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Synergistic effect of tryptamine-urea derivatives to overcome the chromosomally-mediated colistin resistance in *Klebsiella pneumoniae*

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ABSTRACT

Colistin is considered as the last-resort antibiotics to treat multi-drug resistant Gram-negative bacterial infections in humans. However, the clinical use of colistin was limited because of the apparition of chromosomal mutations and mobile colistin resistance genes in bacterial isolates. One promising strategy is to combine existing antibiotics with promising non-antibiotics to overcome the widespread emergence of antibiotic-resistant pathogens. Moreover, colistin resistance would be regulated by two component systems PhoP/PhoQ which leads to permanent synthesis of cationic groups compensating for Mg^{2+} deficiency. In this study, the synthesis of a small library of tryptamine urea derivatives was carried out. In addition, antibiotic susceptibility, antibiotic adjuvant screening and checkerboard assays were used to investigate the antibacterial activity of these synthesized compounds and the potential synergistic activity of their combination with colistin. Conformational analysis of the docked binding modes of the active compound in the predicted binding pocket of bacterial response regulator PhoP were carried out, to see if the active compound inhibits PhoP which is involved in colistin resistance. Finally, hemolytic activity studies have been conducted on the most active compound.

1. Introduction

Antibiotic resistance rates in Gram-negative bacteria are rising worldwide and represent a serious global crisis[1,2]. The emergence of multi-resistant and highly resistant Gram-negative bacteria with multiple resistance mechanisms is a major threat, as these pathogens are resistant to almost all clinically available antibiotics[3,4]. Since there are few alternatives for treating infections caused by drug-resistant pathogens, and the development of new antibiotics is slow, colistin is generally used and considered a last resort treatment[5]. Indeed, colistin (also known as polymyxin E) is prescribed for the treatment of severe human infections caused by multidrug-resistant Gram-negative bacteria in particular *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), which are resistant to carbapenems[6,7]. Unfortunately, the emergence of colistin-resistant bacteria is due to the increased use of colistin without appropriate regulation in livestock[8,9]. Until the end of 2015, resistance to colistin was only associated with changes at the level of genes or regulatory regions located on the bacterial chromosome (known as chromosomally-mediated resistance) [10,11]. At this date, a colistin resistance gene, called *mcr-1*, was described for the first time on a conjugative plasmid from *E. coli* in the veterinary environment (known as plasmid-mediated resistance)[12,13]. Polymyxins target the outer membrane of Gram-negative bacteria that possess lipopolysaccharide (LPS) on their outer surface. LPS consists of lipid A, with 2 glucosamine residues linked to phosphorylated fatty acids and is consequently negatively charged. The negative charges are stabilized by divalent cations (Mg^{2+} and Ca^{2+}). A low Mg^{2+} level leads to the constitutive activation of two component systems (TCS) particularly PhoP/PhoQ which in turn leads to permanent synthesis of cationic groups such as phosphoethanolamine and/or 4-amino-4-deoxy-L-arabinose. These cationic groups bind to lipid A leading to colistin resistance[14]. In this context, drug repurposing, which consists of the use of a non-antibiotic compound to treat resistant bacteria was used as alternative solution.

Therefore, there is an urgent need to identify novel strategies to overcome colistin resistance in *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

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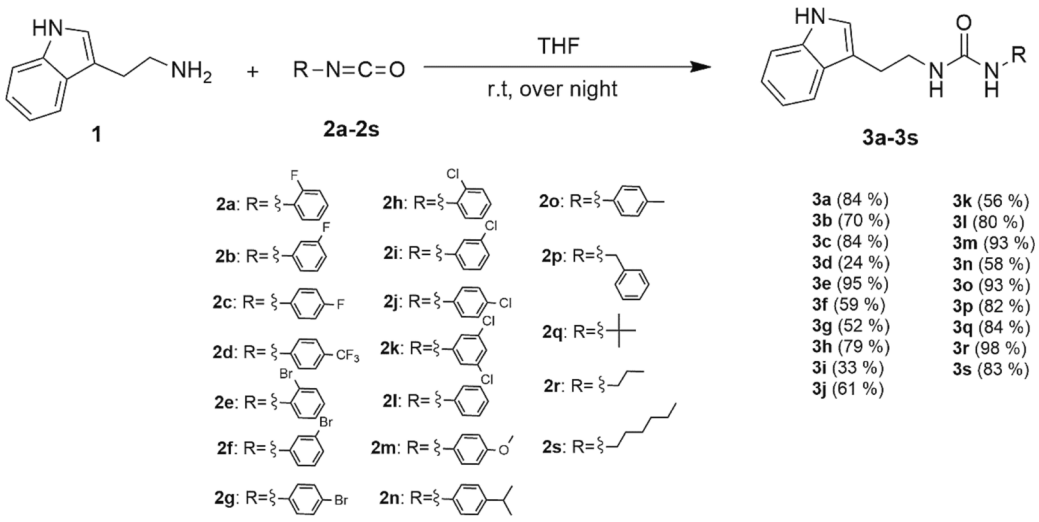


Scheme 1. Synthetic route of compounds **3a-3s.**

One promising strategy is to combine existing antibiotics with non-antibiotic called adjuvant[15]. Several library screenings of non-antibiotic compounds have been carried out to identify antibacterial agents or colistin adjuvants against colistin-resistant strains[16,17]. Tryptamine, an ubiquitous active scaffold comprising an indole ring system, has been identified by screening for its ability to effectively neutralize colistin resistance in Gram-negative pathogenic bacteria[18,19]. In addition, various compounds containing the tryptamine scaffold have been identified as inhibitors of TCS response regulator PhoP[20]. Moreover, urea function was also identified several times as a beneficial function to combat colistin-resistant bacteria[21]. In this context, we synthesized a series of tryptamine-urea derivatives which have been evaluated both as antibacterial agents and as colistin adjuvant against plasmid-mediated *mcr-1* colistin resistance strains of *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Moreover, conformational analysis of the docked binding modes of the active compound in the predicted binding pocket of PhoP were carried out, in order to see if the active compound inhibited PhoP which is involved in colistin resistance. Finally, hemolytic activity studies have been conducted on the most active compound.

2. Results and discussion

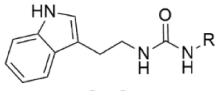
2.1. Chemistry

The synthetic route of compounds **3a-3s** is described in Scheme 1. The compounds of the present work were synthesized following the protocol of S. Zemolka *et al* 2008 and Kanwal *et al* 2019 with some modifications[22,23]. Tryptamine was used as starting material which reacted with the different derivatives of isocyanate to offer compounds **3a-3s** in moderate to a good yield (24–98%). The moderate yields for compounds **3d**, **3g** and **3i** are due to the recrystallization step, which has led to a loss of product mass. We report for the first time the synthesis of compounds **3a**, **3b**, **3e**, **3k**, **3n**, **3o** and **3s** using tryptamine and isocyanate derivatives as starting material. Some of these compounds (**3d, 3i**, **3j**, and **3l**) have been synthesized previously and evaluated for various biological activities[24,25]. The final compounds **3a-3s** were fully characterized by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, HMBC, and infrared (IR) spectroscopy; high-performance liquid chromatography (HPLC); and high-resolution mass spectrometry (HRMS). The spectroscopic data of the compounds are consistent with the assigned structures (see Supporting information).

2.2. In vitro antibacterial activity

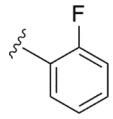
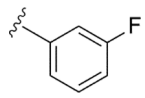
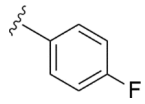
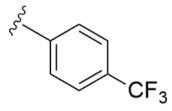
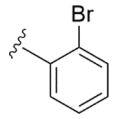
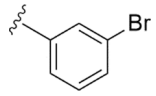
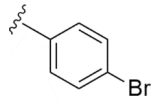
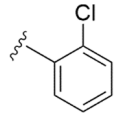
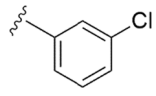
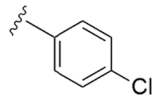
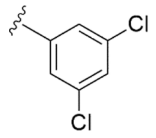
In this work, we studied the antibacterial activity of the synthesized tryptamine-urea derivatives **3a-3s** and the colistin, the adjuvant activity of compounds **3a-3s** and the potential synergistic activity of their combination with colistin. To our knowledge none of the synthesized tryptamine-urea derivatives **3a-3s** has been evaluated for their antibacterial properties or colistin adjuvant activity against plasmid-mediated *mcr-1* colistin resistance strains of *E. coli* (NTCC13846, A50) and *P. aeruginosa* (15080, 10039 and 1002) and chromosomally-mediated resistance strain of *K. pneumoniae* (BA03057) used in this

Table 1
Antibacterial susceptibility testing tryptamine-urea derived **3a-3s** and colistin against reference sensitive *E. coli* ATCC25922, *K. pneumoniae* ATCC700603 and *P. aeruginosa* ATCC27853 (PA) and colistin clinically resistant *EC* NTCC 13846, ECA50, *K. pneumoniae* BA03057 and *P. aeruginosa* (15080, 10039 and 1002).

	MIC[a] (μg/mL)								
	EC[b]	KP[b]	PA[b]	EC NTCC 13846[c]	EC A50[c]	KP BA03057[d]	PA 15080[e]	PA 10039[e]	PA 1002[e]
 **3a-3s**	>128	>128	>128	>128	>128	>128	>128	>128	>128
Colistin	0.25	0.5	2	2	8	64	2	4	2

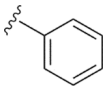
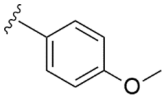
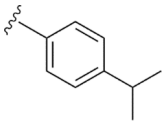
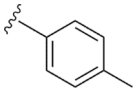
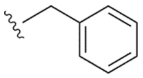
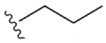
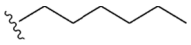
[a] MIC representing mean values of at least three replicates. [b] EC: *E. coli* ATCC25922, KP: *K. pneumoniae* ATCC700603, PA: *P. aeruginosa* ATCC27853. [c] EC NTCC 13846 and ECA50 are the colistin plasmid-mediated *mcr-1* colistin resistance strains. [d] KP BA03057 is chromosomally-mediated resistance *K. pneumoniae* strain. PA 15080, 10039 and 1002 are chromosomally-mediated colistin resistance *P. aeruginosa* strains.

Table 2
Colistin adjuvant activity of compounds **3a**-**3s** at 100 μM against colistin clinically resistant *E. coli* (EC A50) and *K. pneumoniae* (KP BA03057). Calculated lipophilicity of the prepared compounds (cLogP) and fraction of sp3 carbon atoms (Fsp3).

