Journal of Global Antimicrobial Resistance 33 (2023) 114–119

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

# Antimicrobial and anthelmintic activities of aryl urea agents

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ARTICLE INFO

*Article history:*
Received 16 June 2022
Revised 3 January 2023
Accepted 23 February 2023
Available online 10 March 2023

Editor: Prof Fabrizio Bruschi

*Keywords:*
Aryl urea
Antibacterial
Anthelmintic
Carbapenemase
*Caenorhabditis elegans*

ABSTRACT

*Objectives:* This study aimed to characterise compounds with activity against carbapenemase-expressing Gram-negative bacteria and nematodes and evaluate their cytotoxicity to non-cancerous human cells.
*Methods:* The antimicrobial activity and toxicity of a series of phenyl-substituted urea derivatives were evaluated using broth microdilution, chitinase, and resazurin reduction assays.
*Results:* The effects of different substitutions present on the nitrogen atoms of the urea backbone were investigated. Several compounds were active against *Staphylococcus aureus* and *Escherichia coli* control strains. Specifically, derivatives **7b, 11b**, and **67d** exhibited antimicrobial activity against *Klebsiella pneumoniae* 16, a carbapenemase-producing Enterobacteriaceae species, with minimum inhibitory concentration (MIC) values of 100, 50, and 72 µM (32, 64, and 32 mg/L), respectively. In addition, the MICs obtained against a multidrug-resistant *E. coli* strain were 100, 50, and 36 µM (32, 16, and 16 mg/L) for the same compounds, respectively. Furthermore, the urea derivatives **18b, 29b, 50c, 51c, 52c, 55c–59c**, and **62c** were very active towards the nematode *Caenorhabditis elegans*.
*Conclusions:* Testing on non-cancerous human cell lines suggested that some of the compounds have the potential to affect bacteria, especially helminths, with limited cytotoxicity to humans. Given the simplicity of synthesis for this class of compounds and their potency against Gram-negative, carbapenemase-expressing *K. pneumoniae*, aryl ureas possessing the 3,5-dichloro-phenyl group certainly warrant further investigation to exploit their selectivity.



## 1. Introduction

Antibiotic-resistant bacteria, particularly multidrug-resistant (MDR) strains, constitute a considerable threat to global public health. The World Health Organization (WHO) recently published a list of noteworthy pathogens, laying the foundation for the discovery and development of novel antimicrobial agents [1]. For instance, the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.), were categorised as 'high priority' because they are commonly associated with increased hospital stays and burgeoning healthcare costs [2]. These pathogens are distinct from common microorganisms because they demonstrate a high level of antibiotic resistance and virulence via several mechanisms; consequently, they can 'escape' the action of antimicrobials and the immune response [3–5]. Infections related to ESKAPE pathogens pose challenges with regard to selecting efficient therapeutic approaches and account for extensive morbidity and mortality in patients [3]. Among these pathogens, *K. pneumoniae* (carbapenemase-producing Enterobacteriaceae; CPE) stands out because of its ability to produce carbapenemases. These enzymes can hydrolyse not only carbapenems but also numerous other antimicrobial agents, especially $\beta$-lactams, limiting treatment options [6].

Despite advances in the research and development of new antimicrobial drugs and a growing number of new antibacterial

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https://doi.org/10.1016/j.jgar.2023.02.021

molecules, MDR strains continue to spread extensively. Moreover, parasitic nematodes cause debilitating diseases and present a significant problem in medicine [7]. According to WHO estimates, soil-transmitted helminths alone infect more than 1 billion people worldwide. The problem is further exacerbated by a limited repertoire of currently available anthelmintic drugs, and there is a considerable risk that the parasites will quickly develop resistance to these drugs, as is frequently seen in veterinary medicine [8].

In this study, a series of *N,N*-disubstituted urea derivatives, previously synthesised and characterised, were evaluated as potential antimicrobial and anti-nematodal agents [9–11]. Because of the clinical significance of ESKAPE pathogens worldwide, all synthesised compounds were screened in vitro against four different bacterial strains from the 'high priority' list, namely *S. aureus* NTCT 12981, *Escherichia coli* NTCT 10418, *E. coli* G69 (MDR clinical isolate), and *K. pneumoniae* 16 (CPE). The derivatives were also evaluated against *Caenorhabditis elegans*, a free-living model nematode frequently used for screening new potential anthelmintic drugs [12]. Additionally, their toxicity against several human cell lines was assessed.

