

## Characterisation of twelve newly synthesised *N*-(substituted phenyl)-2-chloroacetamides with QSAR analysis and antimicrobial activity tests

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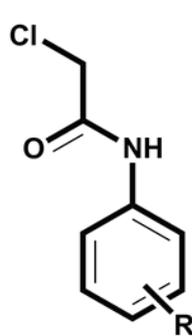
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In this study we screened twelve newly synthesised *N*-(substituted phenyl)-2-chloroacetamides for antimicrobial potential relying on quantitative structure-activity relationship (QSAR) analysis based on the available cheminformatics prediction models (Molinspiration, SwissADME, PreADMET, and PkcSM) and verified it through standard antimicrobial testing against *Escherichia coli*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Candida albicans*. Our compounds met all the screening criteria of Lipinski's rule of five (Ro5) as well as Veber's and Egan's methods for predicting biological activity. In antimicrobial activity tests, all chloroacetamides were effective against Gram-positive *S. aureus* and MRSA, less effective against the Gram-negative *E. coli*, and moderately effective against the yeast *C. albicans*. Our study confirmed that the biological activity of chloroacetamides varied with the position of substituents bound to the phenyl ring, which explains why some molecules were more effective against Gram-negative than Gram-positive bacteria or *C. albicans*. Bearing the halogenated *p*-substituted phenyl ring, *N*-(4-chlorophenyl), *N*-(4-fluorophenyl), and *N*-(3-bromophenyl) chloroacetamides were among the most active thanks to high lipophilicity, which allows them to pass rapidly through the phospholipid bilayer of the cell membrane. They are the most promising compounds for further investigation, particularly against Gram-positive bacteria and pathogenic yeasts.

**KEY WORDS:** *N*-substituted amides; antimicrobial potential; quantitative analysis of chemical structure and activity relationship

The growing spread and resistance of various pathogens call for developing new promising antimicrobial agents. One such group of agents that have received attention due to a wide variety of biological activities (such as analgesic, antipyretic, antimicrobial, bactericidal, fungicidal, hypoglycaemic, and antitumor) and applications in agriculture are chloroacetamides (1–10). Their biological activity is driven primarily by their chemical structure, i.e. the type of functional groups that bind to active sites on receptors of bacterial and fungal strains and promote desired intermolecular interactions. Knowing the relation between specific structures and their activity allows us to predict the biological activity of newly synthesised structures. As there is ample evidence (1–18) that *N*-substituted chloroacetamides are highly effective and selective as microbial reagents, we were encouraged to develop new *N*-(substituted phenyl)-2-chloroacetamide analogues (19) (Figure 1) with an aim to improve their selectivity, lipophilicity, and antimicrobial activity. This study is therefore an extension on a series of

newly synthesised *N*-(substituted phenyl)-2-chloroacetamides with the aim to determine how the chemical structure of their substituted functional residues contributes to their antimicrobial activity.



No.	R
1	H
2	4-CH <sub>3</sub>
3	4-OCH <sub>3</sub>
4	4-Cl
5	4-Br
6	4-F
7	4-I
8	4-COCH <sub>3</sub>
9	4-OH
10	3-CN
11	4-CN
12	3-Br

**Figure 1** Structural formula of the investigated *N*-(substituted phenyl)-2-chloroacetamides

To do that, we applied quantitative structure-activity relationship (QSAR) analysis as well as Lipinski's *rule of five* (Ro5) (20) and its extensions such as Veber's (21) and Egan's (22) methods. The assessment of biological activity took into account molecular descriptors, biophysicochemical properties, and biophysical-kinetic parameters. In addition, the synthesised derivatives were evaluated *in vitro* for antimicrobial activity against some of the most common pathogens.

## MATERIALS AND METHODS

### *Synthesis of N*-(substituted phenyl)-2-chloroacetamides

*N*-(substituted phenyl)-2-chloroacetamides – namely *N*-phenyl chloroacetamide (SP1), *N*-(4-methylphenyl) chloroacetamide (SP2), *N*-(4-methoxyphenyl) chloroacetamide (SP3), *N*-(4-chlorophenyl) chloroacetamide (SP4), *N*-(4-bromophenyl) chloroacetamide (SP5), *N*-(4-fluorophenyl) chloroacetamide (SP6), *N*-(4-iodophenyl) chloroacetamide (SP7), *N*-(4-acetylphenyl) chloroacetamide (SP8), *N*-(4-hydroxyphenyl) chloroacetamide (SP9), *N*-(4-cyanophenyl) chloroacetamide (SP10), *N*-(3-cyanophenyl) chloroacetamide (SP11), and *N*-(3-bromophenyl) chloroacetamide (SP12) – were synthesised following the method described in our earlier article (19).

### *Characterisation methods and spectral analysis*

The chemical structure and purity of the synthesised compounds were verified by melting point, Fourier-transform infrared (FTIR), and <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) spectroscopy. FTIR spectra were recorded in transmission mode using a Bomem MB 100 (ABB Bomem Inc., Quebec, Canada) spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were determined in deuterated

dimethylsulphoxide (DMSO-*d*<sub>6</sub>), used as the solvent, and recorded on a Bruker AC-250 spectrometer (Bruker Corporation, Billerica, MA, USA) at 200 MHz using tetramethylsilane (TMS) as internal standard. Chemical shifts were determined with respect to distortionless enhancement by polarisation transfer (DEPT), two-dimensional <sup>1</sup>H to <sup>13</sup>C heteronuclear correlation (HETCOR), and selective insensitive nuclei enhancement by polarisation transfer (INEPT) long-range experiments and are expressed in ppm with respect to TMS ( $\delta_{\text{H}}=0$  ppm) in the <sup>1</sup>H NMR spectra and to residual solvent signal ( $\delta_{\text{C}}=39.5$  ppm) in the <sup>13</sup>C NMR spectra. Full spectral characterisation of all 12 chloroacetamides is given in Tables 1–3.