Compound				EC A50		KP BA03057	
	R	cLogP	Fsp3	MIC of Colistin (μg/mL)	Potentiation Fold	MIC of Colistin (μg/mL)	Potentiation Fold
	–			8	–	64	–
3a		3.19	0.12	8	1	8	1/8
3b		3.05	0.12	4	1/2	1	1/64
3c		3.01	0.12	4	1/2	8	1/8
3d		3.78	0.17	8	1	2	1/32
3e		3.54	0.12	4	1/2	2	1/32
3f		3.37	0.12	8	1	8	1/8
3g		3.36	0.12	2	1/4	8	1/8
3h		3.46	0.12	8	1	8	1/8
3i		3.33	0.12	8	1	2	1/32
3j		3.24	0.12	4	1/2	4	1/16
3k		3.80	0.12	2	1/4	2	1/32

(*continued on next page*)

Table 2 (*continued*)

Compound	R	cLogP	Fsp3	EC A50		KP BA03057	
				MIC of Colistin (μg/mL)	Potentiation Fold	MIC of Colistin (μg/mL)	Potentiation Fold
3l		2.69	0.12	8	1	8	1/8
3m		2.74	0.17	4	1/2	16	1/4
3n		3.66	0.25	4	1/2	16	1/4
3o		3.07	0.17	4	1/2	8	1/8
3p		2.75	0.17	4	1/2	8	1/8
3q		2.35	0.40	8	1	8	1/8
3r		2.12	0.36	4	1/2	8	1/8
3s		3.16	0.47	4	1/2	2	1/32

work.

2.2.1. Antibiotic susceptibility testing against reference sensitive and colistin clinically resistant strains of E. coli, K. pneumoniae and P. aeruginosa

Tryptamine-derived ureas **3a-3s** were subjected to antibiotic susceptibility testing against reference susceptible strains *E. coli* ATCC25922, *K. pneumoniae* ATCC700603 and *P. aeruginosa* ATCC27853 and colistin resistant clinical *E. coli* NTCC 13846, *E. coli* A50, *K. pneumoniae* BA03057 and *P. aeruginosa* (15080, 10039 and 1002). The minimum inhibitory concentration (MIC) against the studied bacterial strains were determined using the broth microdilution assay according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST)[26]. Resistance to colistin is determined by broth microdilution assay against the epidemiological cut-off (ECOFF) as defined by EUCAST (susceptible $\leq$ 2 μg/mL, resistant > 2 μg/mL). It was observed that none of these compounds **3a-3s** exhibited significant antibacterial activity against all the studied Gram-negative bacterial strains (Table 1). The possible reason may be that compounds do not penetrate the bacterium because of its outer membrane and lack of access to compounds.

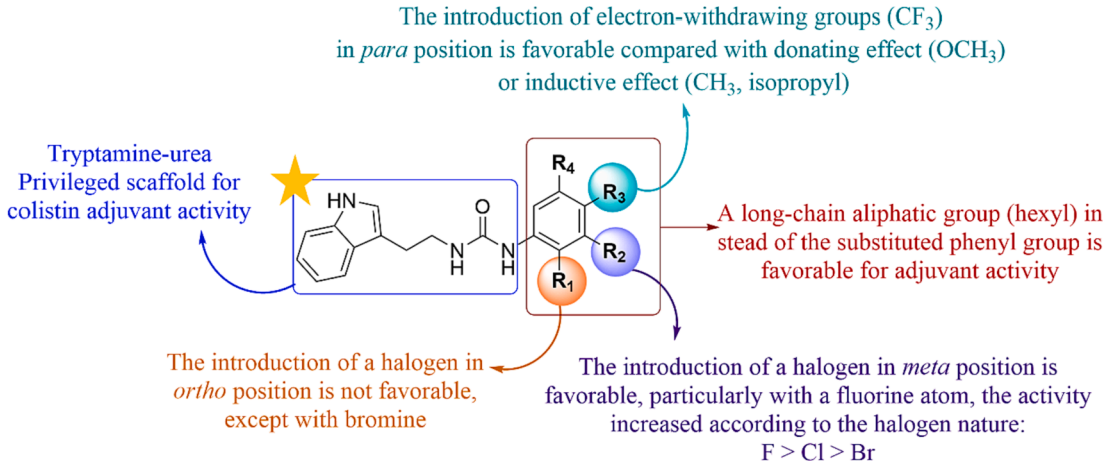


Fig. 1. SARs of tryptamine-urea derivatives against chromosomally-mediated colistin resistance *K.pneumoniae* strain (KP BA03057).

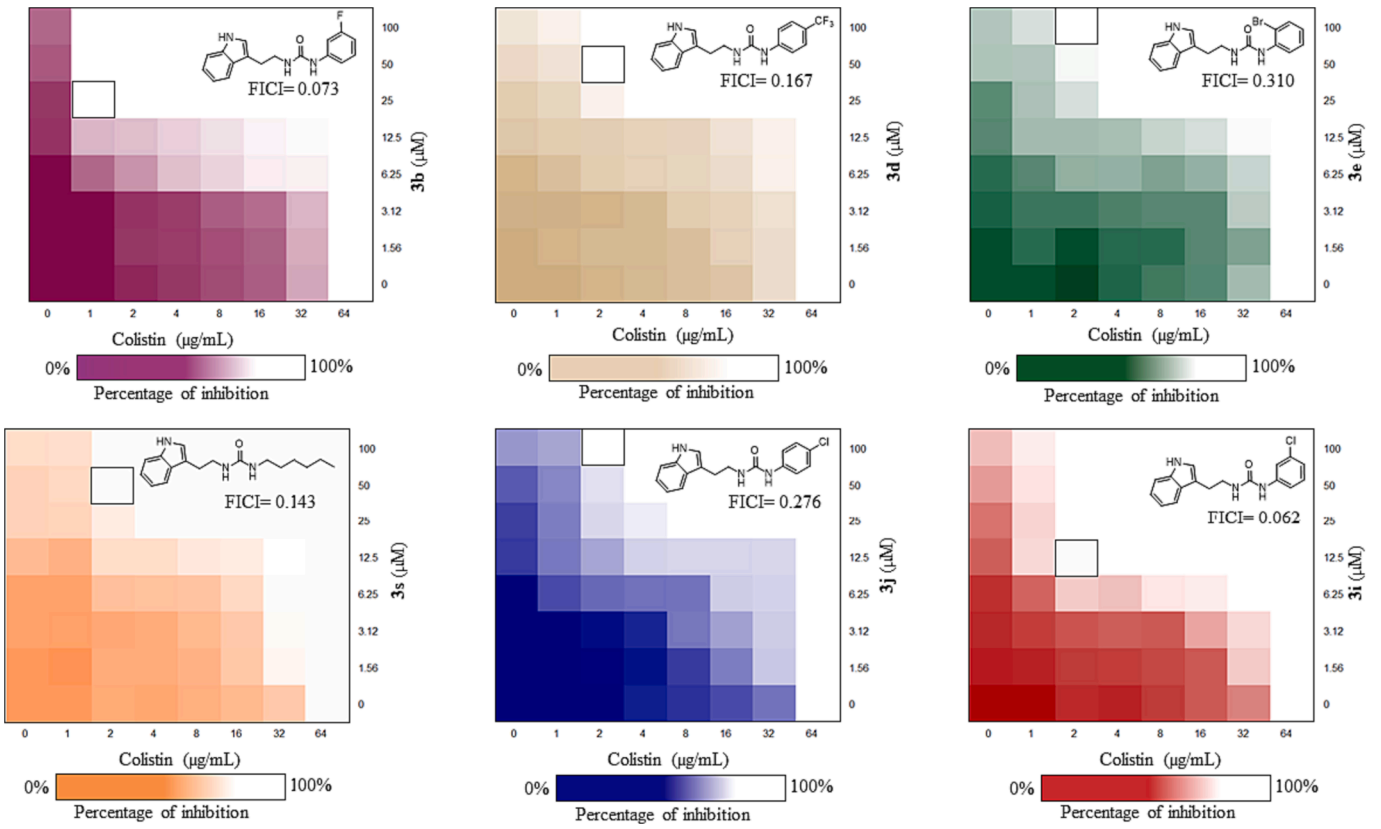


Fig. 2. Representative checkerboard assays for compounds **3d**, **3e**, **3i**, **3j**, **3s** and **3b** in combination with colistin against KP BA03057 strain. In each case, the bounded box in the checkerboard assays indicates the combination of compound and antibiotic resulting in the lowest FICI. The intense color indicates bacterial growth, white color indicates no bacterial growth.

*2.2.2. Colistin adjuvant activity of compounds **3a-3s** against colistin clinically resistant Gram-negative strains and structure–activity relationship (SAR)*

In this work, we evaluated the colistin adjuvant activity of tryptamine- urea derived **3a-3s** against plasmid-mediated *mcr-1* colistin resistance strains of *E. coli* (NTCC13846, A50) and chromosomally-mediated resistance strain of *K. pneumoniae* (BA03057) and *P. aeruginosa* (15080, 10039 and 1002). Compounds **3a-3s** are used in a fixed concentration (100 µM (24.5–35.7 µg/mL); at these concentrations no antibacterial activity was observed) in combination with serial dilution concentrations of colistin (2–0.004 µg/mL against EC NTCC 13846, PA 15080 and 1002, 8–0.015 µg/mL against EC A50, 64–0.125 µg/mL against KP BA03057 and 4–0.008 µg/mL against PA 10039). According to the obtained results, compounds **3a-3s** have shown a low colistin adjuvant activity against plasmid-mediated *mcr-1* colistin resistance EC A50 with colistin MIC potentiation from 8 to 4 µg/mL (2-fold) for compounds **3b**, **3c**, **3e**, **3j**, **3m-3p**, **3r** and **3s**, and from 8 to 2 µg/mL (4-fold) for compounds **3g** and **3k** (Table 2). Compounds **3a**, **3d**, **3f**, **3h**, **3i**, **3l** and **3q** have not shown any colistin adjuvant activity against EC A50 strains. All the synthesized compounds exhibited no colistin adjuvant activity against the plasmid-mediated *mcr-1* colistin resistance *EC* NTCC 13846 and PA 15080, 10039 and 1002. The most interesting colistin potentiation effect was observed against chromosomally-mediated resistance KP BA03057. Compounds **3a**, **3c**, **3f**, **3g**, **3h**, **3l**, **3m-3o** and **3p-3r** exhibited a low colistin adjuvant activity against KP BA03057 strains by reducing the MIC of colistin from 64 to 16 µg/mL (4-fold) for compounds **3m** and **3n**, and from 64 to 8 µg/mL (8-fold) for compounds **3a**, **3c**, **3f**, **3g**, **3h**, **3l**, **3o** and **3p-3r**. The colistin adjuvant activity against KP BA03057 becomes to be remarkable when the MIC of colistin is reduced from 64 to 4 µg/mL (compound **3j**, 16-fold). A significant reduction in MIC of colistin from 64 to 2 µg/mL (32-fold) was achieved when colistin was combined with compounds **3d**, **3e**, **3i**, **3k** and **3s**. Finally, compound **3b** showed the best colistin adjuvant activity in this study, offering the possibility to reduce the MIC of colistin from 64 to 1 µg/mL (64-fold) against KP BA03057.