## 2. Materials and Methods

### 2.1. Synthesis

The synthesis of compounds **1a**–**20a, 1b**–**32b**, and **33c–66c** was described by Nisler et al. in 2016, 2021, and 2022, respectively [9–11]. The synthesis of compound **67d** and a list of all tested compounds is provided in the Supplementary Materials (S1). Generally, all compounds were prepared according to common protocols for the synthesis of diphenylurea derivatives using substituted phenyl isocyanate and substituted aniline. Most of the phenyl isocyanates and substituted anilines are commercially available. If the desired phenyl isocyanate derivative was not available, it was prepared from substituted aniline and diphosgene in tetrahydrofuran.

### 2.2. Antibacterial activity

The antibacterial activity of the synthetic compounds was tested against *E. coli* NCTC 10418, *E. coli* G69 (MDR clinical isolate), *K. pneumoniae* 16 (CPE), and *S. aureus* 12981 using a broth microdilution assay according to Andrews (2001) [13]. Briefly, all bacterial strains were cultured on nutrient agar plates (Sigma-Aldrich, Gillingham, UK) and incubated for 24 h at 37°C prior to minimum inhibitory concentration (MIC) determination. In addition, known quantities of each test sample were dissolved in DMSO and then diluted in Luria-Bertani (LB) broth (Sigma-Aldrich) to yield a concentration range of 128–0 mg/L. At the same concentrations, DMSO showed no inhibitory effect on bacterial growth. Finally, overnight cultures of each of the tested strains were suspended to an inoculum density of approximately $1.0 \times 10^8$ CFU/mL in phosphate-buffered saline (PBS), consisting of 137 mM NaCl, 3 mM KCl, 8 mM $Na_2HPO_4$, and 15 mM $KH_2PO_4$ (Oxoid, Basingstoke, UK). The cell suspensions were standardised by adjusting the optical density to 0.1 at 600 nm, using a UV-Vis spectrophotometer (Thermo Scientific, Cambridge, UK), and then diluted 1:100 in LB broth prior to inoculation. Amoxicillin was used as the positive control for all experiments. The assays were performed by microdilution using 96-well microtiter plates with a final inoculum density of $5 \times 10^5$ CFU/mL. Each sample was tested in duplicate in at least two independent experiments to confirm the reliability of the data. Results were determined by visual inspection of the wells, and the presence of an opaque medium or white pellets was considered indicative of bacterial growth. MIC values were recorded as the lowest concentration at which no bacterial growth was detected.

### 2.3. Toxicity evaluation in *C. elegans*

The wild-type N2 (Bristol) *C. elegans* strain and bacterial *E. coli* strain OP50 were obtained from the *Caenorhabditis* genetic centre and cultivated using standard protocols [14]. The toxicity of all compounds to *C. elegans* and their effect on worm fecundity were measured in a four-day chitinase assay, as described in detail by Nisler et al., 2022 [10].

### 2.4. Cytotoxicity

The effect of 72 h treatments with the test compounds on non-cancerous human cell line viability was evaluated using a resazurin reduction assay to measure the metabolic activity of the cell population. The following cell lines were used: BJ (skin fibroblasts), ARPE-19 (retinal pigment epithelium cells), and HaCaT (keratinocytes). BJ and ARPE-19 were obtained from the American Type Culture Collection (Manassas, VA, USA). HaCaT was obtained from the German Cancer Research Center (DKFZ) (Heidelberg, Germany). The assay was performed according to the methodology reported by Voller et al. [15]. Each experiment was repeated at least three times. $IC_{50}$ values were calculated using the drc package in R software (https://cran.r-project.org/web/packages/drc).

## 3. Results and Discussion

A series of compounds was developed according to general protocols for the synthesis of diphenylurea derivatives, using aromatic isocyanates and the corresponding anilines [16]. Antimicrobial screening of approximately 70 urea derivatives (Fig. 1 and Table S1) revealed that some of these compounds demonstrated excellent to moderate growth inhibition towards the evaluated bacterial strains (Table 1).