### *QSAR analysis*

Molecular descriptors, i.e. molecular weight (MW), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), molecular hydrophobicity/partition coefficient (log*P*), number of rotatable bonds (Nrot), and topological polar surface area (TPSA) of the twelve synthesised chloroacetamides were obtained using the available computational web tools, namely Molinspiration (23) and SwissADME (24), while their biophysical-kinetic parameters related to absorption and metabolism were obtained using SwissADME (24), PreADMET (25), and PkcSM (26) designed to predict absorption, distribution, metabolism, excretion (ADME) and bioactivity of tested molecules.

### *Microbial strains and growth conditions*

Antibacterial activity was tested against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and methicillin resistant *S. aureus* (MRSA) ATCC 33591 and antifungal activity against *Candida albicans* ATCC 10231. The bacterial strains were cultured in the Luria-

**Table 1** Melting point and yield of *N*-(substituted phenyl) chloroacetamides

Compound	Substituent	Melting point (°C)	Yield (%)
SP1	H	136–137	86
SP2	4-CH <sub>3</sub>	160–162	89
SP3	4-OCH <sub>3</sub>	117–119	84
SP4	4-Cl	166–168	65
SP5	4-Br	178–180	88
SP6	4-F	128–130	83
SP7	4-I	192–195	72
SP8	4-CH <sub>3</sub> CO	144–145	64
SP9	4-OH	144–146	76
SP10	4-CN	180–183	56
SP11	3-CN	165–170	61
SP12	3-Br	110–113	83

SP1 – *N*-phenyl chloroacetamide; SP2 – *N*-(4-methylphenyl) chloroacetamide; SP3 – *N*-(4-methoxyphenyl) chloroacetamide; SP4 – *N*-(4-chlorophenyl) chloroacetamide; SP5 – *N*-(4-bromophenyl) chloroacetamide; SP6 – *N*-(4-fluorophenyl) chloroacetamide; SP7 – *N*-(4-iodophenyl) chloroacetamide; SP8 – *N*-(4-acetylphenyl) chloroacetamide; SP9 – *N*-(4-hydroxyphenyl) chloroacetamide; SP10 – *N*-(4-cyanophenyl) chloroacetamide; SP11 – *N*-(3-cyanophenyl) chloroacetamide; SP12 – *N*-(3-bromophenyl) chloroacetamide

**Table 2** Characterisation of investigated *N*-(substituted phenyl)-2-chloroacetamides

Comp	R	IR (KBr) $\nu_{\max}$ (cm <sup>-1</sup> )
SP1	H	3267 (N-H); 3207, 3145, 3098 (C-H aromatic ring); 2947 (C-H); 1671 (C=O); 1618 (C=C); 1557 (N-H deformation); 1498, (C-H bending); 1443 (C-H bending); 1344 (C-H); 1251 (C-N); 749 (N-H).
SP2	4-CH <sub>3</sub>	3273 (N-H); 3204, 3135, 3090 (C-H aromatic ring); 2954 (C-H); 1673 (C=O); 1616 (C=C); 1554 (N-H); 1402 (C-H); 1343 (C-H); 1251 (C-N); 818 (N-H).
SP3	4-OCH <sub>3</sub>	3295 (N-H); 3139, 3073 (C-H aromatic ring); 2957 (C-H); 2909 2835 (C-H); 1663 (C=O); 1612 (C=C); 1547 (N-H); 1510 (N-H); 1465 (C-H); 1413 (C-H); 1247 (C-N); 830 (N-H).
SP4	4-Cl	3264(N-H); 3199, 3131, 3082 (C-H aromatic ring); 3005, 2952(C-H); 1669 (C=O); 1614 (C=C); 1551 (N-H); 1490 (C-H); 1400 (C-H); 1248 (C-N); 825 (N-H).
SP5	4-Br	3263 (N-H); 3194 (C-H); 3125, 3077 (C-H aromatic ring); 3000 2953 (C-H); 1669 (C=O); 1549 (N-H); 1488 (C-H); 1395 (C-H); 1248 (C-N); 822 (N-H).
SP6	4-F	3275, 3221 (N-H); 3165 (C-H aromatic ring); 2947 (C-H); 1668 (C=O); 1508 (N-H); 1406 (C-H); 1292; 1212 (C-N); 832 (N-H).
SP7	4-I	3309, 3270 (N-H); 3194, 3077 (C-H aromatic ring); 2936 (C-H); 2953 (C-H); 1672 (C=O); 1610 (N-H); 1543 (C-H); 1392–1089 (CH); 1245 (C-N); 817 (N-H).
SP8	4-COCH <sub>3</sub>	3325, 3286 (N-H); 3196, 3109 (C-H aromatic ring); 2922, 2857 (C-H); 1707 (C=O); 1655 (C=C); 1599 (N-H); 1539 (C-H); 1283 (C-O); 1252 (C-N); 834 (N-H).
SP9	4-OH	3296 (O-H); 3144 (N-H); 3098 (C-H); 1677 (C=O); 1508 (N-H); 1313 (C-H); 1211 (C-N); 820 (N-H).
SP10	4-CN	3265 (N-H); 3192, 3119 (C-H); 2946 (C-H); 2226 (C?N); 1681 (C=O); 1603 (C=C); 1539 (N-H); 1408, 1345 (C-H); 1256 (C-N); 839 (N-H).
SP11	3-CN	3265 (N-H); 3096 (C-H); 2964 C-H); 2232 (C?N); 1678 (C=O); 1610 (C=C); 1561 (N-H); 1485 (C-H); 1293 (C-N); 1089 (C-H); 799 (N-H).
SP12	3-Br	3268 (N-H); 3193, 3127 (C-H); 2945 (C-H); 1679 (C=O); 1594 (N-H); 1424 (C-H); 1249 (C-N); 779 (N-H).