Some conclusions can be deduced from the previous observations on colistin adjuvant activity of compounds **3a-3s** against chromosomally resistance KP BA03057 (Fig. 1). Firstly, a marked variation in adjuvant activity was observed between the halogen atoms and their respective positions on the phenyl group. Adjuvant activity was low when the substituent is a phenyl (**3l**). The colistin adjuvant activity of the synthesized compounds varies according to the nature and the position of substitution on the phenyl group. The bromine atom showed a favorable adjuvant activity (**3e**: 32-fold) compared to fluorine or chlorine (**3a** or **3h** respectively: 8-fold) when they were introduced in the *ortho* position of the phenyl group. Regarding *meta* position of phenyl group, fluorinated (**3b**), chlorinated (**3i**) and dichlorinated (**3k**) compounds showed the best adjuvant activity with a potentiation fold of 32 (**3i** and **3k**) and 64 (**3b**). Whereas adjuvant activity remained low when bromine (**3f**) occupied the same position with a potentiation fold of 8. Whatever the substituted atom or group (F (**3c**), Br (**3g**), Cl (**3j**), OCH_3 (**3m**), CH_3 (**3o**) or isopropyl (**3n**)) in the *para* position of the phenyl showed a low to medium adjuvant activity with a potentiation fold of 4 for compounds **3m** and **3n**, 8 for compounds **3c**, **3g** and **3o**, except for compound **3d** showing a high adjuvant activity with trifluoromethyl group (32-fold). It was observed that modification of phenyl (**3l**) by benzyl (**3p**) group had no influence on the colistin adjuvant activity which indicated that a conjugated system is not required for the adjuvant activity. This observation is also confirmed by the results obtained for compounds **3q** and **3r** (8-fold) with branched or unbranched aliphatic chains. Interestingly, when the substituent was a hexyl (**3s**), adjuvant activity increased significantly. By comparing the adjuvant activity of compounds **3q** (R =

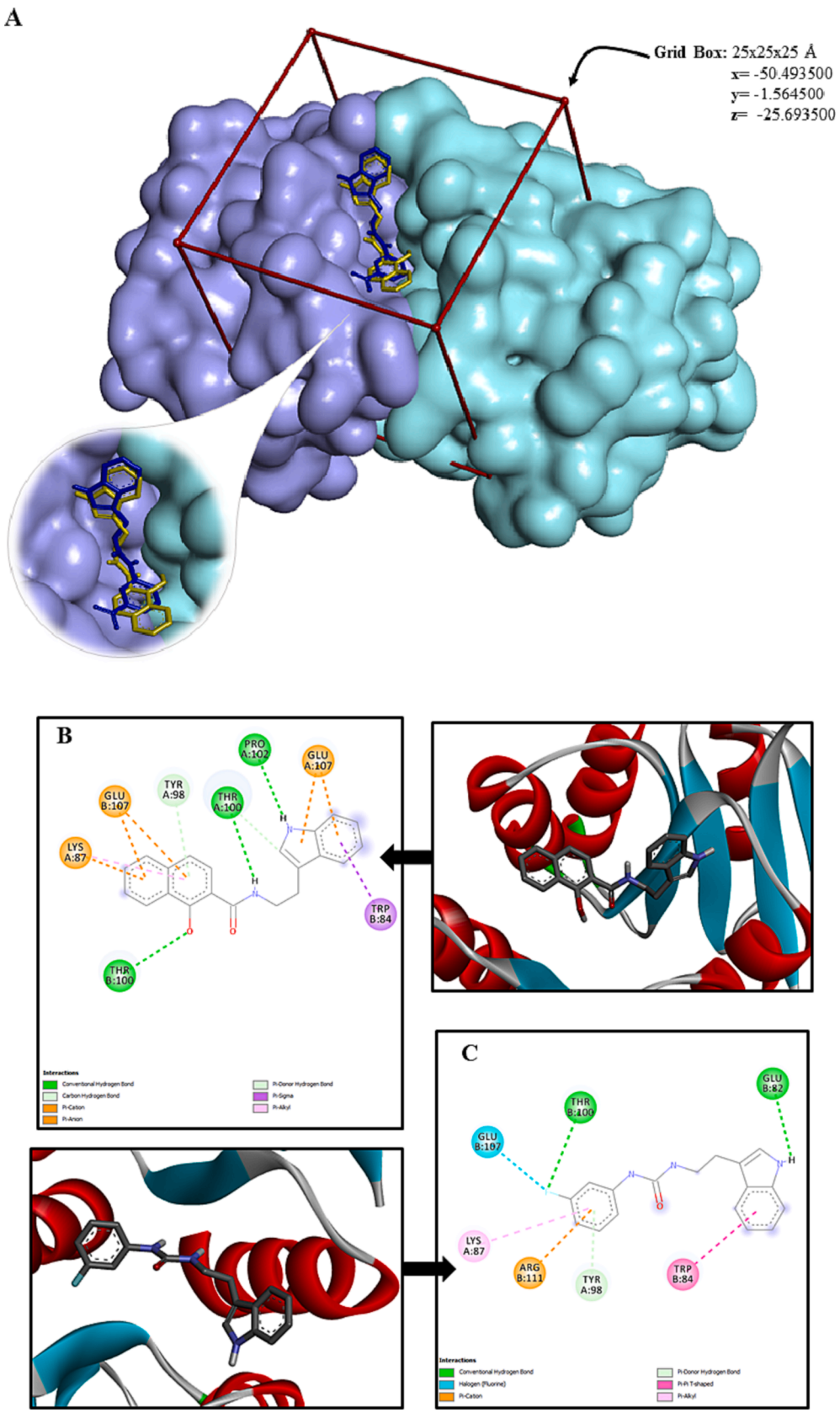


Fig. 3. A) Binding pocket of the homo-dimer PhoP of *E. coli* (PDB: 2PKX), and superimposed bond ligands: **A** (blue color) and **3b** (yellow color). B) and C) present the docked binding mode of compound **A** and **3b** respectively.

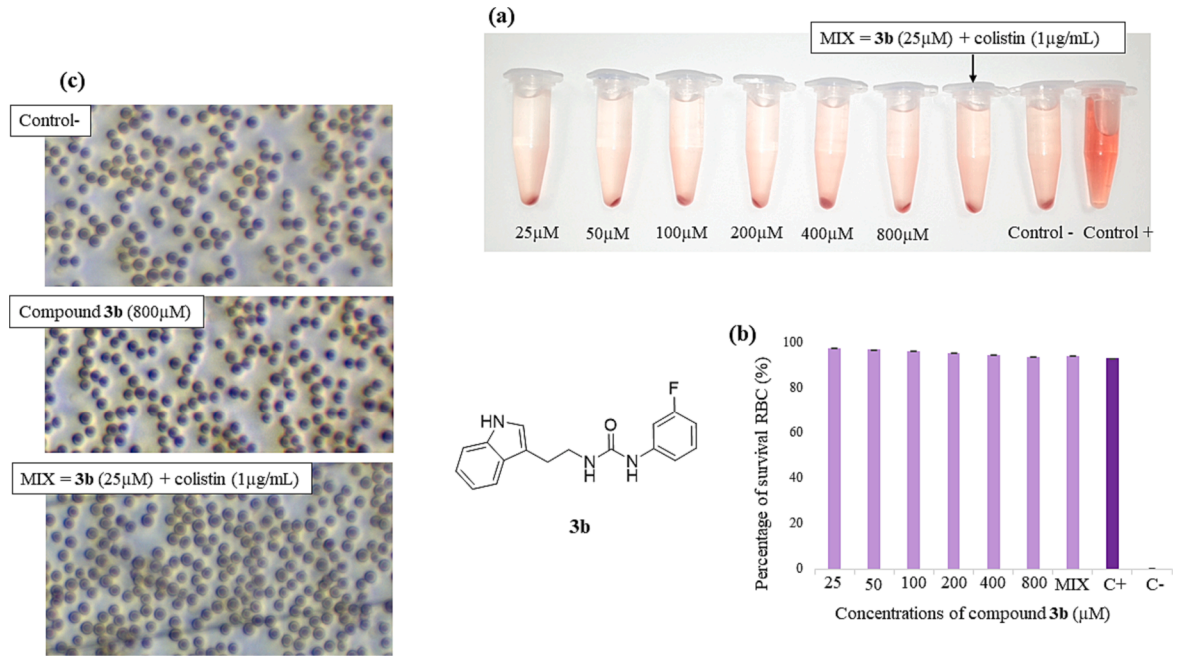


Fig. 4. Red blood cell (RBC) hemolysis results**: (a)** Visual results of hemolytic activity of compound **3b**; **(b)** Percentage of survival RBC (%) when exposed to the different concentrations of compound **3b,** and MIX; **(c)** RBCs observed by optical microscopy.

propyl, 8-fold) and **3s** (R = hexyl, 32-fold), we can deduce that the length of the aliphatic chain (long-chain alkyl) can greatly improve the colistin adjuvant activity. Finally, compound **3b** with no antibacterial activity (MIC > 128 µg/mL) and the best colistin adjuvant compound in this study (colistin MIC reduction from 64 to 1 µg/mL, 64-fold) against chromosomally-mediated resistance *K. pneumoniae* strain (KP BA03057), can be considered a promising adjuvant for colistin, in accordance with the antibiotic adjuvant concept. Computed physico-chemical properties and molecular descriptors can provide an overview of the chance of becoming clinical candidates. Using SwissADME we calculated the lipophilicity of the prepared compounds (cLogP) which provides valuable information about the lipophilic/hydrophobic property of the compound and strongly influences the absorption of the substance and its metabolism[27]. We also calculated the fraction of sp3 carbon atoms (Fsp3), which is another interesting physico-chemical parameter important to characterize the drug-likeness[28,29]. All synthesized compounds showed a cLogP (2.12–3.80) in the ideal range of logP (<5) to be presented in the context of compounds development. Concerning Fsp3, compounds **3q**-**3s** present the highest Fsp3 value which is considered as being favorable[28,29].

2.2.3. Synergy checkerboard assay of compounds 3a-3s and fractional inhibitory concentration index (FICI) measurements

Once the adjuvant activity of synthesized compounds had been confirmed, a further checkerboard trial was carried out on adjuvant compounds showing the best colistin MIC reduction fold (**3d**, **3e**, **3i**, **3j** and **3s**: 32-fold and **3b**: 64-fold). At this stage of testing, the active compounds were evaluated in combination with the colistin only against KP BA03057 strain in which the combination proved to be effective. The highest used concentration for colistin is 64 µg/mL, corresponding to the MIC of colistin against KP BA03057 strain. For compounds **3d**, **3e**, **3i**, **3j**, **3s** and **3b**, the highest used concentration was 100 µM (24.5–35.7 µg/mL), the same as used in the adjuvant test (no antibacterial concentration). Concentrations of both colistin and compounds **3d**, **3e**, **3i**, **3j**, **3s** and **3b** were diluted to identify concentrations of both agents that achieved the most potent interaction. This was followed by the determination of the combinatorial effect between the synthesized compounds and colistin by calculating fractional inhibitory concentration index (FICI) (see material and methods). Synergy is defined when FICI ≤ 0.5. This assay was repeated twice independently yielding similar results. In general, all compounds proved to be capable of synergizing with colistin at different concentrations (Fig. 2). Compounds **3e** and **3g** were able to reduce the MIC of colistin from 64 to 2 µg/mL (32-fold) with a concentration of 100 µM showing a synergy effect with colistin (FICI of 0.310 and 0.276 respectively). Compounds **3d** and **3s** reduced the MIC of colistin from 64 to 2 µg/mL (32-fold) with a concentration of 50 µM and they also provide a synergetic effect with colistin (FICI of 0.167 and 0.143 respectively). Interestingly, compounds **3b** and **3i** reduced the MIC of colistin from 64 to 1 µg/mL at a concentration of 25 µM (**3b**) and from 64 to 2 µg/mL at a concentration of 12.5 µM (**3i**) (FICI of 0.073 and 0.062 respectively). The obtained results showed that the introduction of halogens atoms (F (**3b**) or Cl (**3i**)) in the phenyl meta-position not only enhanced the colistin adjuvant activity with low concentrations, but also had a synergistic effect with colistin with the best FICI values. Compounds **3b** and **3i** were found to be the most effective synergists with FICI values of 0.073 and 0.062, respectively.