### 3.1. Antimicrobial activity against *S. aureus* NCTC 12981

The results indicated that among the tested urea derivatives, compounds **11b, 33c, 41c, 50c, 51c, 55c, 56c, 62c**, and **67d** showed excellent inhibitory microbial growth activity (MIC $\leq$ 50 μM) against *S. aureus*. Compound **11b**, 1-(2-(2-aminoethyl)phenyl)-3-(3,5-dichlorophenyl)urea, was the only compound from the 'b' series that was active against *S. aureus*. Compounds of the 'b' series contained in their structure 2-chloropyridine, 2,6-dichloropyridine, 3,5-dichlorophenyl, or a (trifluoromethoxy)phenyl group. Therefore, the activity of compound **11b** was attributed to a combination of the 2-aminoethyl group and 3,5-dichlorophenyl group, rather than to a 3,5-dichlorophenyl group only. The same principle applied to compounds of the 'c' series. Most of the compounds contained in their structure a 3-(trifluoromethoxy)phenyl group, but only some were active. The activity of compound **33c** provided clear evidence of the key role of the position and nature of the substituent in determining inhibitory activity. All compounds (**33c**–**48c**) were very similar derivatives of 1-phenyl-3-(3-(trifluoromethoxy)phenyl)urea, differing mainly in the type of halogen(s) and its position on the phenyl ring. Compound **33c** featured a chlorine atom in position 3. From a comparison with compounds **41c** and **16b**, it was apparent that the pyridine-phenyl urea derivatives showed higher toxicity than the diphenyl urea derivatives. This was confirmed by the results obtained for the toxicity of compounds **50c** and **51c** (comparing the activity of compound **51c** with that of **52c**). The role of halogen atom size was demonstrated by comparing the toxicity of compounds **56c** and **57c**, which contained bromine instead of chlorine.

Supporting these findings, a recent report revealed that compounds containing the dichlorophenyl group exhibit bactericidal

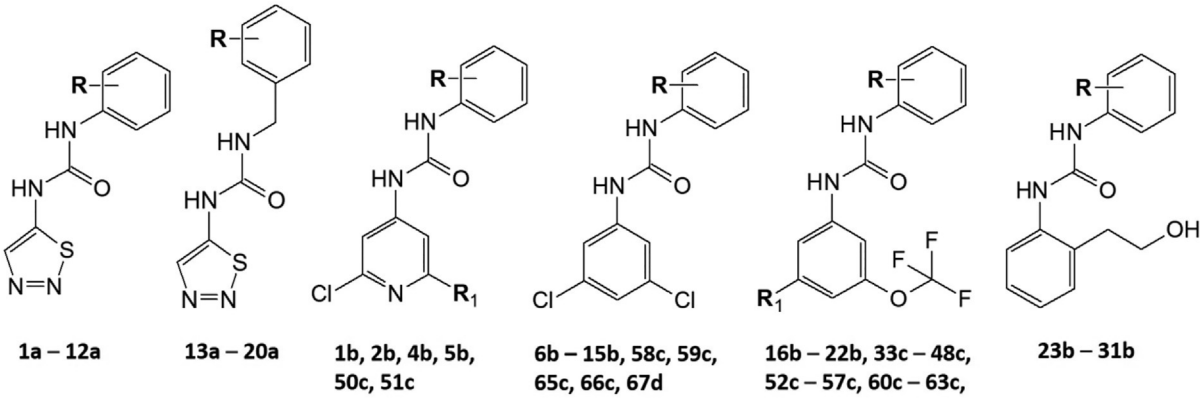


**Fig. 1.** Structures of the urea derivatives tested in this study.

**Table 1**
Minimum inhibitory concentration (MIC; μM) of the urea derivatives against the strains *Staphylococcus aureus*NCTC 12981, *Escherichia coli* NCTC 10418, *E. coli* G69, and *Klebsiella pneumoniae* CPE 16; $IC_{50}$ (μM) of the compounds against the nematode *Caenorhabditis elegans*; and cytotoxicity (μM) of the active derivatives against non-cancerous human cells (ARPE-19, BJ, and HaCaT).

