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Antimicrobial and anthelmintic activities of aryl urea agents

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Highlights

- Multidrug-resistant strains constitute a relevant threat to global public health
- The antimicrobial and anthelmintic effects of new urea derivatives were evaluated
- It was demonstrated significant activity against a multi-drug resistant *E. coli*
- Carbapenemase-Producing Enterobacteriaceae strain was inhibited by urea derivatives

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ABSTRACT

Objectives: The study aimed to characterise compounds with activity against carbapenemase-expressing Gram-negative bacteria and nematodes, with the intention that the compounds would lack cytotoxicity against non-cancer human cells.

Methods: The antimicrobial activity and toxicity to nematodes and human cell lines of a series of phenyl substituted urea derivatives were evaluated using Minimum Inhibitory Concentration (MIC), 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 (CPE) 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 multi-drug resistant *E. coli* strain were 100, 50 and 36 μM (32, 16 and 16 mg/L), for the same compounds. 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-cancer human cell lines suggested that some of the compounds have potential to affect bacteria and especially helminths with limited cytotoxicity for humans. Given the simplicity of synthesis for this class of compound and the potency against a Gram-negative carbapenemase-expressing *Klebsiella pneumoniae*, aryl ureas possessing the 3,5-dichloro-phenyl group certainly warrant further investigation to exploit their selectivity.

Keywords:
Aryl urea
Antibacterial
Anthelmintic
Carbapenemase
Caenorhabditis elegans

1. Introduction

Antibiotic-resistant bacteria, particularly multidrug-resistant (MDR) strains, constitute a considerable threat to global public health. The World Health Organization (WHO) recently revealed a list of pathogens, laying the foundation for the discovery and development of novel antimicrobial agents [1]. For instance, the ESKAPE pathogens (*Enterococcus faecium, S. aureus, K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.), were categorised as high priority because they are commonly related with increased hospital stay, burgeoning healthcare costs and are categorised as of the utmost priority [2]. These pathogens are distinct from common microorganisms for demonstrating a high level of antibiotic resistance and virulence via several mechanisms, and consequently can 'escape' the action of antimicrobials and the immune response [3]–[5]. Infections related with ESKAPE became a significant problem in the choice of efficient therapeutic approaches, and accounts for extensive morbidity and mortality in patients [3]. Among these pathogens, *K. pneumoniae* (CPE) stands out due to its ability to produce carbapenemases. These enzymes have the capacity of hydrolysing not just carbapenems but also numerous other antimicrobial agents, especially of the class of β-lactams, hindering treatment options [6].

Despite advances in research and development for new antimicrobial drugs and a rising number of new antibacterial molecules, MDR strains continue to spread extensively. Moreover, parasitic nematodes also cause debilitating diseases and present a significant problem in medicine [7]. According to WHO estimates, just soil-transmitted helminths alone infect more than 1 billion people worldwide. The problem is further exacerbated by a limited repertoire of currently available anti-helminthic drugs, and a considerable risk is that the parasites will quickly develop resistance to these, as can frequently be seen in veterinary medicine [8].

In this context, a series of *N,N*-disubstituted urea derivatives, previously synthesised and characterised, were evaluated as potential antimicrobial and antinematodal agents [9]–[11]. Due to the clinical significance of ESKAPE pathogens worldwide, all synthesised compounds were screened *in vitro* against 4 different bacterial strains from the high priority list namely *S. aureus* NTCT 12981, *E. coli* NTCT 10418, *E. coli* G69 (MDR-clinical isolate) and *K. pneumoniae* 16 (CPE). The derivatives were also evaluated against *C. elegans*, a free-living model nematode which is frequently used for screening of new potential anthelmintic drugs [12]. Additionally, their toxicity against several human cell lines was also assessed.