2.3. Molecular modeling

Chromosomal colistin resistance is typically mediated by two-component systems, such as PmrB / PmrA and PhoQ / PhoP. These two TCSs mediated the chromosomal mutation by modifying the lipid A moiety of LPS, which is the main target of colistin. However, it is important to note that colistin adjuvants can also reverse colistin resistance allowing restoration of colistin activity against resistant bacteria[30–32]. In this context, Qing *et al* 2018 have identified a number of compounds that inhibit the PhoP response regulator[20]. Different compounds possessing an amide linked to a tryptamine core have shown strong PhoP inhibitory activity. Among these tryptamine inhibitors of PhoP, compound **A** (Fig. 3, B) showed a strong *in vitro* inhibition with IC_{50} = 32.3 µM. Compound **A** has the same basic core as our active compound **3b** (chemical structure presented in Fig. 4, B), which is very interesting and may shed light on the mode of action of compound **3b** in that it could be a potential inhibitor of response regulator PhoP, allowing the restoration of colistin activity against clinically resistant *K. pneumoniae*. Molecular docking was performed using the crystal

structure of *E. coli* (PDB: 2PKX) to study the *in silico* interaction of compound **3b** with the binding pocket of PhoP. The protein structure was carefully treated in Discovery studio (DS) and prepared for the docking in AutoDockTools (ADT). In order to study protein–ligand interactions, we first performed a blind molecular docking of compound **A** and **3b** using a grid box determined in AutoDockTools comprising the entire targeted protein (homodimeric PhoP) in order to identify the most favorable docking site(s), meaning the best energy area(s). The different interaction modes of compound **A** and **3b** with PhoP generated by Vega ZZ are predominantly located at the protein–protein interface (PPI) of PhoP (hydrophobic interactions) and is equally comprised by residues from the monomers forming the homo-dimer, including Glu82, Ser83, Trp84, Lys87, Tyr98, Val99, Thr100, Pro102, Phe103, His104, Glu107, Arg111 as its shown by Qing *et al* in 2018. We then performed a targeted docking in the identified binding pocket (PPI) using a much smaller grid box allowing a more accurate prediction (Fig. 3, A). The lowest energy pose of **A** establishes different interactions with some conserved residues on PPI in particular with Trp84 (Pi-sigma bond), Lys87 (Pi-cation/anion and Pi-alkyl bonds), Typ98 (carbon hydrogen or/and Pi-donor hydrogen bond), Thr100 located on both monomers (Hydrogen bond), Pro102 (Hydrogen bond) and Glu107 located on both monomers (Pi-cation / anion bond) (Fig. 3, B). The lowest energy pose of compound **3b** is similar to that found for compound **A** (Fig. 3, A). Compound **3b** bonds to some residues of the binding pocket and most of them are the same as those observed for **A**: Glu82 (Hydrogen bond), Trp84 (Pi-Pi *T*-shaped bond), Lys87 (Pi-alkyl bond), Typ98 (Pi-donor hydrogen bond), Thr100 (Hydrogen bond), Glu107 (halogen (fluorine) bond) and Arg111 (Pi-cation bond) (Fig. 3, C). As compound **3b** perfectly fits the PPI of PhoP response regulator, we suggest that **3b** may act as a stabilizer or inhibitor of PhoP's homo-dimeric conformation by binding to both monomers of PhoP. This stabilizing or inhibiting effect can lead to induced dimerization of the two monomers and subsequently enhance or prevent the expression of PhoP-dependent genes. This hypothesis is consistent with the concept of small-molecule-based modulation of PPIs validated by P. Thiel *et al* 2011[33]. To conclude, we hypothesize that compound **3b** acts indirectly against clinically resistant *K. pneumoniae* as a potential inhibitor of the PhoP response regulator, conferring the adjuvant activity of colistin (MIC reduction from 64 to 1 μg/mL) without imposing bacterial stress (MIC > 128 μg/mL).

2.4. Red blood cell (RBC) hemolysis effect

The erythrocytes of mammals represent an excellent model to perform an *in vitro* evaluation of the cytotoxicity of compound **3b**. The possibility of using the resistance of mammalian red blood cell (RBC) membranes as a method of toxicity evaluation studies have been documented [34]. Here, we use RBCs to investigate the cytotoxicity of compound **3b** alone at the lowest concentration showing the best colistin adjuvant activity (25 μM), at higher doses (2×, 4×, 8×, 16×, 32× and 64× 25 μM) and in combination with colistin at therapeutic doses (1 μg/mL: the concentration at which colistin MIC was reduced). Interestingly, 25 μM of compound **3b** showed no RBCs hemolytic activity (no erythrocytes breakdown) even at high concentration 64 × 25 μM. This was observed visually (Fig. 4, (a)) and quantitatively based on the percentage of survival RBCs (%) (Fig. 4, (b)). The erythrocytes exposed to 1×, 2×, 4×, 8×, 16× and 32× 25 μM of compound **3b** and a combination between colistin and **3b** (MIX = **3b** (25 μM) + colistin (1 μg/mL)) showed a survival rate of 96 ± 1 %.

Sickle cell anemia is a form of blood disorder that affects RBCs, turning them from round flexible discs into stiff and sticky sickled cells. Besides the fact that anemia can be inherited, this problem has also been associated with drugs [35]. Drug overdose precipitated sickle cell crisis leading to death. In this study, after exposing and incubating RBCs to the combination (MIX = **3b** (25 μM) + colistin (1 μg/mL)) and to the different concentrations (1×, 2×, 4×, 8×, 16×, 32× 25 μM) of compound **3b**, we observed the RBCs shape using optical microscopy (Fig. 4, atmosphere). no RBCs deformation was observed at MIX concentrations and even at the highest concentration (800 μM).

3. Conclusion

Tryptamine scaffold has been recognized as privilege scaffold for antibiotic treatment and mainly found in various antibiotic adjuvant small compounds. In this study, we investigated the antibacterial, the colistin adjuvant and possible synergistic effects for a library of tryptamine-urea derivatives. None of the synthesized tryptamine-urea derivatives exhibited an antibacterial activity (MIC > 128 μg/mL). Furthermore, among the synthesized compounds, **3d**, **3e**, **3j**, **3i** and **3s** showed a high adjuvant effect with 32-fold potentiation of colistin activity against chromosomally-mediated colistin resistance *K. pneumoniae* (KP BA03057). Interestingly, **3b** with a fluorine atom in *meta* position of phenyl group displayed the strongest colistin potentiation effect against KP BA03057 with 64-fold potentiation of colistin activity reducing the MIC from 64 μg/mL to 1 μg/mL. A study of SAR showed that the introduction of halogen atom especially fluorine in *meta* position or long-chain alkyl can greatly improve the colistin adjuvant activity against KP BA03057. Moreover, 25 μM of compound **3b** was sufficient to potentiate colistin activity synergistically with the best FICI value (0.073). Molecular docking indicates that compound **3b** may be a potential inhibitor of PhoP response regulator enabling a reversal colistin resistance and restoring colistin's activity against resistant *K. pneumoniae*. Cytotoxicity assay showed that compound **3b** displayed no toxicity on RBC at combinatorial therapeutic concentrations (**3b** (25 μM) + colistin (1 μg/mL)) and very high concentration of compound **3b** (32 × 25 μM). Thus, compound 3b can be a promising lead compound for further improvement and pharmacokinetics evaluations.

4. Experimental section

4.1. Chemistry

All reagents used for chemical synthesis were purchased from commercially available sources (Sigma Aldrich or Thermo scientific, Apollo Scientific) and used without further purification. (NMR) spectra were recorded with a Bruker Avance 400 spectrometer (300/400, 75 and 377 MHz for ^{1}H, ^{13}C NMR and ^{19}F respectively). NMR solvents were obtained from Eurisotop and used as is. Chemical shifts are given on the δ-scale in parts per million (ppm) relative to the respective NMR solvent; coupling constants (J) are in hertz (Hz). Solvent signals used as an internal reference for the ^{1}H and ^{13}C. In reporting spectral data, the following abbreviations were used: s = singlet; bs = broad singlet; d = doublet; dd = doublet of doublets; t = triplet; q = quartet; sext = sextuplet; dt = doublet of triplets; td = triplet of doublets; m = multiplet. For structure elucidation, ^{1}H, ^{13}C spectra were provided. For NMR assignment data an arbitrary numbering was applied for each compound. Spectra are edited in Mestrenova. Mass spectra (HRMS) were obtained by electrospray using a TOF analyzer Platform. Infrared (IR) spectra were obtained using an IRSpirit FTIR with QATR-S. Only structurally important peaks ($\bar{\upsilon}$) are presented in cm^{-1}. Reactions were monitored by Merck Kieselgel 60F254 precoated aluminum silica gel plates (0.25 mm thickness). Melting points were determined on a Stuart scientific SMP10 apparatus and are uncorrected. Flash chromatography experiments were carried out on Silica Gel premium R_f grade (40–63 μm) or were performed on a Grace Reveleris X2 using a 40 μm packed silica cartridge. HPLC analyses were obtained on a Waters Alliance 2795 system using the following conditions: a Thermo Hypersil C18 column (3 μm, 50 mm L × 2.1 mm ID), 20 °C column temperature, 1 mL/min flow rate, photodiode array detection (210–400 nm), and mobile phase consistent of a gradient of water and acetonitrile (each containing 0.1% formic acid). The purity of all synthetic compounds was determined by HPLC analysis and was ≥ 97%.

4.2. Synthesis procedure

The synthesis of tryptamine-derived ureas **3a**-**3 s** was accomplished using the following protocol: In a dry round bottom flask, a solution of tryptamine (1.2 mmol) in 10 mL of dry THF was stirred at room temperature, then a solution of isocyanate in dry THF (1.4 mmol) was added successively under argon atmosphereher. The resulting mixture was subsequently stirred at room temperature overnight. Reaction progress was monitored with TLC. The mixture was concentrated and extracted with AcOEt (4 × 70 mL), washed twice with brine, dried with sodium sulfate, and then concentrated *in vacuo*. The obtained residue was then purified via flash chromatography (eluent: 0–60% PE/EtOAc) for all compounds, followed by recrystallisation in DCM or Et_2O for some compounds.