| Compound | *S. aureus* NCTC 12981 MIC (μM) | *E. coli* NCTC 10418 MIC (μM) | *E. coli* G69 MIC (μM) | *K. pneumoniae CPE 16* MIC (μM) | *C. elegans* $IC_{50}$ (μM) | ARPE-19 $IC_{50}$ (μM) | BJ $IC_{50}$ (μM) | HaCaT $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|---|
| **7a** | > 500 | 250–500 | > 500 | > 500 | *n.t.* | *n.t* | *n.t* | *n.t* |
| **14a** | > 500 | 500 ± 2 | 500 ± 15 | > 500 | *n.t.* | *n.t* | *n.t* | *n.t* |
| **16a** | > 500 | 250–500 | 500 ± 17 | > 500 | *n.t.* | *n.t* | *n.t* | *n.t* |
| **17a** | > 450 | 450 ± 13 | > 450 | > 450 | *n.t.* | *n.t* | *n.t* | *n.t* |
| **1b** | > 450 | 450 ± 10 | > 450 | > 450 | *N.A.* | > 50 | > 50 | > 50 |
| **4b** | > 400 | 200–400 | > 400 | > 400 | 40 - 50 | > 50 | > 50 | > 50 |
| **5b** | 100 ± 3 | 12–24 | > 100 | > 100 | *N.A.* | > 50 | 31 ± 6 | 17 ± 3 |
| **7b** | 200 ± 3 | 100–200 | 100–200 | 200 ± 14 | *N.A.* | 47 ± 2 | 34 ± 3 | 27 ± 3 |
| **11b** | 50 ± 1 | 50–100 | 50–100 | 100 ± 9 | > 50 | 25 ± 4 | 19 ± 3 | 18 ± 2 |
| **18b** | > 350 | 350 ± 8 | > 350 | > 350 | > 5 | 46 ± 10 | 44 ± 11 | 37 ± 8 |
| **29b** | > 350 | 200–350 | > 350 | > 350 | 24 ± 14 | > 50 | > 50 | > 50 |
| **32b** | > 350 | 175–350 | > 350 | > 350 | *N.A.* | > 50 | > 50 | > 50 |
| **33c** | 11 ± 1 | > 350 | > 350 | > 350 | *N.A.* | > 50 | > 50 | 29 ± 14 |
| **35c** | > 350 | 350 ± 12 | > 350 | > 350 | *N.A.* | > 50 | 35 ± 2 | 16 ± 5 |
| **36c** | > 350 | 350 ± 5 | > 350 | > 350 | *N.A.* | > 50 | > 50 | > 50 |
| **39c** | > 350 | 185–370 | > 350 | > 350 | *N.A.* | *> 50* | 48 ± 5 | 26 ± 3 |
| **40c** | > 300 | 300 ± 8 | > 300 | > 300 | *N.A.* | 45 ± 3 | 34 ± 5 | 14 ± 3 |
| **41c** | 50 ± 2 | 100–200 | 200–400 | > 400 | 37 ± 4 | > 50 | 49 ± 2 | 50 ± 1 |
| **43c** | > 350 | 350 ± 13 | > 350 | > 350 | *N.A.* | 42 ± 12 | 40 ± 9 | 11 ± 1 |
| **45c** | > 350 | 350 ± 4 | > 350 | > 350 | *N.A.* | > 50 | > 50 | > 50 |
| **50c** | 50 ± 1 | 100–200 | > 200 | > 200 | 0.6 ± 0.3 | *> 50* | 27 ± 5 | 16 ± 2 |
| **51c** | 20 ± 1 | 5–10 | > 350 | > 350 | 0.34 ± 0.01 | 19 ± 1 | 13 ± 4 | 4.8 ± 0.7 |
| **52c** | > 350 | 1.4–2.7 | > 350 | > 350 | 1.1 ± 1.0 | 20 ± 3 | 13 ± 4 | 5.7 ± 2.7 |
| **55c** | 45 ± 2 | 180–370 | > 350 | > 350 | 20 ± 7 | 34 ± 2 | 24 ± 4 | 13 ± 3 |
| **56c** | 20 ± 1 | 5–10 | > 300 | > 300 | 0.7 ± 0.2 | 12 ± 3 | 10 ± 1.5 | 7.1 ± 2.8 |
| **57c** | > 250 | 18–36 | > 300 | > 300 | 0.9 ± 0.3 | 9.1 ± 1.5 | 3.3 ± 1.4 | 4.3 ± 2.2 |
| **62c** | 2.5 ± 0.2 | 5.2–10.5 | > 350 | > 350 | 4.6 ± 0.7 | 27 ± 6 | 17 ± 4 | 6.0 ± 1.5 |
| **67d** | 35 ± 1 | 18 ± 4 | 36 ± 4 | 72 ± 5 | 18 ± 7 | 17 ± 3 | 14 ± 2 | 12 ± 1 |
| **Amoxicillin** | 0.35 ± 0.05 | 5 ± 0.8 | > 350 | > 350 | *n.t* | *n.t* | *n.t* | *n.t* |
| **Ivermectin** | *n.t* | *n.t* | *n.t* | *n.t* | < 0.1 | *n.t* | *n.t* | *n.t* |