SP1 – *N*-phenyl chloroacetamide; SP2 – *N*-(4-methylphenyl) chloroacetamide; SP3 – *N*-(4-metoxylphenyl) chloroacetamide; SP4 – *N*-(4-chlorophenyl) chloroacetamide; SP5 – *N*-(4-bromophenyl) chloroacetamide; SP6 – *N*-(4-fluorophenyl) chloroacetamide; SP7 – *N*-(4-iodophenyl) chloroacetamide; SP8 – *N*-(4-acetylphenyl) chloroacetamide; SP9 – *N*-(4-hydroxyphenyl) chloroacetamide; SP10 – *N*-(4-cyanophenyl) chloroacetamide; SP11 – *N*-(3-cyanophenyl) chloroacetamide; SP12 – *N*-(3-bromophenyl) chloroacetamide

Bertani (LB) medium (HiMedia, Mumbai, India) and *C. albicans* in tryptic soy broth (TSB) (Biomedics, Madrid, Spain). The bacterial strains and the yeast were cultured overnight at 37 °C. Suspensions were adjusted to 0.5 McFarland standard turbidity (BioMérieux, Marcy-l'Étoile, France), which corresponds to 10<sup>8</sup> CFU/mL.

#### MIC assay

Minimum inhibitory (MIC), minimum bactericidal (MBC), and minimum fungicidal concentrations (MFC) for the 12 *N*-(substituted phenyl)-2-chloroacetamides (SP1–12) were determined using the broth microdilution method. The final concentration of each sample in the first well was 4000 µg/mL, while the concentration of the solvent dimethyl sulphoxide (DMSO) was 5 %. Twofold serial dilutions of the chloroacetamide samples were made with LB and TSB in 96-well microtitre plates in the concentration range from 32 to 4000 µg/mL. Besides negative control (untreated bacteria and fungi) we also used sterility control (containing only the culture medium) and positive control, treated with rifampicin and nystatin. The final concentration of rifampicin and nystatin in the first well was 400 and 2000 µg/mL, respectively. Each well, except for the sterility

control, was inoculated with 20 µL of bacterial and yeast culture (1×10<sup>8</sup> CFU/mL), reaching a final volume of 200 µL. At the end, 22 µL of resazurin (oxidation-reduction indicator of cell growth) was added to each well. The plates were incubated at 37 °C for 24 h. All tests were performed in a lighted environment, but the plates were incubated in the dark. Resazurin is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases from viable cells (27). MIC was determined as no change in colour. MBC and MFC, which were obtained by sub-culturing test dilutions from each well without colour change on agar plates and incubating them for 24 h, corresponded to the lowest concentration that showed no bacterial or yeast growth. The results were expressed in µg/mL.

#### Statistical analysis

For the analysis of variance (ANOVA) we used the Kolmogorov-Smirnov test for the normality of residuals and Levene's test for homogeneity of variance. For mean separation for MIC, MBC, and MFC we used Tukey's honest significant difference (HSD) test. Significance was set at *P*<0.05. All dilutions were tested in duplicate with