2. Materials and Methods

2.1 Synthesis

Synthesis of compounds **1a** – **20a**, **1b** – **32b** and **33c – 66c** were described by Nisler et al., 2016, 2021 and 2022, respectively [9]–[11]. Synthesis of compound **67d** and a list of all tested compounds is provided in the supplementary materials file (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 the broth microdilution assay according to Andrews (2001) [13]. Briefly, all bacterial strains were cultured on nutrient agar plates (Sigma-Aldrich, UK) and incubated for 24 hours at 37°C prior to MIC determination. In addition, known quantities of each test sample were dissolved in DMSO and then diluted in LB (Luria-Bertani Broth, Sigma-Aldrich, UK) to give a concentration range of 128 – 0 mg/L. At the same concentrations, DMSO showed no inhibitory effect towards bacterial growth. Finally, overnight cultures of each of the tested strains were suspended to an inoculum density of approximately 1.0×10^8 CFU/mL in the Phosphate Buffered Saline (PBS), consisting of 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, and 15 mM KH_2PO_4 (Oxoid, UK). The cell suspensions were standardized by adjusting the optical density to 0.1 at 600 nm (Thermo Scientific UV-Vis Spectrophotometer, UK) before being diluted 1:100 in LB 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 of $5x10^5$ CFU/mL and each sample was tested in duplicate in at least two independent experiments in order 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 were indicative of bacterial growth. The MIC values were recorded as the lowest concentration at which no bacterial growth was detected.

2.3 Toxicity evaluation in Caenorhabditis 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 was measured in a 4-day chitinase assay, as described in detail by Nisler et al., 2022 [10].

2.4 Cytotoxicity Activity

The effect of 72-hour treatments with the test compounds on human non-cancer cell line viability was evaluated using a resazurin reduction assay, measuring 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. Only HaCaT were obtained from the German Cancer Research Center (DKFZ), Heidelberg, Germany. The assay was carried out according to Voller and co-workers [15]. Each experiment was repeated at least three times. IC_{50} values were calculated using the drc package for R software (https://cran.r-project.org/web/packages/drc).

3. Results and Discussion

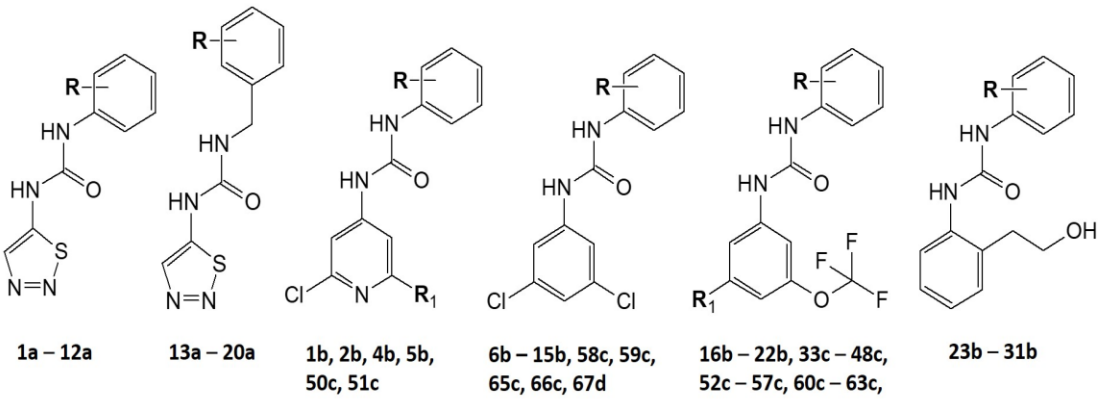

Fig. 1. Structures of the urea derivatives tested in this study.

The series of compounds were developed according to general protocols for the synthesis of diphenylurea derivatives using aromatic isocyanates and the corresponding anilines [16].

The antimicrobial screening of approximately 70 urea derivatives (Figure 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, **11b, 33c, 41c, 50c, 51c, 55c, 56c 62c, and 67d,** showed excellent inhibitory microbial growth activity (MIC ≤ 50 μM) against *S. aureus*. Compound **11b** is 1-(2-(2-aminoethyl)phenyl)-3-(3,5-dichlorophenyl)urea and is the only compound from the "b" series which was active against *S. aureus*. Compounds of the b-series contain in their structure 2-chloropyridine, 2,6-dichloropyridine, 3,5-dichlorophenyl or a (trifluoromethoxy)phenyl group. This indicated that the activity of compound **11b** was attributed to a combination of 2-aminoethyl group with 3,5-dichlorophenyl group rather than to a 3,5-dichlorophenyl group only. The same principle applied in the case of compounds of the c-series. Most of the compounds contain 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 substituent on

inhibitory activity. All compounds (**33c** – **48c**) are 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** bears a chlorine atom in position 3. From a comparison of the results with compounds **41c** and **16b**, it is further apparent that pyridine-phenyl urea derivatives show higher toxicity than the diphenyl urea derivatives. This was confirmed by results obtained for toxicity of compounds **50c** and **51c** (compare the activity of compound **51c** with that of **52c**). The role of the size of a halogen atom can be demonstrated by comparison of the toxicity of compounds **56c** and **57c**, which bear bromine instead of chlorine.