CPE, carbapenemase-producing Enterobacteriaceae; N.A., not active (viability of *C. elegans* was higher than 75% in the presence of 50 μM compound); n.t., not tested.

and anti-biofilm activities against *S. aureus* [17]. However, the chlorine atoms are located at positions 3 and 4 on the urea backbone. Another study showed the importance of the different key moieties, such as the dichlorophenyl group, in inhibitors of *S. aureus* RnpA [18]. RnpA has been hypothesised to be one of the main players in RNA degradation. The authors suggested that the combination of a small aliphatic amine with a 3,5-dichlorophenyl moiety is required for RnpA inhibition.

### 3.2. Antimicrobial activity against *E. coli* NCTC 10418

Compounds **5b, 51c, 52c, 56c, 57c, 62c**, and **67d** showed remarkable growth inhibition activity towards *E. coli* NCTC 10418. In this case, suitably substituted diphenyl urea derivatives appeared to exhibit higher toxicity than similar pyridyl-phenyl urea derivatives (compare the activity of **51c** with that of **52c**). Compared with the MIC of **56c, 57c**, and **62c**, the MIC of **52c** indicated that the addition of another halogen atom or the substitution of the 3,5-dichlorophenyl group by another 3-(trifluoromethoxy)phenyl group did not improve the antimicrobial activity against *E. coli* NCTC 10418. In addition, derivatives **7a, 14a, 16a, 1b, 4b, 18b, 29b, 32b, 39c, 35c, 40c, 36c, 43c**, and **45c** were found to be moderate antimicrobial agents against *E. coli* NCTC 10418.

### 3.3. Antimicrobial activity against *E. coli* G69 and *K. pneumoniae* CPE 16

In contrast, most of the compounds screened exhibited little or no activity against *E. coli* G69 (an MDR strain) and *K. pneumoniae* CPE 16 (a carbapenemase-producing bacterial strain). Notwith-

standing, compounds **7b, 11b**, and **67d** exhibited exceptional activity against both these strains, showing MIC values of 200, 100, and 72 μM (64, 32, and 32 mg/L) against *K. pneumoniae* CPE 16, respectively (Table 1). Furthermore, amoxicillin, the standard compound used in this study, displayed no effect against these two MDR strains, emphasising the significant activity of these urea-derived compounds.

The three urea derivatives **7b, 11b**, and **67d** bear aminomethyl or aminoethyl moieties in their structures. Interestingly, the 2-aminoethyl moiety linked via an amide group to the phenyl ring had greater antimicrobial effects than the 2-aminoethyl moiety attached directly to the phenyl ring. Furthermore, multiple studies have at least partially attributed antimicrobial properties to the presence of aminoalkyl groups in the compound structures [19–21]. For instance, a peptide bearing an aminoethyl moiety was identified as the most potent compound tested against both *S. aureus* and *E. coli*, among other derivatives [21]. Another study using variable aminoalkyl chains on cellulose nanofibers demonstrated potent antibacterial effects [22]. These effects were significantly affected by the position, length, and quantity of aminoalkyl moieties in the structures, revealing the importance of this functional group on antibacterial activity.