**Table 3** <sup>1</sup>H and <sup>13</sup>C NMR spectral data

<i>N</i> -phenyl chloroacetamide ( <b>SP1</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.272 (2H, s, Cl-CH <sub>2</sub> ), 7.057–7.130 (1H, t, <i>J</i> <sub>HH</sub> = 7.4 Hz, Ar-4H), 7.302–7.380 (2H, t, <i>J</i> <sub>HH</sub> = 7.8 Hz, Ar-H), 7.597–7.636 (2H, d, <i>J</i> <sub>HH</sub> = 7.8 Hz, Ar-H), 10.321 (1H, s, NH). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.833 (Cl-CH <sub>2</sub> ), 119.651 (C <sub>2</sub> , C <sub>5</sub> ), 124.130 (C <sub>4</sub> ), 129.119 (C <sub>3</sub> , C <sub>6</sub> ), 138.751 (C <sub>1</sub> ), 164.934 (C=O).
<i>N</i> -(4-methylphenyl) chloroacetamide ( <b>SP2</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 2.255 (2H, s, CH <sub>3</sub> ), 4.421 (1H, s, Cl-CH <sub>2</sub> ), 7.111–7.153 (2H, d, <i>J</i> <sub>HH</sub> = 8.2 Hz, Ar-H), 7.473–7.515 (2H, d, <i>J</i> <sub>HH</sub> = 8.2 Hz, Ar-H), 10.222 (1H, s, NH). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 20.655 (CH <sub>3</sub> ), 43.797 (Cl-CH <sub>2</sub> ), 119.614 (C <sub>2</sub> , C <sub>6</sub> ), 129.483 (C <sub>3</sub> , C <sub>5</sub> ), 133.088 (C <sub>1</sub> ), 136.238 (C <sub>4</sub> ), 164.643 (C=O).
<i>N</i> -(4-methoxyphenyl) chloroacetamide ( <b>SP3</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 3.729 (2H, s, OCH <sub>3</sub> ), 4.229 (1H, s, Cl-CH <sub>2</sub> ), 6.886–6.948 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 7.481–7.560 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 10.177 (1H, s, NH). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.742 (Cl-CH <sub>2</sub> ), 55.359 (OCH <sub>3</sub> ), 114.189 (C <sub>3</sub> , C <sub>5</sub> ), 121.217 (C <sub>2</sub> , C <sub>6</sub> ), 131.814 (C <sub>1</sub> ), 155.885 (C <sub>4</sub> ), 164.424 (C=O).
<i>N</i> -(4-chlorophenyl) chloroacetamide ( <b>SP4</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.280 (1H, s, Cl-CH <sub>2</sub> ), 7.358–7.431 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 7.613–7.686 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 10.445 (1H, s, NH). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.741 (Cl-CH <sub>2</sub> ), 121.162 (C <sub>2</sub> , C <sub>6</sub> ), 129.010 (C <sub>3</sub> , C <sub>5</sub> ), 137.677 (C <sub>1</sub> ), 165.061 (C=O).
<i>N</i> -(4-bromophenyl) chloroacetamide ( <b>SP5</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.274 (1H, s, Cl-CH <sub>2</sub> ), 7.495–7.616 (4H, m, Ar-H), 10.447 (1H, s, N-H). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.742 (Cl-CH <sub>2</sub> ), 115.736 (C <sub>4</sub> ), 121.526 (C <sub>2</sub> , C <sub>6</sub> ), 131.923 (C <sub>3</sub> , C <sub>5</sub> ), 138.095 (C <sub>1</sub> ), 165.061 (C=O).
<i>N</i> -(4-fluorophenyl) chloroacetamide ( <b>SP6</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.369 (1H, s, Cl-CH <sub>2</sub> ), 7.122–7.226 (2H, t, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 7.588–7.675 (2H, m, Ar-H), 10.337 (1H, s, NH). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.688 (Cl-CH <sub>2</sub> ), 115.463–115.900 (C <sub>3</sub> , C <sub>5</sub> ), 121.381 (C <sub>2</sub> , C <sub>6</sub> ), 135.073 (C <sub>1</sub> ), 160.983 (C <sub>4</sub> ), 164.861 (C=O).
<i>N</i> -(4-iodophenyl) chloroacetamide ( <b>SP7</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.263 (1H, s, Cl-CH <sub>2</sub> ), 7.425–7.4709 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 7.658–7.701 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 10.416 (1H, s, NH). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.760 (Cl-CH <sub>2</sub> ), 87.732 (C <sub>4</sub> ), 121.745 (C <sub>2</sub> , C <sub>6</sub> ), 137.750–138.551 (C <sub>3</sub> , C <sub>5</sub> ), 165.043 (C=O).
<i>N</i> -(4-acetylphenyl) chloroacetamide ( <b>SP8</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 2.544 (3H, s, CH <sub>3</sub> ), 4.328 (1H, s, Cl-CH <sub>2</sub> ), 7.723–7.768 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 7.945–7.990 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 10.646 (1H, s, NH). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 26.645 (CH <sub>3</sub> ), 43.833 (Cl-CH <sub>2</sub> ), 118.868 (C <sub>2</sub> , C <sub>6</sub> ), 129.793 (C <sub>3</sub> , C <sub>5</sub> ), 132.451 (C <sub>4</sub> ), 143.030 (C <sub>1</sub> ), 165.462 (C=O), 196.798 (COCH <sub>3</sub> ).
<i>N</i> -(4-hydroxyphenyl) chloroacetamide ( <b>SP9</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.280 (2H, s, Cl-CH <sub>2</sub> ), 4.684 (1H, s, OH), 7.139–7.184 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 7.625–7.686 (2H, d, <i>J</i> <sub>HH</sub> = 8.8 Hz, Ar-H). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.706 (Cl-CH <sub>2</sub> ), 120.671 (C <sub>3</sub> , C <sub>5</sub> ), 122.091 (C <sub>2</sub> , C <sub>6</sub> ), 136.7489 (C <sub>1</sub> ), 146.180 (C <sub>4</sub> ), 164.989–166.791 (C=O).
<i>N</i> -(4-cyanophenyl) chloroacetamide ( <b>SP10</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.319 (2H, s, Cl-CH <sub>2</sub> ), 7.552–7.619 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 7.782–7.877 (2H, d, <i>J</i> <sub>HH</sub> = 9.0 Hz, Ar-H), 10.745 (1H, s, 2-H). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.669 (Cl-CH <sub>2</sub> ), 111.985 (C <sub>3</sub> ), 118.668 (CN), 122.309 (C <sub>2</sub> ), 124.203 (C <sub>6</sub> ), 127.662 (C <sub>4</sub> ), 130.321–130.594 (C <sub>5</sub> ), 139.516 (C <sub>1</sub> ), 165.589 (C=O).
<i>N</i> -(3-cyanophenyl) chloroacetamide ( <b>SP11</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.339 (1H, s, Cl-CH <sub>2</sub> ), 7.552–7.619 (2H, d, <i>J</i> <sub>HH</sub> = 5.6 Hz, Ar-H), 7.782–7.813 (1H, m, Ar-H), 8.094 (1H, s, Ar-H), 10.745 (1H, s, NH). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.669 (Cl-CH <sub>2</sub> ), 111.985 (C <sub>3</sub> ), 118.668 (CN), 122.309 (C <sub>2</sub> ), 124.203 (C <sub>6</sub> ), 127.662 (C <sub>4</sub> ), 130.321–130.594 (C <sub>5</sub> ), 139.516 (C <sub>1</sub> ), 165.589 (C=O).
<i>N</i> -(3-bromophenyl) chloroacetamide ( <b>SP12</b> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> ): δ 4.286 (3H, s, Cl-CH <sub>2</sub> ), 7.285–7.358 (2H, m, Ar-H), 7.470–7.571 (1H, m, Ar-H), 7.962 (1H, s, Ar-H), 10.489 (1H, s, N-H). <sup>13</sup> C NMR (CDCl <sub>3</sub> ): δ 43.688 (Cl-CH <sub>2</sub> ), 118.376 (C <sub>2</sub> ), 121.836 (C <sub>5</sub> ), 121.927 (C <sub>6</sub> ), 126.697 (C <sub>4</sub> ), 131.067 (C <sub>3</sub> ), 140.262 (C <sub>1</sub> ), 165.243 (C=O).

three repetitions. Statistical analyses were run on STATISTICA v.7 (StatSoft, Inc., Tulsa, OK, USA) and IBM SPSS Statistics v.20 (SPSS, Inc., Chicago, IL, USA).

## RESULTS

### Biological profile of *N*-(substituted phenyl)-2-chloroacetamides

Table 4 shows that compounds containing the 4-COCH<sub>3</sub> (SP8), 4-OH (SP9), 3-CN (SP10), and 4-CN (SP11) groups within the phenyl core had their TPSA in the optimal interval from 46.17 to 52.89 Å<sup>2</sup>, which is the most favourable for high permeability. *N*-(4-bromophenyl)-2-chloroacetamide (SP5) showed the highest lipophilicity, and the compound carrying the *p*-OH-substituent (SP9) the lowest (Table 5).