4.2.1. 1-(2-(1H-indol-3-yl)ethyl)-3-(2-fluorophenyl)urea (3a)

Compound **3a** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 2-fluorophenylisocyanate (205.5 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3a** was isolated in 84% yield (310.2 mg) as a white powder. mp 177–180 °C; R_f: 0.25 (PE/ACOEt: 8/2); IR (cm^{-1}): 3394 (ν_{NH}), 3388 (ν_{NH}), 3328 (ν_{NH}), 1642 ($\nu_{C=O}$), 1559–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.86 (s, 1H, H_1 Indole), 8.33 (d, J = 2.1 Hz, 1H, H_{14} Urea), 8.17 (td, J = 8.3, 1.5 Hz, 1H, H_{20}), 7.57 (d, J = 7.8 Hz, 1H, H_4), 7.35 (d, J = 8.0 Hz, 1H, H_7), 7.18 (d, J = 2.0 Hz, 1H, H_2), 7.17 – 7.12 (m, 1H, H_{17}), 7.08 (t, J = 7.5 Hz, 2H, H_6 and H_{19}), 6.98 (t, J = 7.0 Hz, 1H, H_5), 6.95 – 6.86 (m, 1H, H_{18}), 6.66 (t, J = 5.6 Hz, 1H, H_{12} Urea), 3.42 (q, J = 6.0 Hz, 2H, H_{11}), 2.87 (t, J = 7.0, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.9 (C_{13}), 151.5 (d, $^1J_{CF}$ = 240.4 Hz, C_{16}), 136.3 (C_8), 128.4 (d, $^2J_{CF}$ = 10.2 Hz, C_{15}), 127.2 (C_9), 124.4 (d, $^4J_{CF}$ = 2.6 Hz, C_{19}), 122.8 (C_2), 121.3 (d, $^3J_{CF}$ = 7.1 Hz, C_{18}), 120.9 (C_6), 120.1 (C_{20}), 118.3, 118.2 (C_4 and C_5), 114.7 (d, $^2J_{CF}$ = 19.0 Hz, C_{17}), 111.6 (C_3), 111.4 (C_7), 39.5 (C_{11}), 25.7 (C_{10}). ^{19}F NMR (377 MHz, DMSO-d_6) δ = -130.62. HPLC: t_R: 15.21 min; purity: 97.07%. HRMS: $[M + H]^+$ calcd for C17H17ON3F: 298.13502; found: 298.13504.

4.2.2. 1-(2-(1H-indol-3-yl)ethyl)-3-(3-fluorophenyl)urea (3b)

Compound **3b** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 3-fluorophenylisocyanate (205.5 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3b** was isolated in 70% yield (261.5 mg) as a white powder. mp 130–133 °C; R_f: 0.30 (PE/ACOEt: 8/2); IR (cm^{-1}): 3385 (ν_{NH}), 3333 (ν_{NH}), 3198 (ν_{NH}), 1682 ($\nu_{C=O}$), 1599–1455 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.86 (s, 1H, H_1, Indole), 8.75 (s, 1H, H_{14} Urea), 7.57 (d, J = 7.8 Hz, 1H, H_4), 7.48 (dt, J = 12.4, 2.2 Hz, 1H, H_{18}), 7.35 (d, J = 8.0 Hz, 1H, H_7), 7.23 (dd, J = 15.4, 8.2 Hz, 1H, H_{19}), 7.18 (d, J = 2.3 Hz, 1H, H_2), 7.08 (td, J = 8.0, 1.0 Hz, 1H, H_6), 7.04 – 6.95 (m, 2H, H_5 and H_{20}), 6.68 (td, J = 8.4, 2.4 Hz, 1H, H_{16}), 6.22 (t, J = 5.6 Hz, 1H, H_{12} Urea), 3.41 (q, J = 6.0 Hz, 2H, H_{11}), 2.87 (t, J = 7.1 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 162.4 (d, $^1J_{CF}$ = 240.1 Hz, C_{17}), 155.0 (C_{13}), 142.5 (d, $^3J_{CF}$ = 11.5 Hz, C_{15}), 136.3 (C_8), 130.1 (d, $^3J_{CF}$ = 9.8 Hz, C_{19}), 127.2 (C_9), 122.8 (C_2), 120.9 (C_6), 118.3, 118.2 (C_4 and C_5), 113.2 (C_{20}), 111.7 (C_3), 111.4 (C_7), 107.1 (d, $^2J_{CF}$ = 21.3 Hz, C_{16}), 104.20 (d, $^2J_{CF}$ = 26.6 Hz, C_{18}), 39.52 (C_{11}), 25.79 (C_{10}). ^{19}F NMR (377 MHz, DMSO-d_6) δ = -112.48. HPLC: t_R: 15.46 min; purity: 99.31%. HRMS: $[M + H]^+$ calcd for C17H17ON3F: 298.13502; found: 298.13507.

4.2.3. 1-(2-(1H-indol-3-yl)ethyl)-3-(4-fluorophenyl)urea (3c)

Compound **3c** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 4-fluorophenylisocyanate (205.5 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3c** was isolated in 84% yield (310.4 mg) as a white powder. mp 172–175 °C; R_f: 0.27 (PE/ACOEt: 8/2); IR (cm^{-1}): 3282 (ν_{NH}), 3178 (ν_{NH}), 3127 (ν_{NH}), 1674 ($\nu_{C=O}$), 1559–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.85 (s, 1H, H_1, Indole), 8.53 (s, 1H, H_{14} Urea), 7.57 (d, J = 7.8 Hz, 1H, H_4), 7.39 (dd, J = 9.1, 5.0 Hz, 2H, H_{16} and H_{20}), 7.35 (d, J = 8.0, 1H, H_7), 7.18 (d, J = 2.1 Hz, 1H, H_2), 7.11 – 7.01 (m, 3H, H_6, H_{17} and H_{19}), 6.98 (t, J = 7.0 Hz, 1H, H_5), 6.12 (t, J = 5.6 Hz, 1H, H_{12} Urea), 3.40 (q, J = 6.0 Hz, 2H, H_{11}), 2.86 (t, J = 7.1 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 156.8 (d, $^1J_{CF}$ = 235.5 Hz, C_{18}), 155.2 (C_{13}), 137.0 (C_8), 136.3 (C_{15}), 127.2 (C_9), 122.7 (C_2), 120.9 (C_6), 119.1 (d, $^3J_{CF}$ = 7.4 Hz, C_{20} and C_{16}), 118.3, 118.2 (C_4 and C_5), 115.0 (d, $^2J_{CF}$ = 22.0 Hz, C_{17} and C_{19}), 111.76 (C_3), 111.38 (C_7), 39.52 (C_{11}), 25.87 (C_{10}). ^{19}F NMR (377 MHz, DMSO-d_6) δ = -121.50. HPLC: t_R: 15.22 min; purity: 99.35%. HRMS: $[M + H]^+$ calcd for C17H17ON3F: 298.1350; found: 298.1351.

4.2.4. 1-(2-(1H-indol-3-yl)ethyl)-3-(4-fluoromethylphenyl)urea (3d)

Compound **3d** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 4-trifluoromethylphenylisocyanate (280.53 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3d** was isolated in 24% yield (103.5 mg) as a white powder. mp 183–186 °C; R_f: 0.23 (PE/ACOEt: 8/2); IR (cm^{-1}): 3379 (ν_{NH}), 3357 (ν_{NH}), 3239 (ν_{NH}), 1622 ($\nu_{C=O}$), 1568–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.86 (s, 1H, H_1 Indole), 8.95 (s, 1H, H_{14} Urea), 7.63–7.52 (m, 5H, H_4, H_{16}, H_{17}, H_{19} and H_{20}), 7.34 (d, J = 8.1 Hz, 1H, H_7), 7.18 (d, J = 2.1 Hz, 1H, H_2), 7.07 (td, J = 7.0, 1.1 Hz, 1H, H_6), 6.98 (td, J = 7.1, 1.0 Hz, 1H, H_5), 6.30 (t, J = 5.7 Hz, 1H, H_{12} Urea), 3.41 (q, J = 6.0 Hz, 2H, H_{11}), 2.87 (t, J = 7.1 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154 (C_{13}), 144.3 (C_{15}), 136.3 (C_8), 127.2 (C_9), 126.0 (q, $^3J_{CF3}$ = 3.5 Hz, C_{17} and C_{19}), 124.7 (q, $^1J_{CF3}$ = 270.9 Hz, C_{21}), 122.8 (C_2), 121.01 (C_6), 120.8 (q, $^2J_{CF3}$ = 32 Hz, C_{18}), 118.4, 118.2 (C_4 and C_5), 117.1 (C_{16} and C_{20}), 111.6 (C_3), 111.4 (C_7), 39.5 (C_{11}), 25.7 (C_{10}). ^{19}F NMR (377 MHz, DMSO-d_6) δ = -59.89. HPLC: t_R: 17.33 min; purity: 98.88%. HRMS: $[M + H]^+$ calcd for C18H17ON3F3: 348.13182; found: 348.13187.

4.2.5. 1-(2-(1H-indol-3-yl)ethyl)-3-(2-bromophenyl)urea (3e)

Compound **3e** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 2-bromophenylisocyanate (295.4 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3e** was isolated in 95% yield (422 mg) as a white powder. mp 176–179 °C; R_f: 0.21 (PE/ACOEt: 8/2); IR (cm^{-1}): 3394 (ν_{NH}), 3319 (ν_{NH}), 1645 ($\nu_{C=O}$), 1579–1430 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.86 (s, 1H, H_1 Indole), 8.09 (dd, J = 8.3, 1.5 Hz, 1H, H_4), 7.87 (s, 1H, H_{14} Urea), 7.57 (d, J = 8.4 Hz, 1H, H_{20}), 7.54 (dd, J = 8.1, 1.4 Hz, 1H, H_{17}), 7.35 (d, J = 8.0 Hz, 1H, H_7), 7.28 (td, J = 8.5, 1.4 Hz, 1H, H_{19}), 7.19 (d, J = 2.2 Hz, 1H, H_2), 7.14 (t, J = 5.5 Hz, 1H, H_{12} Urea), 7.08 (td, J = 8.0, 1.1 Hz, 1H, H_{18}), 6.99 (td, J = 7.7, 1.0 Hz, 1H, H_5), 6.88 (td, J = 8.0, 1.6 Hz, 1H, H_6), 3.42 (q, J = 6.0 Hz, 1H, H_{11}), 2.88 (t, J = 7.1 Hz, 1H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.8 (C_{13}), 137.9 (C_{15}), 136.3 (C_8), 132.3 (C_{17}), 127.9 (C_{19}), 127.2 (C_9), 123.1 (C_6), 122.8 (C_2), 121.7 (C_4), 120.9 (C_{18}), 118.3, 118.2 (C_{20} and C_5), 112.2 (C_{16}), 111.7 (C_7), 111.4 (C_3), 39.5 (C_{11}), 25.7 (C_{10}). HPLC: t_R: 16.24 min; purity: 97.61%. HRMS: $[M + H]^+$ calcd for C17H17ON3Br: 358.05495; found: 358.05502.