Moreover, compounds **7b, 11b**, and **67d** (as well as other compounds used in this study that exhibited antimicrobial activity) carry not only aminoalkyl moieties but also a 3,5-dichlophenyl group, which may also play an important role in determining their antimicrobial properties. Comparable results for compounds containing (3,4-dichlorophenyl)urea were demonstrated by Patil et al. [23]. Patil et al. synthesised a series of urea compounds containing a dichlorophenyl moiety that showed significant to moderate antimicrobial activity towards Gram-positive and Gram-negative species, including *S. aureus* and *E. coli* strains. Likewise, a thiourea derivative bearing a dichlorophenyl moiety in its structure showed appreciable activity against *S. aureus* and *Pseudomonas aeruginosa* [24]. In the same way, another study using dichlorophenyl compounds also detailed significant antimicrobial and antifungal activity against similar bacterial strains [25]. Therefore, linking a phenyl group containing an aminoalkyl moiety with a 3,5-dichlorophenyl group is a promising strategy for the development of new antimicrobial compounds.

Compound **52c** 1-(3,5-dichlorophenyl)-3-(3-(trifluoromethoxy)phenyl)urea exhibited an MIC of 1.4 μM (0.5 mg/L) against *E. coli*, which was lower than that of the standard amoxicillin. Similarly, derivative **62c** (1,3-bis(3-(trifluoromethoxy)phenyl)urea) exhibited the lowest MIC (2.6 μM; 1 mg/L) against *S. aureus* but also demonstrated potent activity towards *E. coli* (MIC = 5.2–10.5 μM; 2–4 μg/mL). The trifluoromethoxy-phenyl group appeared to play a substantial role in these effects as well, when combined with a halogenated phenyl or pyridyl group. These findings are in line with those of prior studies. A study using trifluoromethoxy-substituted chalcone derivatives revealed potent antimicrobial activities against Gram-positive and Gram-negative bacteria [26]. For the future development of potent antimicrobial compounds derived from diphenyl urea or pyridyl-phenyl urea, introducing combinations of substitutions, such as aminoalkyl groups with trifluoromethoxy groups and/or chlorines, is promising.

### 3.4. Toxicity to *C. elegans*

The anti-nematodal activity of all compounds against *C. elegans* was assessed via chitinase assay (Table 1, Fig. 2A and B) combined with microscopic evaluation of the populations. Compounds **4b, 8b, 9b, 18b, 29b, 41c**, and **55c** inhibited the growth of the nematodes by more than 50% at the higher concentration tested (50 μM), although healthy populations were observed in wells treated with 5 μM of the compounds. Compounds **58c, 59c, 62c**, and **67d** were more active than the compounds mentioned above, showing a negative effect on *C. elegans* at both the 50 and 5 μM concentrations, although the 5 μM concentration did not cause complete inhibition of *C. elegans* development. The highest toxicity was exhibited by compounds **50c, 51c, 52c, 56c**, and **57c**; both tested concentrations completely inhibited the growth of nematodes and significantly delayed their development and fecundity. The $IC_{50}$ values of these compounds were determined (Table 1). Some of these results were reported and discussed recently [10].

Generally, compounds of the 'b' series were less toxic than compounds of the 'c' series. From the results obtained for 'b' series compounds, we can conclude that the hydroxymethyl group in compound **8b** inhibited *C. elegans* development more severely than other moieties (such as hydroxyethyl, aminomethyl, aminoethyl, chloroethyl, and carboxylic acid groups) attached at the same position on the same compound. A similar effect could be attributed to the methoxymethyl moiety in compound **18b**. Other chemical groups were not active, even though all the derivatives possessed a 3-(trifluoromethoxy)phenyl group (compounds **16b**–**22b**). The addition of another halogen atom did not increase the toxicity of diphenyl urea derivatives with a 3-(trifluoromethoxy)phenyl group (compounds **33c**–**48c**). Interestingly, the study of positional isomers (compounds **19b, 28b**, and **29b**), in which the trifluoromethoxy group was attached to the same molecule in the *ortho, meta*, or *para* position, revealed that this group exhibited the greatest toxic effect when in the *para* position (**29b**).