The best predisposition for optimal intestinal absorption was seen in the derivatives containing electron-donor substituent (compound SP3) and strong electron-acceptor/halogen substituents (compounds SP8 and SP10–12), and these properties were significantly higher than those observed for commercial drugs levetiracetam and piracetam (Table 6).

The above mentioned web tools Molinspiration (23), SwissADME (24), PreADMET (25), and PkcSM (26) predicted that the investigated chloroacetamides would not significantly inhibit the activity of P-glycoprotein (P-gp or ABCB1) (Table 7). Regarding CYP450 inhibition, CYP1A2 showed the highest probability to be inhibited by all tested chloroacetamides.

### Antimicrobial activity

Table 8 shows the results of antibacterial and antifungal activity of the tested chloroacetamides. DMSO, which was used as negative/solvent control, did not show any inhibitory effect on the tested strains. The most sensitive strains, with MIC mainly lower than 100 µg/mL, were *S. aureus* and MRSA. The MIC of most compounds ranged between 40 and 130 µg/mL for these pathogens and did not significantly differ from rifampicin. *N*-(4-iodophenyl) chloroacetamide (SP7) showed the strongest activity against both Gram-positive strains (MIC 40 µg/mL). *E. coli* was the most resistant strain, as the MIC of half of the tested compounds (SP1, SP2, SP4, SP5, SP7, and SP11) ranged from 920 to 4000 µg/mL. All compounds save for SP1 and SP11 were significantly less effective than rifampicin.

The yeast strain *C. albicans* showed moderate susceptibility to chloroacetamides compared to Gram-positive bacterial strains, with MICs mostly below 500 µg/mL, but much higher than to positive control nystatin, with the MIC of 2000 µg/mL. Only *N*-(4-hydroxyphenyl) chloroacetamide (SP9) had a higher MIC of 2660 µg/mL. The best and statistically significant inhibitory activity vs nystatin against *C. albicans* was observed for SP4, SP6, and SP12, with MICs ranging from 60 to 100 µg/mL and similar to the one against Gram-positive bacterial strains. In general, *N*-(4-cyanophenyl) chloroacetamide (SP10) showed significant inhibitory activity (lowest MICs) against all tested bacterial strains and yeast taken together. SP4 and SP12 showed the strongest inhibitory activity against *C. albicans* and Gram-positive strains. It is interesting to note

**Table 4** Physicochemical properties of the studied chloroacetamides

Compound	Molecular weight (g/mol)	Number of atoms	Number of rotatable bonds	Number of hydrogen bond donors	Number of hydrogen bond acceptors	Molar refractivity	Topological polar surface area (Å <sup>2</sup> )
SP1	169.61	11	3	1	2	45.55	29.10
SP2	183.63	12	3	1	2	50.52	29.10
SP3	199.63	13	4	1	3	52.04	38.33
SP4	204.05	12	3	1	2	50.56	29.10
SP5	248.50	12	3	1	2	53.25	29.10
SP6	187.60	12	3	1	2	45.51	29.10
SP7	295.50	12	3	1	2	58.27	29.10
SP8	211.64	14	4	1	3	55.75	46.17
SP9	185.61	12	3	2	3	47.57	49.33
SP10	194.62	13	3	1	3	50.27	52.89
SP11	194.62	13	3	1	3	50.27	52.89
SP12	248.50	12	3	1	3	53.25	29.10
Levetiracetam	156.23	11	3	1	2	48.17	46.33
Piracetam	142.16	10	2	1	2	38.76	63.40

SP1 – *N*-phenyl chloroacetamide; SP2 – *N*-(4-methylphenyl) chloroacetamide; SP3 – *N*-(4-methoxyphenyl) chloroacetamide; SP4 – *N*-(4-chlorophenyl) chloroacetamide; SP5 – *N*-(4-bromophenyl) chloroacetamide; SP6 – *N*-(4-fluorophenyl) chloroacetamide; SP7 – *N*-(4-iodophenyl) chloroacetamide; SP8 – *N*-(4-acetylphenyl) chloroacetamide; SP9 – *N*-(4-hydroxyphenyl) chloroacetamide; SP10 – *N*-(4-cyanophenyl) chloroacetamide; SP11 – *N*-(3-cyanophenyl) chloroacetamide; SP12 – *N*-(3-bromophenyl) chloroacetamide

**Table 5** Partition coefficients of the studied chloroacetamides

Compound	log $P$ (22)	log $P_{o/w}$ (XLOGP3) (23)	log $P_{o/w}$ (WLOGP) (23)	log $P_{o/w}$ (MLOGP) (23)
SP1	1.72	1.63	1.67	1.84
SP2	2.17	1.99	1.98	2.15
SP3	1.78	1.65	1.68	1.54
SP4	2.40	2.26	2.33	2.42
SP5	2.53	2.32	2.44	2.56
SP6	1.89	1.73	2.23	2.27
SP7	2.81	2.28	2.28	2.71
SP8	1.62	1.86	1.88	1.47
SP9	1.24	1.27	1.38	1.23
SP10	1.45	1.82	1.54	1.18
SP11	1.48	1.35	1.54	1.18
SP12	2.51	2.93	2.44	2.56
Levetiracetam	0.69	0.62	-0.03	0.28
Piracetam	-1.32	-1.54	-1.29	-0.96