Supporting these findings, a recent report revealed bactericidal and anti-biofilm activities against *S. aureus* with compounds containing the dichlorophenyl group [17]. However, the positions of the chlorines are 3 and 4 on the urea backbone. Another study showed the importance of the different key moieties, such as the dichlorophenyl, in RnpA inhibitors in *S. aureus* [18]. RnpA has been hypothesized 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 NTCT 10418

Compounds **5b**, **51c, 52c, 56c, 57c, 62c,** and **67d** showed remarkable growth inhibition towards *E. coli* NTCT 10418. In this case it seems that suitably substituted diphenyl urea derivatives exhibit higher toxicity than similar pyridyl-phenyl urea derivatives (compare the activity of **51c** with that of **52c**). The comparison of the MIC of **52c** with those of **56c**, **57c** and **62c** indicated that an additional halogen atom or the substitution of the 3,5-dichlorophenyl group by another 3-(trifluoromethoxy)phenyl group, do not improve the antimicrobial activity against *E. coli* NTCT 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* NTCT 10418.

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

In contrast, most of the compounds screened exhibited poor or no activity against *E. coli* G69 (a multi-drug resistant strain) and *K. pneumoniae* CPE 16 (the carbapenemase-producing bacterial strain). Notwithstanding, 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, stressing the significant activity of these urea derived compounds, specifically.

These three urea derivatives **7b**, **11b** and **67d** bear aminomethyl or aminoethyl moieties in their structures. Interestingly, the 2-aminoethyl linked via an amide group to a phenyl ring had higher antimicrobial effects than a 2-aminoethyl attached directly to phenyl ring. Furthermore, multiple studies showing antimicrobial properties attributed, at least partially, 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]. This activity was significantly affected by the position, length, and quantity of aminoalkyl moieties in the structures, revealing the importance of this functional group on this particular activity.

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

Notwithstanding, 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*, lower than the standard amoxicillin. Similarly, derivative **62c** (1,3-bis(3-(trifluoromethoxy)phenyl)urea) exhibited the lowest MIC of 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 former studies. A report 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, it seems promising to combine substitutions such as aminoalkyl groups with trifluoromethoxy groups and/or chlorines.

3.2 Toxicity to the nematode C. elegans

The antinematodal activity of all compounds against *C. elegans* was assessed by the chitinase assay (Table 1, Figure 2A and 2B) combined with microscopic evaluation of the populations. Compounds **4b**, **8b**, **9b**, **18b**, **29b**, **41c**, and **55c** inhibited the growth of 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 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**, where 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). Part of these results was shown and discussed recently [10].

Generally, compounds of the b-series were less toxic than compounds of the c-series. From the results obtained with b-series compounds, we can conclude that hydroxymethyl group in compound **8b** caused inhibition of *C. elegans* development more severely than other moieties (such as hydroxyethyl, aminomethyl, aminoethyl, chloroethyl, carboxylic acid) attached in the same position on the same compound. A similar effect could be attributed to methoxymethyl 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 a further 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**), where the trifluoromethoxy group is attached on the same molecule in the *ortho*, *meta*, or *para* position, revealed that this group exhibited the highest toxic effect when *para* (**29b**).

The results with compounds of the c-series further showed that these derivatives exhibited a significantly larger toxic effect on the vitality of the nematodes than the compounds of the previous series. It was apparent that the presence of other group(s) (e.g., hydroxyethyl group) rather than halogen or trifluoromethoxy-phenyl, 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** (1B). Again, the detrimental influence of the trifluoromethoxy-phenyl group was also evident from a comparison of the effects 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* [29]. Once again, these findings corroborate and reinforce the importance of the presence of trifluoromethoxy-phenyl groups on the antinematode effect.

3.3 Toxic effects in noncancer human cells

In order to evaluate the cytotoxicity of the derivatives active against any of the tested bacteria strains to human non-cancer cells (ARPE-19, BJ and HaCaT), a resazurin reduction assay measuring the metabolic activity of the cell population was used after 3 days 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 to 32 µM against all human cell lines used (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 the cytotoxicity for human cells, suggesting selectivity and a potential therapeutic application.