The results for compounds of the 'c' series further showed that these derivatives exhibited a significantly greater toxic effect on the viability of the nematodes than the compounds of the previous series. It was apparent that the presence of other group(s) (e.g., the hydroxyethyl group), rather than halogens or trifluoromethoxy-phenyl groups, significantly decreased the toxicity of the compounds (compare the toxicity of **47d** with that of **52c**). Additionally, compounds **56c** and **57c**, possessing a trifluoromethoxy-phenyl group, were more lethal than the analogous derivatives **58c** and **59c** (Fig. 1B). Again, the influence of the trifluoromethoxy-phenyl group was also evident from an analysis of compounds containing two trifluoromethoxy-phenyl groups (**62c**) rather than one [10]. Likewise, in a further study, the authors suggested that the presence of dichlorophenyl and trifluoromethoxy-phenyl groups in their new compounds could be closely related to this effect [27]. A series of novel synthetic disulfonylmethane compounds, also possessing trifluoromethoxy-phenyl groups, showed anthelmintic and insecticidal activity [28]. Several analogues have shown activity against the internal nematode *Haemonchus contortus*, a very common parasite and one of the most pathogenic nematodes of ruminants. In a similar way, a series of 2,6-dichloro-4-(trifluoromethyl)phenyl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide derivatives were revealed to be potent nematocidal agents against the tomato root-knot nematode *Meloidogyne incognita* [28]. Once again, these findings corroborate and reinforce the important role of trifluoromethoxy-phenyl groups in the anti-nematode effect.

### 3.5. Toxic effects on non-cancerous human cells

To evaluate the cytotoxicity of the derivatives active against any of the tested bacteria strains to non-cancerous human cells (ARPE-19, BJ, and HaCaT), a resazurin reduction assay measuring the metabolic activity of the cell population was used after 3 d of compound exposure [15]. Derivative **11b** exhibited the lowest $IC_{50}$ values (7 μM and 8 μM) against the K562 and HaCaT cell lines, respectively. Furthermore, compound **67d** showed an $IC_{50}$ against RPE-1 cells of 8 μM. Conversely, urea derivative **7b** had higher $IC_{50}$ values, with a range of 26–32 μM, against all human cell lines used