SP1 – *N*-phenyl chloroacetamide; SP2 – *N*-(4-methylphenyl) chloroacetamide; SP3 – *N*-(4-metoxylphenyl) chloroacetamide; SP4 – *N*-(4-chlorophenyl) chloroacetamide; SP5 – *N*-(4-bromophenyl) chloroacetamide; SP6 – *N*-(4-fluorophenyl) chloroacetamide; SP7 – *N*-(4-iodophenyl) chloroacetamide; SP8 – *N*-(4-acetylphenyl) chloroacetamide; SP9 – *N*-(4-hydroxyphenyl) chloroacetamide; SP10 – *N*-(4-cyanophenyl) chloroacetamide; SP11 – *N*-(3-cyanophenyl) chloroacetamide; SP12 – *N*-(3-bromophenyl) chloroacetamide

**Table 6** QSAR pharmacokinetic profiles of the selected compounds related to absorption properties

Compound	SwissADME	pkCSM	SwissADME	PreADMET	SwissADME	PreADMET
	Gastrointestinal absorption	Intestinal absorption (%)	the compound penetrates the blood-brain barrier	The compound penetrating the blood-brain barrier ( $c_{\text{brain}}/c_{\text{blood}}$ )	the compound is a P-gp inhibitor	the compound is a P-gp inhibitor
SP1	High	91.156	Yes	0.902206	No	No
SP2	High	91.692	Yes	2.16896	No	No
SP3	High	93.810	Yes	0.612824	No	No
SP4	High	91.969	Yes	1.65555	No	No
SP5	High	91.902	Yes	1.79202	No	No
SP6	High	91.217	Yes	1.07913	No	No
SP7	High	90.802	Yes	1.52595	No	No
SP8	High	92.635	Yes	0.546121	No	No
SP9	High	90.745	Yes	0.975597	No	No
SP10	High	92.986	Yes	0.975597	No	No
SP11	High	92.817	Yes	0.975597	No	No
SP12	High	92.405	Yes	1.79204	No	No
Levetiracetam	High	86.852	No	0.440234	No	No
Piracetam	High	86.061	No	0.165163	No	No

SP1 – *N*-phenyl chloroacetamide; SP2 – *N*-(4-methylphenyl) chloroacetamide; SP3 – *N*-(4-metoxylphenyl) chloroacetamide; SP4 – *N*-(4-chlorophenyl) chloroacetamide; SP5 – *N*-(4-bromophenyl) chloroacetamide; SP6 – *N*-(4-fluorophenyl) chloroacetamide; SP7 – *N*-(4-iodophenyl) chloroacetamide; SP8 – *N*-(4-acetylphenyl) chloroacetamide; SP9 – *N*-(4-hydroxyphenyl) chloroacetamide; SP10 – *N*-(4-cyanophenyl) chloroacetamide; SP11 – *N*-(3-cyanophenyl) chloroacetamide; SP12 – *N*-(3-bromophenyl) chloroacetamide

that SP7 and SP9, which strongly inhibited Gram-positive strains, were completely ineffective against *C. albicans*.

MICs for rifampicin control were in the range evidenced for most chloroacetamides against Gram-positive strains, and quite lower against *E. coli*. Chloroacetamide MBCs and MFCs were at least twice as high as their MICs, varying from 120 to over 4000 µg/mL in the case of MBCs for SP4 and SP5 against *E. coli*, which was the highest concentration we applied in testing (Table 8).

## DISCUSSION

Predicting biological activity of newly synthesised small molecules to be screened for medicinal use takes into account simple molecular properties such as molecular weight and the number of hydrogen/rotatable bonds, which determine the size, polarity, and flexibility of a compound (28, 29). The most common screening criterion is Lipinski's rule of five (Ro5). Its name stems from the following cheminformatics filters:  $MW \leq 500$  g/mol, number of HBD  $\leq 5$ , number of HBA  $\leq 10$ , and  $\log P \leq 5$ , whose aim is to filter out compounds that do not satisfy the most common oral absorption parameters. Although it does not predict whether a compound will be biologically active, Ro5 does not allow more than one deviation from the set parameters (20). This rule was later extended by other empirical thresholds for potential bioactivity, such as those proposed by Veber (21) ( $Nrot \leq 10$ ,  $TPSA \leq 140 \text{ \AA}^2$ , number of HBD/HBA  $\leq 12$ ) and Egan (22) ( $WlogP \leq 5.88$  and  $TPSA \leq 131.6 \text{ \AA}^2$ ).

Chloroacetamides synthesised and tested in our study met all of these criteria (Tables 4 and 5). The introduction of a *p*-substituted phenyl ring and the chlorine atom into the acetamide fragment increased the molecular weight of the synthesised compounds compared to commercial levetiracetam and piracetam, and their topological polar surface area in the range of 29.10–52.89 Å<sup>2</sup> and the number of rotatable bonds not exceeding 4 promise good biological activities (30). Veber (31) demonstrated that molecules with  $TPSA \leq 140 \text{ \AA}^2$  display efficient permeability. Higher partition coefficient  $\log P$  will allow biologically active chloroacetamides higher efficiency by passive diffusion as well as effective binding to the active receptor sites (32). Besides, almost no divergence in  $\log P$  (-Br, -CN) was observed for compounds with the same substituent in different positions (3). Likewise, all analysed chloroacetamide molecules showed higher absorption probability (Table 3) thanks to small size and low polarity (29).

In antimicrobial activity tests, all *N*-(substituted phenyl)-2-chloroacetamides were effective against Gram-positive bacteria, less effective against the Gram-negative *E. coli*, and moderately effective against the *C. albicans* yeast (Table 8). This was expected, given the differences in the cell wall structure and composition of these species. Ertan et al. (5) reported good bactericidal and fungicidal

Table 7 QSAR biophysical-kinetic profiles of the compounds related to metabolism properties