Overall, the results indicated that the urea derivative compounds, especially those comprising 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 using the same concentrations, it still remains an interesting observation, and suggests the possibility of further development and biological evaluation of other urea derivatives comprising these groups. Additionally, the crucial compounds of these urea derivatives are obtainable via easy synthesis from low-cost and easy-to-access 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 antinematode activity towards *C. elegans*. Testing on non-cancer human cell lines suggested that the compounds have potential to affect bacteria and 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 present an increasing challenge in medicine, urea derivatives may offer a useful alternative in the field of new antimicrobial and anthelmintic development.

5. Conflict of interest

The authors have declared that there are no conflicts of interest.

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7. Ethical Approval

Not required.

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Table 1. Minimum Inhibitory Concentrations (μM**)** of the urea derivatives against the strains *S. aureus* NCTC 12981, *E. coli* NCTC 10418, *E. coli* G69 and *K. pneumoniae* CPE 16, the IC_{50} (μM**)** of the compounds against the nematode *C. elegans*, and the cytotoxicity (μM**)** of the active derivatives against human non-cancer cells (ARPE-19, BJ and HaCaT).

Compound	***S. aureus* NCTC 12981**	***E. coli* NCTC 10418**	***E. coli* G69**	***K. pneumoniae CPE 16***	***C. elegans***	**ARPE-19**	**BJ**	**HaCaT**
	MIC (μM)	**MIC (μM)**	**MIC (μM)**	**MIC (μM)**	**IC_{50} (μM)**	**IC_{50} (μM)**	**IC_{50} (μM)**	**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*

n.t. – not tested; *N.A.* – not active (viability of *C. elegans* was higher than 75% in the presence of 50 μM compound).

Figure 2A. The effect of tested compounds on the reproductive capacity of *C. elegans*. Values are means ± SD of at least the results from two independent assays.

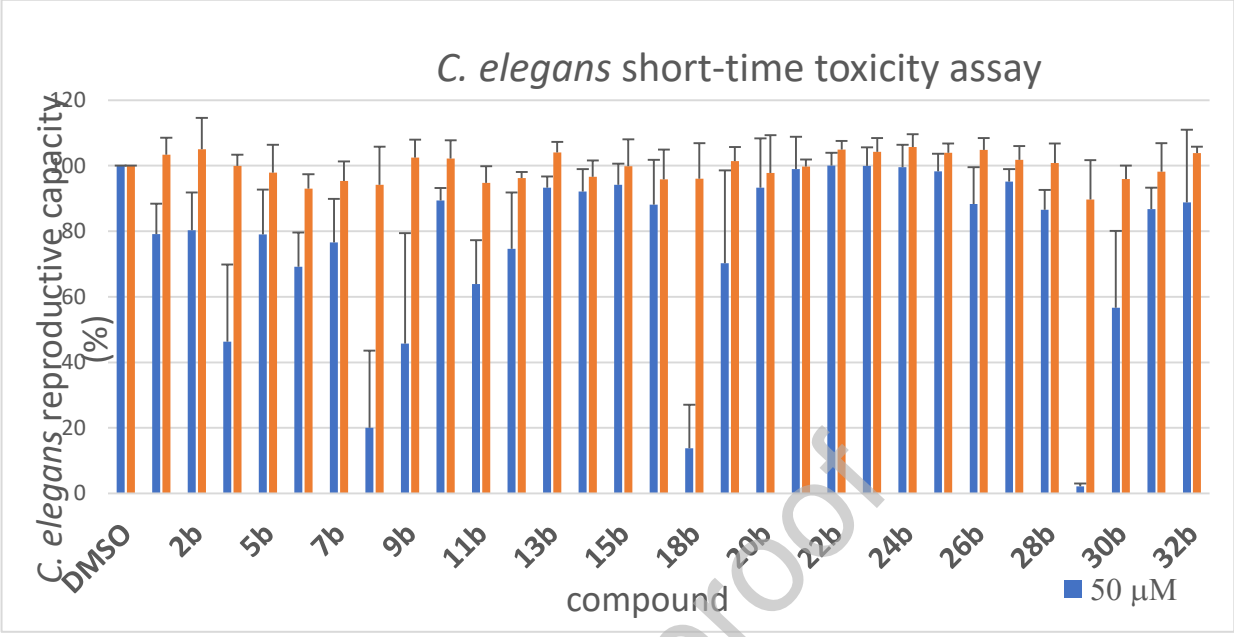

Figure 2B. The effect of tested compounds on the reproductive capacity of *C. elegans*. Values are means ± SD of at least the results from two independent assays.