kinetics profiles with the different organisms. Time kill assays measures the effects on a population of bacteria over a period following exposure to an antimicrobial agent. Considering the time kill kinetics of the active compounds on the *K. pneumoniae* strain (Fig. 2), the amino based compounds **L2** and **L3** were observed to be growing exponentially for the first 6 h but stopped after 24 h at their MIC. This led to the conclusion that they were bacteriostatic. The nitro-based counterparts **L1** and **L4** initially showed a bactericidal trend at their MIC for 5 h, but after 24 h, we observed an exponential growth which signified bacteriostatic activity. The observed trends of **L1** and **L4** were consistent with conventional drugs bearing a nitro substituent which are associated with cell membrane disruption as a mode of action [16]. Amine-containing drugs have previously been shown to inhibit bacterial growth by competitive inhibition of DHPS, an enzyme involved in folic acid biosynthetic pathway [13]. Because of the presence of the amino substituent in **L2** and **L3**, the bacteriostatic effects were suspected to be due to the antagonism of the folic acid biosynthesis pathway, thus slowing the reproduction of the bacteria. Comparatively, the effects observed for **L2** and **L3** were similar to bacteriostatic effects of the control drug, **AgSD**, on *K. pneumoniae* after a 4-hour exposure to the drug. Of note, the control drug **AgSD** is known to be bactericidal due to the presence of the silver (I) ion, while the free sulfadiazine moiety is

Table 1
MIC of the compounds.

		MIC (mM)			
Compound↓	*Microbe→*	*K. pneumoniae*	MDRKP	*S. aureus*	MRSA
AgSD		0.18 ± 0.002	0.13 ± 0.02	0.13 ± 0.002	0.26 ± 0.06
4PY		0.19 ± 0.006	0.22 ± 0.003	0.57 ± 0.011	0.45 ± 0.04
L1		0.23 ± 0.03	0.23 ± 0.0022	0.52 ± 0.007	0.43 ± 0.03
L2		0.16 ± 0.02	0.24 ± 0.0008	0.37 ± 0.07	0.44 ± 0.007
L3		0.16 ± 0.02	0.23 ± 0.0005	0.37 ± 0.03	0.54 ± 0.05
L4		0.26 ± 0.05	0.22 ± 0.0008	0.51 ± 0.02	0.45 ± 0.05

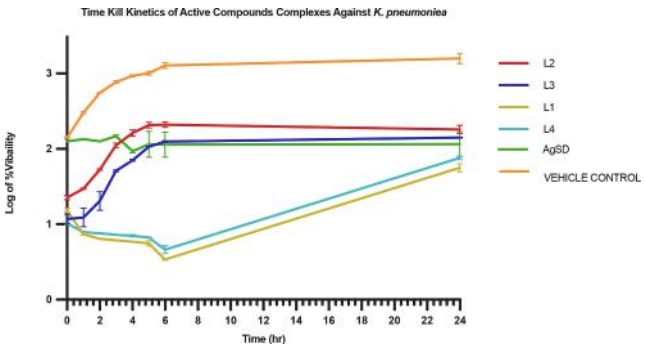

Fig. 3. Time kill kinetics of the compounds against *K. pneumoniae*.

bacteriostatic [25].

The time kill study of the active compounds on MDRKP shown in Fig. 3 were different compared to the non-drug resistant strain. The assay showed that all the active compounds were bactericidal against MDRKP at their measured MIC, unlike against *K. pneuoniae pneumoniae* where **L2** and **L3** were bacteriostatic. During the experiment, the activity of all the compounds started at 1 h, showing that the compounds had strong bactericidal effects towards MDRKP. Furthermore, this observation suggests that these compounds may have a different mode of action towards the drug resistant strain as opposed to the non-drug resistant strain. Even though the amino based compounds showed bacteriostatic activity towards the non-drug resistant strain, amino based combination therapy (Trimethoprim–sulfamethoxazole) have previously been shown to be bactericidal when tested on clinical isolates of *K. pneumoniae* [27]. The kinetics of the control drug **AgSD** towards MDRKP showed that it was bactericidal just as the other compounds (Fig. 4). Similarly to *K. pneumoniae,* the bactericidal activity started at 4 h of the experiment. This bactericidal observation against MDRKP suggests that indeed the bioactivities of **L2** and **L3** were similar to **AgSD**. In conclusion, more indepth studies are required to verify the mode of action of the compounds tested herein.

Conclusions

Compounds of a pyridyl moiety bridged by an ether or ester groups and derivatized by either a nitro or amino substituent were synthesized, characterized, and assessed for their potential antibacterial properties. This study was done to evaluate potential lead compounds in the fight against antibiotic resistance. The compounds were more active towards Gram-negative bacteria (i.e., *K. pneumoniae* and MDRKP) than Gram-positives (i.e., *S. aureus* ATCC 25931 and MRSA) with **L2** and **L3** being more potent against *K. pneumoniae* than **AgSD**. Furthermore, the time kill kinetics of the Gram-negative bacteria showed converse results, that is, for the drug resistant bacteria all the compounds were observed to be bactericidal. However, for the non-drug resistant bacteria, the amino group compounds **L2**, **L3** and the control **AgSD** were observed to be bacteriostatic while the nitro group compounds **L1** and **L4** were bactericidal. Finally, the reported potent concentrations of the compounds qualify them as good candidates or lead compounds for the development of new antimicrobials. We wish to further study these compounds' toxicity towards normal mammalian cells, their folic acid biosynthesis inhibition, as well as cell membrane disruption against the *K. pneumoniae* strains, to determine their possible mode of action.

Supplementary materials

The characterization and biological assay data used to support the findings of this study are summarized within the article in tables and graphs. While the Supplementary Materials include the spectral data of the compounds.

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CRediT authorship contribution statement

James T.P. Matshwele: Writing – original draft, Conceptualization, Data curation. **Mosimanegape Jongman:** Methodology. **Moses O. Koobotse:** Visualization. **Ofentse Mazimba:** Conceptualization. **Daphne Mapolelo:** Writing – review & editing. **David O. Nkwe:** Writing – review & editing. **Florence Nareetsile:** Writing – review & editing. **Sebusi Odisitse:** Supervision, Validation.

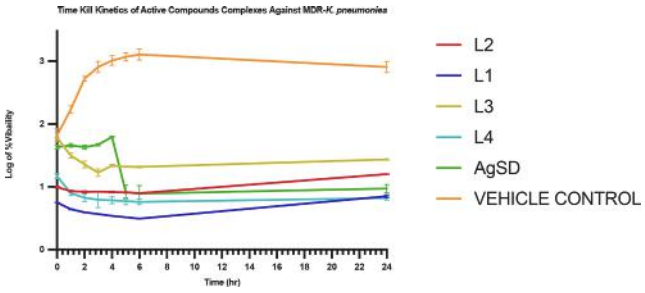

Fig. 4. Time kill kinetics of the compounds against MDR-*K. pneumoniae*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2022.100401.

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