Prediction tool	SwissADME	pkCSM	SwissADME	pkCSM	SwissADME	pkCSM	SwissADME	pkCSM	SwissADME	pkCSM	SwissADME	pkCSM
Compound	Inhibits CYP1A2	Inhibits CYP1A2	Inhibits CYP2C19	Inhibits CYP2C19	Inhibits CYP2C9	Inhibits CYP2C9	Inhibits CYP2D6	Inhibits CYP2D6	Inhibits CYP2D6	Inhibits CYP3A4	Inhibits CYP3A4	Inhibits CYP3A4
SP1	Yes	No	No	No	No	No	No	No	No	No	No	No
SP2	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP3	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP4	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP5	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP6	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP7	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP8	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP9	No	No	No	No	No	No	No	No	No	No	No	No
SP10	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP11	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SP12	Yes	Yes	No	No	No	No	No	No	No	No	No	No
Levetiracetam	No	No	No	No	No	No	No	No	No	No	No	No
Piracetam	No	No	No	No	No	No	No	No	No	No	No	No

SP1 – *N*-phenyl chloroacetamide; SP2 – *N*-(4-methylphenyl) chloroacetamide; SP3 – *N*-(4-methoxyphenyl) chloroacetamide; SP4 – *N*-(4-chlorophenyl) chloroacetamide; SP5 – *N*-(4-bromophenyl) chloroacetamide; SP6 – *N*-(4-fluorophenyl) chloroacetamide; SP7 – *N*-(4-iodophenyl) chloroacetamide; SP8 – *N*-(4-acetylphenyl) chloroacetamide; SP9 – *N*-(4-hydroxyphenyl) chloroacetamide; SP10 – *N*-(4-cyanophenyl) chloroacetamide; SP11 – *N*-(3-cyanophenyl) chloroacetamide; SP12 – *N*-(3-bromophenyl) chloroacetamide

**Table 8** Minimum inhibitory, bactericidal, and fungicidal concentrations of *N*-(substituted phenyl)-2-chloroacetamides (means ± standard errors)

Tested substances	<i>R</i>	MIC (µg/mL)			
		<i>C. albicans</i>	<i>E. coli</i>	<i>S. aureus</i>	MRSA
SP1	4-H	190±40 <sup>c</sup>	920±80 <sup>c</sup>	90±20 <sup>c</sup>	50±0 <sup>cd</sup>
SP2	4-CH <sub>3</sub>	330±110 <sup>c</sup>	3330±330 <sup>ab</sup>	60±0 <sup>c</sup>	60±0 <sup>cd</sup>
SP3	4-OCH <sub>3</sub>	190±40 <sup>c</sup>	540±110 <sup>c</sup>	110±10 <sup>c</sup>	190±40 <sup>bc</sup>
SP4	4-Cl	60±0 <sup>c</sup>	3670±330 <sup>ab</sup>	60±0 <sup>c</sup>	90±20 <sup>bcd</sup>
SP5	4-Br	330±80 <sup>c</sup>	4000±0 <sup>a</sup>	60±0 <sup>c</sup>	60±0 <sup>cd</sup>
SP6	4-F	110±10 <sup>c</sup>	500±140 <sup>c</sup>	150±50 <sup>bc</sup>	110±10 <sup>bcd</sup>
SP7	4-I	830±170 <sup>c</sup>	2670±330 <sup>b</sup>	40±10 <sup>c</sup>	40±10 <sup>d</sup>
SP8	4-COCH <sub>3</sub>	330±80 <sup>c</sup>	330±80 <sup>c</sup>	190±40 <sup>bc</sup>	90±20 <sup>bcd</sup>
SP9	4-OH	2660±670 <sup>a</sup>	270±20 <sup>c</sup>	130±0 <sup>c</sup>	40±10 <sup>d</sup>
SP10	3-CN	290±40 <sup>c</sup>	190±40 <sup>c</sup>	40±10 <sup>c</sup>	90±20 <sup>bcd</sup>
SP11	4-CN	230±20 <sup>c</sup>	1000±290 <sup>c</sup>	750±140 <sup>a</sup>	220±20 <sup>ab</sup>
SP12	3-Br	100±20 <sup>c</sup>	500±140 <sup>c</sup>	80±20 <sup>c</sup>	90±20 <sup>bcd</sup>
Ant/Myc	–	2000±0 <sup>ab</sup>	90±10 <sup>c</sup>	40±10 <sup>c</sup>	70±20 <sup>cd</sup>
MBC/MFC (µg/mL)					
SP1	4-H	500±0 <sup>c</sup>	2000±0 <sup>b</sup>	250±0 <sup>bcd</sup>	120±0 <sup>c</sup>
SP2	4-CH <sub>3</sub>	670±170 <sup>c</sup>	4000±0 <sup>a</sup>	170±40 <sup>cd</sup>	310±110 <sup>c</sup>
SP3	4-OCH <sub>3</sub>	330±80 <sup>c</sup>	1000±0 <sup>c</sup>	250±0 <sup>bcd</sup>	330±80 <sup>c</sup>
SP4	4-Cl	2000±0 <sup>b</sup>	Nd	130±0 <sup>d</sup>	750±140 <sup>bc</sup>
SP5	4-Br	4000±0 <sup>a</sup>	Nd	420±80 <sup>bcd</sup>	750±140 <sup>bc</sup>
SP6	4-F	330±80 <sup>c</sup>	1000±0 <sup>c</sup>	750±140 <sup>bc</sup>	250±0 <sup>c</sup>
SP7	4-I	3000±580 <sup>ab</sup>	4000±0 <sup>a</sup>	130±0 <sup>d</sup>	290±110 <sup>c</sup>
SP8	4-COCH <sub>3</sub>	670±170 <sup>c</sup>	670±170 <sup>d</sup>	750±140 <sup>bc</sup>	330±80 <sup>c</sup>
SP9	4-OH	4000±0 <sup>a</sup>	500±0 <sup>d</sup>	330±80 <sup>bcd</sup>	170±40 <sup>c</sup>
SP10	3-CN	500±0 <sup>c</sup>	420±80 <sup>d</sup>	130±0 <sup>d</sup>	250±0 <sup>c</sup>
SP11	4-CN	500±0 <sup>c</sup>	2000±0 <sup>b</sup>	2330±330 <sup>a</sup>	1330±330 <sup>ab</sup>
SP12	3-Br	670±170 <sup>c</sup>	1000±0 <sup>c</sup>	210±40 <sup>cd</sup>	330±80 <sup>c</sup>
Ant/Myc	–	Nd	130±30 <sup>e</sup>	100±0 <sup>d</sup>	100±0 <sup>c</sup>