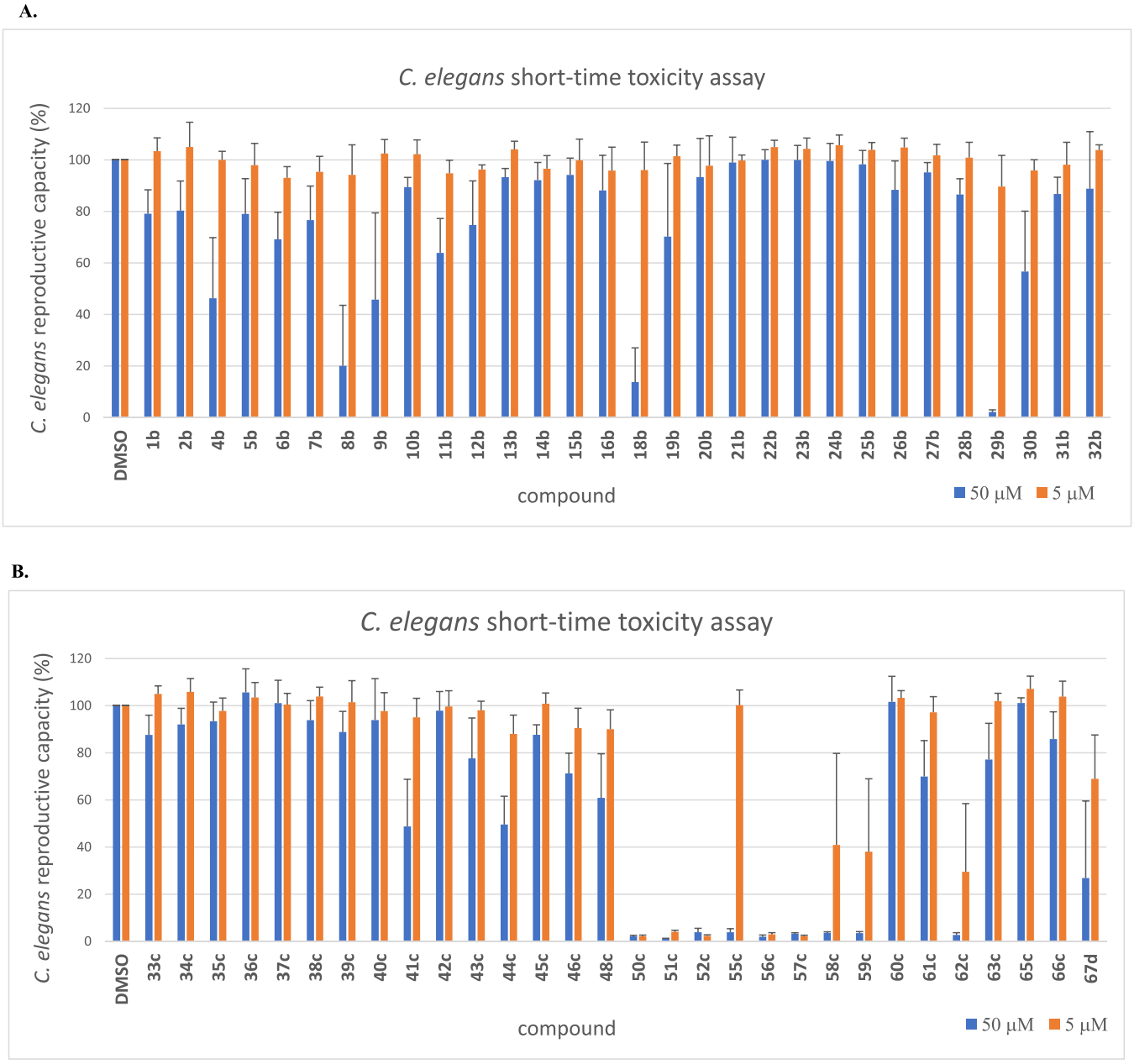


**Fig. 2.** (A) Effect of tested compounds on the reproductive capacity of *Caenorhabditis elegans*. Values represent the mean ± standard deviation (SD) of the results from at least two independent assays. (B) Effect of tested compounds on the reproductive capacity of *C. elegans*. Values represent the mean ± SD of the results from at least two independent assays.

(Table 1). A noticeable selective toxicity for worms vs. human cells was observed for derivatives **18b, 29b, 41c, 50c, 51c, 52c, 56c**, and **57c**. This indicated that the concentrations required for nematocidal activity were significantly lower than cytotoxic concentrations for human cells, suggesting selectivity and potential therapeutic applications.

Overall, the results indicated that the urea derivative compounds, especially those containing 3,5-dichlorophenyl and trifluoromethoxy-phenyl groups, were able to inhibit growth of the control and MDR bacteria, as well as nematodes. Even though some derivatives were shown to be cytotoxic at the same concentrations, these observations are interesting, and the findings suggest the possibility of further development and biological evaluation of other urea derivatives containing these groups. Additionally, the crucial compounds of these urea derivatives are obtainable via easy synthesis from low-cost, accessible reagents, making their production price comparatively affordable.

## 4. Conclusions

Novel urea derivatives were shown to have potent antimicrobial activity against significant pathogens, especially MDR strains. Furthermore, these compounds showed significant anti-nematode activity towards *C. elegans*. Testing on non-cancerous human cell lines suggested that the compounds have the potential to affect bacteria, especially helminths, with limited cytotoxicity against human cell lines. Since ureas are adaptable compounds that can easily be modified, further development of this series could prospectively result in active compounds with a better selectivity and therapeutic index. As antibiotic and anthelmintic resistance

increasingly presents challenges in medicine, urea derivatives may serve as useful alternatives in the field of antimicrobial and anthelmintic development.

## Funding

This research was conducted in the framework of COST action COMBAR (CA16230) and was supported by an INTER-COST grant (project LTC19030) provided by the Czech Ministry of Education and Sports.

## Competing interests

None declared.

## Ethical Approval

Not required.

## Acknowledgements

The multidrug-resistant bacterial strains *E. coli* G69 and *K. pneumoniae* CPE 16 were kindly donated by Dr. Paul Stapleton (UCL–School of Pharmacy) and Dr. Michelle Buckner (Institute of Microbiology and Infection–University of Birmingham), respectively. We are grateful to Ms. Kateřina Faksová and Mr. Tomáš Jirsa for their excellent technical assistance.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.02.021.

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