4.2.6. 1-(2-(1H-indol-3-yl)ethyl)-3-(3-bromophenyl)urea (3f)

Compound **3f** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 3-bromophenylisocyanate (295.4 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3f** was isolated in 59% yield (262.4 mg) as a white powder. mp 156–159 °C; R_f: 0.37 (PE/ACOEt: 8/2); IR (cm^{-1}): 3389 (ν_{NH}), 3320 (ν_{NH}), 1649 ($\nu_{C=O}$), 1557–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.85 (s, 1H, H_1 Indole), 8.72 (s, 1H, H_{14} Urea), 7.84 (t, J = 1.8 Hz, 1H, H_{16}), 7.57 (d, J = 7.7 Hz, 1H, H_4), 7.35 (d, J = 8.0 Hz, 1H, H_7), 7.21 – 7.12 (m, 3H, H_2, H_{19} and H_{20}), 7.02 – 6.93 (m, 2H, H_6 and H_{18}), 6.98 (td, J = 7.9, 1.0 Hz, 1H, H_5), 6.23 (t, J = 5.6 Hz, 1H, H_{12} Urea), 3.40 (q, J = 6.0 Hz, 2H, H_{11}), 2.86 (t, J = 7.1 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.9 (C_{13}), 142.3 (C_{15}), 136.3 (C_8), 130.5 (C_{19}), 127.2 (C_9), 123.4 (C_6), 122.7 (C_2), 121.7 (C_{17}), 120.9 (C_{18}), 119.7 (C_{16}), 118.3, 118.2 (C_4 and C_5), 116.3 (C_{20}), 111.6 (C_3), 111.3 (C_7), 39.5 (C_{11}), 25.7 (C_{10}). HPLC: t_R: 18.57 min; purity: 97.32%. HRMS: $[M + H]^+$ calcd for

C17H17ON3Br: 358.05495; found: 358.05496.

4.2.7. 1-(2-(1H-indol-3-yl)ethyl)-3-(4-bromophenyl)urea (3g)

Compound **3g** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (178.7 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3g** was isolated in 52% yield (231 mg) as a white powder. mp 197–200 °C; R_f: 0.25 (PE/ACOEt: 8/2); IR (cm^{-1}): 3371 (ν_{NH}), 3290 (ν_{NH}), 1622 ($\nu_{C=O}$) and 1588–1453 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.84 (s, 1H, H_1 Indole), 8.66 (s, 1H, H_{12} Urea), 7.57 (d, J = 7.8 Hz, 1H, H_4), 7.37 (s, 4H, H_{16}, H_{17}, H_{19} and H_{20}), 7.35 (d, J = 8.1 Hz, 1H, H_7), 7.17 (d, J = 2.2 Hz, 1H, H_2), 7.07 (td, J = 7.1, 1.1 Hz, 1H, H_6), 6.98 (td, J = 7.9, 1.0 Hz, 1H, H_5), 6.19 (t, J = 5.7 Hz, 1H, H_{12} Urea), 3.40 (dd, J = 13.0, 7.0 Hz, 2H, H_{11}), 2.86 (t, J = 7.1 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 155.0 (C_{13}), 140.0 (C_{15}), 136.3 (C_8), 131.3 (2C, C_{17} and C_{19}), 127.2 (C_9), 122.7 (C_2), 120.9 (C_6), 119.4 (2C, C_{16} and C_{20}), 118.3, 118.2 (C_4 and C_5), 112.1 (C_{18}), 111.6 (C_3), 111.3 (C_7), 39.5 (C_{11}), 25.7 (C_{10}). HPLC: t_R: 19.26 min; purity: 97.37%. HRMS: $[M + H]^+$ calcd for C17H17ON3Br: 358.05495; found: 358.05493.

4.2.8. 1-(2-(1H-indol-3-yl)ethyl)-3-(2-chlorophenyl)urea (3h)

Compound **3h** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 2-chlorophenylisocyanate (229.5 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3h** was isolated in 79% yield (307.4 mg) as a white powder. mp 169–172 °C; R_f: 0.23 (PE/ACOEt: 8/2); IR (cm^{-1}): 3394 (ν_{NH}), 3342 (ν_{NH}), 3305 (ν_{NH}), 1648 ($\nu_{C=O}$), 1585–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.86 (s, 1H, H_1 Indole), 8.18 (dd, J = 8.3, 1.4 Hz, 1H, H_4), 8.05 (s, 1H, H_{14} Urea), 7.57 (d, J = 7.8 Hz, 1H, H_{20}), 7.39 (dd, J = 8.1, 1.4 Hz, 1H, H_{17}), 7.35 (d, J = 7.8 Hz, 1H, H_7), 7.24 (td, J = 8.5, 1.4 Hz, 1H, H_{19}), 7.19 (d, J = 2.1 Hz, 1H, H_2), 7.08 (t, J = 6.9 Hz, 2H, H_{12} Urea and H_{18}), 6.99 (td, J = 7.9, 1.0 Hz,1H, H_5), 6.94 (td, J = 7.5, 1.5 Hz,1H, H_6), 3.43 (q, J = 6.0 Hz, 2H, H_{11}), 2.88 (t, J = 7.0 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.8 (C_{13}), 136.8 (C_{15}), 136.3 (C_8), 129.0 (C_{17}), 127.4 (C_{19}), 127.2 (C_9), 122.8 (C_6), 122.3 (C_2), 121.1 (C_{16}), 120.9 (C_4), 120.8 (C_{18}), 118.3, 118.2 (C_{20} and C_5), 111.7 (C_3), 111.4 (C_7), 39.5 (C_{11}), 25.7 (C_{10}). HPLC: t_R: 16.11 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C17H17ON3Cl: 314.10547; found: 314.10553.

4.2.9. 1-(2-(1H-indol-3-yl)ethyl)-3-(2-chlorophenyl)urea (3i)

Compound **3i** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 3-chlorophenylisocyanate (229.5 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3i** was isolated in 33% yield (127.3 mg) as a white powder. mp 142–145 °C; R_f: 0.36 (PE/ACOEt: 8/2); IR (cm^{-1}): 3383 (ν_{NH}), 3339 (ν_{NH}), 3295 (ν_{NH}), 16,261 ($\nu_{C=O}$), 1592–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO) δ = 10.85 (s, 1H, H_1 Indole), 8.74 (s, 1H, H_{14} Urea), 7.69 (t, J = 1.9 Hz, 1H, H_{16}), 7.57 (d, J = 7.8 Hz, 1H, H_4), 7.35 (d, J = 8.0 Hz, 1H, H_7), 7.26 – 7.14 (m, 3H, H_2, H_{19} and H_{20}), 7.07 (t, J = 7.5 Hz, 1H, H_6), 6.98 (t, J = 7.4 Hz, 1H, H_5), 6.92 (dt, J = 7.7, 1.3 Hz, 1H, H_{18}), 6.24 (t, J = 5.6 Hz, 1H, H_{12} Urea), 3.40 (q, J = 6.0 Hz, 1H, H_{11}), 2.86 (t, J = 7.1 Hz, 1H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.9 (C_{13}), 142.1 (C_{15}), 136.3 (C_8), 133.1 (C_{17}), 130.2 (C_{19}), 127.2 (C_9), 122.7 (C_2), 120.9 (C_6), 120.5 (C_{18}), 118.3, 118.2 (C_5 and C_4), 116.9 (C_{16}), 115.9 (C_{20}), 111.6 (C_3), 111.3 (C_7), 39.5 (C_{11}), 25.7 (C_{10}). HPLC: t_R: 16.48 min; purity: 97%. HRMS: $[M + H]^+$ calcd for C17H17ON3Cl: 314.10547; found: 314.10553.

4.2.10. 1-(2-(1H-indol-3-yl)ethyl)-3-(4-chlorophenyl)urea (3j)

Compound **3j** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 4-chlorophenylisocyanate (229.5 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3j** was isolated in 61% yield (238.1 mg) as a white powder. mp 190–193 °C; R_f: 0.30 (PE/ACOEt: 8/2); IR (cm_1^-): 3371 (ν_{NH}), 3290 (ν_{NH}), 1622 ($\nu_{C=O}$), 1591–1436 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.85 (s, 1H, H_1 Indole), 8.65 (s, 1H, H_{14} Urea), 7.57 (d, J = 7.6 Hz, 1H, H_4), 7.42 (d, J = 8.9 Hz, 2H, H_{16} and H_{20}), 7.35 (d, J = 8.0 Hz, 1H, H_7), 7.25 (d, J = 8.8 Hz, 2H, H_{17} and H_{19}), 7.18 (d, J = 1.9 Hz, 1H, H_2), 7.07 (t, J = 6.9 Hz, 1H, H_6), 6.98 (t, J = 7.0 Hz, 1H, H_5), 6.18 (t, J = 5.2 Hz, 1H, H_{12} Urea), 3.40 (q, J = 6.0 Hz, 2H, H_{11}), 2.86 (t, J = 7.0 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 155.0 (C_{13}), 139.6 (C_{15}), 136.3 (C_8), 128.4 (2C, C_{17} and C_{19}), 127.2 (C_9), 124.3 (C_{18}), 122.7 (C_2), 120.9 (C_6), 119.0 (2C, C_{16} and C_{20}), 118.3, 118.2 (C_5 and C_4), 111.7 (C_3), 111.3 (C_7), 39.5 (C_{11}) 25.8 (C_{10}). HPLC: t_R: 16.28 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C17H17ON3Cl: 314.10547; found: 314.10553.

4.2.11. 1-(2-(1H-indol-3-yl)ethyl)-3-(3,5-dichlorophenyl)urea (3k)

Compound **3k** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with 4-chlorophenylisocyanate (280.4 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3k** was isolated in 56% yield (240.7 mg) as a white powder. mp 166–169 °C; R_f: 0.25 (PE/ACOEt: 8/2); IR (cm^{-1}): 3399 (ν_{NH}), 3388 (ν_{NH}), 1654 ($\nu_{C=O}$), 1596 – 1453 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.85 (s, 1H, H_1 Indole), 8.92 (s, 1H, H_{14} Urea), 7.57 (d, J = 7.8 Hz, 1H, H_4), 7.47 (s, 2H, H_{16} and H_{20}),7.35 (d, J = 7.9 Hz, 1H, H_7), 7.17 (d, J = 1.5 Hz, 1H, H_2), 7.11 – 7.03 (m, 2H, H_6 and H_{18}), 6.98 (t, J = 7.4 Hz, 1H, H_5), 6.36 (t, J = 5.5 Hz, 1H, H_{12} Urea), 3.40 (q, J = 6.0 Hz, 2H, H_{11}), 2.86 (t, J = 7.1 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.70 (C_{13}), 143.15 (C_{15}), 136.31 (C_8), 133.99 (2C, C_{17} and C_{19}), 127.22 (C_9), 122.79 (C_2), 120.97 (C_6), 119.94 (C_{18}), 118, 35, 118.26 (C_4 and C_5), 115.58 (2C, C_{16} and C_{20}), 111.61 (C_3), 111.40 (C_7), 39.52 (C_{11}), 25.68 (C_{10}). HPLC: t_R: 18.59 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C17H16ON3Cl2: 348.06649; found: 348.06662.

4.2.12. 1-(2-(1H-indol-3-yl)ethyl)-3-phenylurea (3l)

Compound **3l** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (178.7 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3l** was isolated in 80% yield (294 mg) as a white powder. mp 178–181 °C; R_f: 0.32 (PE/ACOEt: 8/2); IR (cm^{-1}): 3388 (ν_{NH}), 3349 (ν_{NH}), 1642 ($\nu_{C=O}$) and 1596–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.85 (s, 1H, H_1 Indole), 8.49 (s, 1H, H_{14} Urea), 7.58 (d, J = 7.7 Hz, 1H, H_4), 7.39 (d, J = 7.7 Hz, 2H, H_{16} and H_{20}), 7.35 (d, J = 8.1 Hz, 1H, H_7), 7.21 (t, J = 7.5 Hz, 2H, H_{17} and H_{19}), 7.19 (s, 1H, H_2), 7.08 (t, J = 7.0 Hz, 1H, H_6), 6.98 (t, J = 7.1 Hz, 1H, H_5), 6.88 (t, J = 7.3 Hz, 1H, H_{18}), 6.14 (t, J = 5.6 Hz, 1H, H_{12} Urea), 3.41 (q, J = 6.0 Hz, 2H, H_{11}), 2.86 (t, J = 7.1 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 155.2 (C_{13}), 140.6 (C_{15}), 136.3 (C_8), 128.6 (2C, C_{17} and C_{19}), 127.2 (C_9), 122.7 (C_2), 120.9 (C_{18}), 120.9 (C_6), 118.4, 118.2 (C_5 and C_4), 117.5 (2C, C_{16} and C_{20}), 111.7 (C_3), 111.3 (C_7), 39.5 (C_{11}), 25.8 (C_{10}). HPLC: t_R: 15.22 min; purity: 99%. HRMS: $[M + H]^+$ calcd for C17H183ON3: 280.14444; found: 280.14453.

4.2.13. 1-(2-(1H-indol-3-yl)ethyl)-3-(4-methoxyphenyl)urea (3m)

Compound **3m** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (223.6 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3m** was isolated in 93% yield (358 mg) as a white powder. mp 200–203 °C; R_f: 0.30 (PE/ACOEt: 8/2); IR (cm^{-1}): 3368 (ν_{NH}), 3287 (ν_{NH}), 1622 ($\nu_{C=O}$), 1596–1461 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.84 (s, 1H, H_1 Indole), 8.29 (s, 1H, H_{14} Urea), 7.57 (d, J = 7.7 Hz, 1H, H_4), 7.35 (d, J = 8.0 Hz, 1H, H_7), 7.28 (d, J = 9.0 Hz, 2H, H_{16} and H_{20}), 7.17 (d, J = 2.2 Hz, 1H, H_2), 7.07 (td, J = Hz, 1H, H_6), 6.98 (td, J = Hz, 1H, H_5), 6.80 (d, J = 9.0 Hz, 2H, H_{17} and H_{19}), 6.03 (t, J = 5.7 Hz, 1H, H_{12} Urea), 3.69 (s, 3H, H_{21}), 3.39 (q, J = 6.0 Hz, 2H, H_{11}), 2.85 (t, J = 7.1 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 155.4 (C_{13}), 153.8 (C_{18}), 136.3 (C_8), 133.7 (C_{15}), 127.2 (C_9), 122.7 (C_2), 120.9 (C_6), 119.3 (2C, C_{16} and C_{20}), 118.4, 118.2 (C_5 and C_4), 113.8 (2C, C_{17} and C_{19}), 111.8 (C_3), 111.3 (C_7), 55.1 (C_{21}), 39.5 (C_{11}), 25.9 (C_{10}). HPLC: t_R: 13.94 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C18H20O2N3: 310.15500; found: 310.15506.

4.2.14. 1-(2-(1H-indol-3-yl)ethyl)-3-(4-isopropylphenyl)urea (3n)

Compound **3n** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (241.6 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3n** was isolated in 58% yield (231 mg) as a white powder. mp 188–191 °C; R_f: 0.27 (PE/ACOEt: 8/2); IR (cm^{-1}): 3374 (ν_{NH}), 3282 (ν_{NH}), 1625 ($\nu_{C=O}$), 1593–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.84 (s, 1H, H_1 Indole), 8.38 (s, 1H, H_{14} Urea), 7.57 (d, J = 7.7 Hz, 1H, H_4), 7.34 (d, J = 8.0 Hz, 1H, H_7), 7.29 (d, J = 8.5 Hz, 2H, H_{16} and H_{20}), 7.17 (d, J = 2.1 Hz, 1H, H_2), 7.12–7.03 (m, 3H, H_6, H_{17} and H_{19}), 6.98 (td, J = 6.9, 1.1 Hz, 1H, H_5), 6.08 (t, J = 5.6 Hz, 1H, H_{12} Urea), 3.40 (q, J = 6.0 Hz, 2H, H_{11}), 2.85 (t, J = 7.1 Hz, 2H, H_{10}), 2.77 (m, 1H, H_{21}), 1.16 (d, J = 6.9 Hz, 6H, H_{22} and H_{23}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 155.3 (C_{13}), 140.9 (C_{18}), 138.3 (C_{15}), 136.3 (C_8), 127.2 (C_9), 126.3 (2C, C_{17} and C_{19}), 122.7 (C_2), 120.9 (C_6), 118.3, 118.2 (C_5 and C_4), 117.7 (2C, C_{16} and C_{20}), 111.8 (C_3), 111.3 (C_7), 39.5 (C_{11}), 32.7 (C_{21}), 25.9 (C_{10}), 24.0 (2C, C_{22} and C_{23}). HPLC: t_R: 17.50 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C20H24ON3: 322.19139; found: 322.19144.

4.2.15. 1-(2-(1H-indol-3-yl)ethyl)-3-(4-methylphenyl)urea (3o)

Compound **3o** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (199.6 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3o** was isolated in 93% yield (340 mg) as a white powder. mp 202–205 °C; R_f: 0.30 (PE/ACOEt: 8/2); IR (cm^{-1}): 3368 (ν_{NH}), 3273 (ν_{NH}), 1622 ($\nu_{C=O}$), 1591 – 1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.84 (s, 1H, H_1 Indole), 8.37 (s, 1H, H_{14} Urea), 7.57 (d, J = 7.7 Hz, 1H, H_4), 7.34 (d, J = 8.0 Hz, 1H, H_7), 7.27 (d, J = 8.4 Hz, 2H, H_{16} and H_{20}), 7.17 (d, J = 2.2 Hz, 1H, H_2), 7.07 (td, J = 6.9, 1.0 Hz, 1H, H_6), 7.01 (d, J = 7.9, 2H, H_{17} and H_{19}), 6.96 (td, J = 6.9, 1.0 Hz, 1H, H_5), 6.08 (t, J = 5.6 Hz, 1H, H_{12} Urea), 3.39 (q, J = 6.0 Hz, 2H, H_{11}), 2.85 (t, J = 7.1 Hz, 2H, H_{10}), 2.21 (s, 3H, H_{21}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 155.3 (C_{13}), 138.0 (C_{15}), 136.3 (C_8), 129.5 (C_{18}), 129.0 (2C, C_{17} and C_{19}), 127.2 (C_9), 122.7 (C_2), 120.9 (C_6), 118.4, 118.2 (C_5 and C_4), 117.6 (2C, C_{16} and C_{20}), 111.8 (C_3), 111.3 (C_7), 39.5 (C_{11}), 25.9 (C_{10}), 20.3 (C_{21}). HPLC: t_R: 15.38 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C18H20ON3: 294.16009; found: 294.16013.

4.2.16. 1-(2-(1H-indol-3-yl)ethyl)-3-benzylurea (3p)

Compound **3p** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (199.6 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3p** was isolated in 82% yield (294 mg) as a white powder. mp 137–140 °C; R_f: 0.35 (PE/ACOEt: 8/2); IR (cm^{-1}): 3394 (ν_{NH}), 3360 (ν_{NH}), 3308 (ν_{NH}), 1622 ($\nu_{C=O}$), 1591–1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.82 (s, 1H, H_1 Indole), 7.55 (d, J = 7.8 Hz, 1H, H_4), 7.36 – 7.28 (m, 3H, H_7, H_{16} and H_{20}), 7.27 – 7.20 (m, 3H, H_{17}, H_{18} and H_{19}), 7.13 (d, J = 2.2 Hz, 1H, H_2), 7.06 (td, J = Hz, 1H, H_6), 6.97 (td, J = Hz, 1H, H_5), 6.38 (t, J = 6.0 Hz, 1H, H_{14} Urea), 5.97 (t, J = 5.7, 1H, H_{12} Urea), 4.21 (d, J = 6.0 Hz, 2H, H_{21}), 3.32 (q, J = 6.0 Hz, 2H, H_{11}), 2.80 (t, J = 7.2 Hz, 2H, H_{10}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 158.0 (C_{13}), 141.0 (C_{15}), 136.2 (C_8), 128.2 (2C, C_{17} and C_{19}), 127.3 (C_9), 127.0 (2C, C_{16} and C_{20}), 126.5 (C_{18}), 122.6 (C_2), 120.8 (C_6), 118.4, 118.1 (C_5 and C_4), 111.9 (C_3), 111.3 (C_7), 42.8 (C_{21}), 39.5 (C_{11}), 26.1 (C_{10}). HPLC: t_R: 14.01 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C18H20ON3: 294.16009; found: 294.16013.

4.2.17. 1-(2-(1H-indol-3-yl)ethyl)-3-(tert-butyl)urea (3q)

Compound **3q** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (148.6 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3q** was isolated in 84% yield (272.7 mg) as a white powder. mp 150–153 °C; R_f: 0.41 (PE/ACOEt: 8/2); IR (cm^{-1}): 3308 (ν_{NH}), 3213 (ν_{NH}), 1619 ($\nu_{C=O}$), 1559–1453 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.82 (s, 1H, H1 Indole), 7.54 (d, J = 7.6 Hz, 1H, H_4), 7.34 (d, J = 8.0 Hz, 1H, H_7), 7.12 (d, J = 2.1 Hz, 1H, H_2), 7.06 (t, J = 7.1 Hz, 1H, H_6), 6.97 (t, J = 7.4 Hz, 1H, H_5), 5.67 (s, 1H, H_{14} Urea), 5.65 (t, J = 5.9 Hz, 1H, H_{12} Urea), 3.25 (q, J = 6.0 Hz, 2H, H_{11}), 2.77 (t, J = 7.1 Hz, 2H, H_{10}), 1.22 (s, 9H, H_{16}, H_{17} and H_{18}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 157.4 (C_{13}), 136.2 (C_8), 127.3 (C_9), 122.6 (C_2), 120.8 (C_6), 118.4, 118.1 (C_5 and C_4), 112.0 (C_3), 111.3 (C_7), 48.9 (C_{15}), 39.5 (C_{11}), 29.3 (3C, C_{16}, C_{17} and C_{18}), 26.1 (C_{10}). HPLC: t_R: 17.50 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C15H22ON3: 260.17574; found: 260.17575.

4.2.18. 1-(2-(1H-indol-3-yl)ethyl)-3-(propyl)urea (3r)

Compound **3r** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (127.6 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3r** was isolated in 98% yield (300.5 mg) as a white powder. mp 193–196 °C; R_f: 0.43 (PE/ACOEt: 8/2); IR (cm^{-1}): 3362 (ν_{NH}), 3285 (ν_{NH}), 1605 ($\nu_{C=O}$), 1565 – 1456 ($\nu_{C=CAr}$). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.82 (s, 1H, H_1 Indole), 7.54 (d, J = 7.7 Hz, 1H, H_4), 7.33 (d, J = 8.0 Hz, 1H, H_7), 7.13 (d, J = 2.2 Hz, 1H, H_2), 7.06 (td, J = 5.7 Hz, 1H, H_6), 6.97 (td, J = 5.7 Hz, 1H, H_5), 5.88 (t, J = 5.7 Hz, 1H, H_{14} Urea), 5.81 (t, J = 5.7 Hz, 1H, H_{12} Urea), 3.29 (q, J = 6.0 Hz, 2H, H_{11}), 2.95 (q, J = 6.0 Hz, 2H, H_{15}), 2.78 (t, J = 7.2 Hz, 2H, H_{10}), 1.37 (sext, J = 7.2 Hz, 2H, H_{16}), 0.83 (t, J = 7.4 Hz, 3H, H_{17}). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 158.1 (C_{13}), 136.2 (C_8), 127.3 (C_9), 122.6 (C_2), 120.8 (C_6), 118.3, 118.1 (C_5 and C_4), 112.0 (C_3), 111.3 (C_7), 41.1 (C_{15}), 39.5 (C_{11}), 26.1 (C_{10}), 23.2 (C_{16}), 11.4 (C_{17}). HPLC: t_R: 12.05 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C14H20ON3: 246.16009; found: 246.16013.

4.2.19. 1-(2-(1H-indol-3-yl)ethyl)-3-(hexyl)urea (3s)

Compound **3 s** was synthesized according to general procedure. Tryptamine (200 mg, 1.25 mmol) was reacted with phenylisocyanate (127.6 mg, 1.5 mmol) in dry THF (10 mL) over night. Product **3 s** was isolated in 83% yield (297.2 mg) as a white powder. mp 184–187 °C; R_f: 0.42 (PE/ACOEt: 8/2); IR (cm^{-1}): 3388 (ν_{NH}), 3351 (ν_{NH}), 1619 ($\nu_{C=O}$), 1588–1456 ($\nu_{C=CAr}$). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 10.82 (s, 1H, H_1 Indole), 7.53 (d, J = 7.9 Hz, 1H, H_4), 7.33 (d, J = 8.1 Hz, 1H, H_7), 7.12 (d, J = 2.2 Hz, 1H, H_2), 7.06 (td, J = 7.0, 1.1 Hz, 1H, H_6), 6.96 (td, J = 7.0, 1.1 Hz, 1H, H_5), 5.85 (t, J = 5.6 Hz, 1H, H_{14} Urea), 5.79 (t, J = 5.7 Hz, 1H, H_{12} Urea), 3.27 (q, J = 6.0 Hz, 2H, H_{11}), 2.97 (q, J = 6.0 Hz, 2H, H_{15}), 2.77 (t, J = 7.2 Hz, 2H, H_{10}), 1.38 – 1.20 (m, 8H, H_{16}, H_{17}, H_{18} and H_{19}), 0.86 (t, J = 7.0 Hz, 3H, H_{20}). ^{13}C NMR (126 MHz, DMSO-d_6) δ = 158.1 (C_{13}), 136.2 (C_8), 127.3 (C_9), 122.6 (C_2), 120.9 (C_6), 118.4, 118.1 (C_4 and C_5) 112. 0 (C_3), 111.3 (C_7), 39.8 (C_{15}), 39.0 (C_{11}), 31.1 (C_{18}), 30.0 (C_{17}), 26.2 (C_{10}), 26.1 (C_{16}), 22.1 (C_{19}), 14.0 (C_{20}). HPLC: t_R: 16.13 min; purity: 100%. HRMS: $[M + H]^+$ calcd for C14H20ON3: 288.20704; found: 288.20712.

4.3. In vitro biological studies

4.3.1. Minimum inhibitory concentrations (MICs)

All used bacteria were donated by Nîmes University Hospital and were cultivated in Mueller–Hinton broth (MHB) in this study. Three reference sensitive Gram-negative strains: *E. coli* ATCC25922, *K. pneumoniae* ATCC700603 and *P. aeruginosa* ATCC27853 were used as susceptible and quality control strains. Six clinical colistin resistant isolates were used in this study: two plasmid-mediated *mcr-1* colistin resistance *E. coli* (EC NTCC 13846 and EC A50), one chromosomally-mediated resistance *K. pneumoniae* (KP BA03057) and chromosomally-mediated colistin resistance *P. aeruginosa* (PA 15080, PA 10039 and PA 1002). According to the EUCAST guidelines [36], 5.12 mg of all compounds were solubilized in a 15-ml flask with 2 mL of DMSO and 8 mL of MHB (*Cm* = 512 μg/mL). Then two-fold serial dilutions were realized from this solution (0.5–256 μg/ml). The final concentration in the wells (U-base TPP 96-well plate) was 0.25–128 μg/ml. Briefly, each well contained a cell density of 5.10^5 CFU/mL and compound concentrations ranging from 0.25 to 128 μg/mL. After incubation for 20 h at 37 °C under aerobic conditions, the MIC values were recorded as the lowest concentration of compound at which there was no visible bacterial

$$FICI = \frac{\text{MIC of compound } \mathbf{3} \text{ in combination with colistin}}{\text{MIC of compound } \mathbf{3} \text{ tested alone}} + \frac{\text{MIC of colistin in combination with compound } \mathbf{3}}{\text{MIC of colistin tested alone}}$$

Fig. 5. FICI calculation formula.

growth. Three independent experiments were performed for each bacterial strain.

4.3.2. Assessment of colistin potentiating effect of compounds 3a-3s

The adjuvant activity of compounds **3a-3s** was evaluated in combination with the colistin against six clinically resistant Gram-negative bacteria: EC NTCC 13846, EC A50, KP BA03057, PA 15080, PA 10039 and PA 1002. According to the obtained MICs of colistin against different clinically resistant Gram-negative bacteria, colistin was added to each well in a two-fold serial dilution ranging from 2 to 0.004 μg/mL against EC NTCC 13846, PA 15080 and PA 1002, from 8 to 0.015 μg/mL against EC A50, from 4 to 0.008 μg/mL against PA 10039 and from 64 to 0.125 μg/mL against KP BA03057. To each well, the tested compounds **3a-3s** was added to yield a final concentration of 100 μM (24.5–35.7 μg/mL). Finally, each well of the lane contains a different concentration of colistin, 100 μM of the tested compounds and a cell density of 5.10^5 CFU/mL of the tested strains. Then the plates were incubated at 37 ∘C for 24 h under aerobic conditions. The MIC values were recorded as the lowest concentration of colistin in combination with each compound **3a-3 s** at which there was no visible bacterial growth. The experiment was relayed in duplicate.

4.3.3. Checkerboard assay (determination of the fractional inhibitory concentration index (FICI))

After selecting the compounds (**3b**, **3d**, **3e**, **3j**, **3i** and **3s**) showing the best colistin adjuvant activity against KP BA03057, a more detailed checkerboard assay was performed. Colistin was serially diluted along the ordinate (columns) in a two-fold serial dilution ranging from 0 to 64 μg/mL, while the test compounds were diluted along the abscissa (rows) in a two-fold serial dilution ranging from 0 to 100 μM. Then each well was inoculated with KP BA03057 inoculum of 5.10^5 CFU/mL, and the plates were incubated at 37 ∘C for 24 h under aerobic conditions. All the plates were realized in duplicates. Absorption at 600 nm of each well was read with Tecan Infinite 200PRO MNano + using I-control software. The negative control average was considered as 100% inhibition, and the positive control average was considered as 0% inhibition. Heat maps were realized with RStudio software. The synergistic effect was calculated with the fractional inhibitory concentration index (FICI) (Fig. 5). The results were analyzed as follows: synergy, FICI of < 0.5; additivity, FICI of ≥ 0.5 to ≤ 1; no interaction (indifference), FICI of > 1 to ≤ 4; antagonism, FICI of > 4.

4.4. Hemolysis assay

After washing and resuspending in phosphate-buffered saline (PBS), 250 μl of 5% (v/v) of sheep erythrocyte solution was added to Eppendorf tubes, and then the same volume of compound **3b** was added at various concentrations (25 μM, 50 μM, 100 μM, 200 μM, 400 μM and 800 μM). Each hemolysis reaction contained 5% (v/v) RBCs, 5% (v/v) DMSO, and a specific concentration of the respective compounds. The samples were incubated for 1 h at 37 °C under agitation at 100 rpm. 0.5% Triton X-100 and PBS were used as positive control and negative control, respectively. After incubation, the RBCs were separated from the reaction mixture by centrifugation at 1000 g and 4 °C. The experiments were performed in triplicate. The hemolytic activities of compound **3b** were determined visually and quantitatively by reading absorbance (750–600 nm) using a plate reader of Tecan Infinite 200PRO MNano + and I-control software. The deformation effect of RBC by compound **3b** was determined using an optical microscope (Motic, model BA410E) equipped with color matrix camera (Stemmer Immaging, modèle TIS DFK 33UX226).

4.5. Molecular modeling

The co-crystal structure of PhoP of *E. coli* (PDB: 2PKX) was used for docking study. The protein structure was carefully prepared in Discovery studio (DS) before docking simulation and the ligand was processed by full minimization in DS. Molecular docking was limited to the pocket on the homo-dimer interface using a grid box created in AutoDockTools. Molecular simulation was performed using Vina docking on Vega ZZ software. The simulated conformations and their binding mode within the PPI site of PhoP were visualized using DS.

Author contributions

ZB was responsible for the management of the work, overall supervision. ZB and CM wrote the manuscript. CM carried out all the chemistry synthesis, the molecular docking and biological evaluations with the help of DB. ZB, CDR, AP, JD and PM contributed to proofreading and critical revision.

CRediT authorship contribution statement

Chaimae Majdi: Writing – original draft, Investigation, Formal analysis, Writing – review & editing. **Jean Dessolin:** Validation, Writing – review & editing. **David Bénimélis:** Investigation. **Catherine Dunyach-Rémy:** Validation, Writing – review & editing. **Alix Pantel:** Validation, Writing – review & editing. **Patrick Meffre:** Writing – review & editing. **Zohra Benfodda:** Conceptualization, Project administration, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Majdi Chaimae reports financial support was provided by Occitanie region. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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