\*Values followed by the same letter in each column and isolate were not significantly different ( $P < 0.05$ , Tukey's HSD test). Ant/Myc – rifampicin or nystatin. Nd – not determined (above the highest concentration applied of 4000 µg/mL)

effects of organic acetamide derivatives *N*-(2-hydroxy-4(or5)-nitro/aminophenyl) benzamides and phenylacetamides, whose structure differs from our compounds. Their MICs (around 250 µg/mL) were higher than ours against *C. albicans* and *E. coli* and similar against *S. aureus*. Similar findings were reported by a study of biologically active 2-chloro-*N*-alkyl/aryl acetamide derivatives (12). However, as neither study tested their respective compounds against MRSA, ours seems to be the first in this respect. More recently, Sharma et al. (33) identified 2-((4-bromophenyl)amino)-*N*-(4-(4-bromophenyl)thiazol-2-yl)acetamide, *N*-(4-(4-bromophenyl)thiazol-2-yl)-2-((4-chloro-3-nitrophenyl)amino)acetamide, and *N*-(4-(4-bromophenyl)thiazol-2-yl)-2-((2-chloro-4-nitrophenyl)amino)acetamide as effective against *S. aureus*, *E. coli*, and *C. albicans*, with the lowest MICs ranging from 13 to 27 µmol/L. They also reported that the improved antimicrobial activity was owed to the

presence of electron withdrawing groups (–Br, –Cl, –NO<sub>2</sub>) at the *ortho*, *meta*, and *para*-position of the ring B and to *N*-acylation with the synthesised compounds. Another study (34) indicated high activity of 2-(2-methylquinoxalin-3-ylthio)-*N*-(benzo[d]thiazol-2-yl)acetamide and 2-(2-methylquinoxalin-3-ylthio)-*N*-cyclohexylacetamide against *E. coli*, *S. aureus*, and *C. albicans*.

Compounds SP1–SP6, SP9, SP10, and SP12 showed the highest antimicrobial activity in our study, much thanks to their structure (lipophilicity/hydrophobicity) and no more than one hydrogen or nitrogen atom (35). Higher log $P$  values may have facilitated their penetration through the bacterial/fungal cell membrane and microbial death. Good biological activity of SP4–SP7 and SP12 can also be attributed to the presence of halogenated substituents (3, 36).

Jablonkai et al. (37) demonstrated that the biological activity of chloroacetamides varies with the position of

substituents bound to the phenyl ring. This may explain different activity of the compounds SP5 and SP12 or SP10 and SP11 against the tested strains. Furthermore, different susceptibility of the tested pathogens to compound SP10 may be the consequence of their morphological characteristics determining compound penetration into the microbial/fungal cell.

## CONCLUSION

Judging from the cheminformatics prediction models such as Molinspiration, SwissADME, PreADMET, and PkcSM, our twelve newly synthesised *N*-(substituted phenyl)-2-chloroacetamides met all the empirical criteria for good biological activity. Standard antimicrobial activity tests showed they were highly effective against Gram-positive bacteria, less effective against the Gram-negative *E. coli*, and moderately effective against the *C. albicans* yeast. Bearing the halogenated *p*-substituted phenyl ring, *N*-(4-chlorophenyl) chloroacetamide, *N*-(4-fluorophenyl) chloroacetamide, and *N*-(3-bromophenyl) chloroacetamide were among the most active thanks to high lipophilicity, which allows them to pass rapidly through the phospholipid bilayer of the cell membrane. They are the most promising compounds for further investigation, particularly against Gram-positive bacteria and yeasts. Our findings have set the path for the preparation of new, improved *N*-(*p*-substituted phenyl)-2-chloroacetamides and for better understanding of the structure-activity relationship, which should extend research to more different bacterial and fungal strains in the future.

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### Conflicts of interest

None to declare.

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### Karakterizacija dvanaest novosintetiziranih *N*-(supstituiranih fenil)-2-kloroacetamida s QSAR analizom i utvrđivanje njihovih antimikrobnih aktivnosti

U ovom istraživanju analizirano je dvanaest novosintetiziranih *N*-(supstituiranih fenil)-2-kloroacetamida radi determinacije njihova antimikrobnog potencijala u korelaciji s kvantitativnom analizom aktivnosti spojeva i njihove molekularne strukture (QSAR analiza). QSAR analiza omogućena je primjenom predikcijskih modela (Molinspiration, SwisADME, PreADMET i PkCSM) te je verificirana potvrđenom antimikrobnom aktivnošću sintetiziranih spojeva prema bakterijama *Escherichia coli*, *Staphylococcus aureus*, na meticilin otporan *S. aureus* (MRSA) i prema gljivici *Candida albicans*. Novosintetizirani spojevi zadovoljavaju sve predikcijske modele za značajnu potencijalnu biološku aktivnost prema kriterijima Lipinskog, Vebera i Egana. Na osnovi određene antimikrobne aktivnosti spojeva, svi kloroacetamidi pokazali su učinkovitost prema sojevima gram-pozitivnih bakterija *S. aureus* i MRSA, neznatno manju učinkovitost prema gram-negativnoj *E. coli* i umjerenu učinkovitost prema gljivici *C. albicans*. Naše je istraživanje potvrdilo potencijal biološke aktivnosti kloroacetamida, koji je bilo uvjetovan intenzitetom manifestirane aktivnosti u zavisnosti od pozicije supstituenata vezanih za fenilni prsten, što je i razlog značajnije učinkovitosti pojedinih spojeva prema gram-negativnima u odnosu na gram-pozitivne bakterije ili gljivicu *C. albicans*. Budući da imaju halogenirani *p*-supstituirani fenilni prsten, *N*-(4-klorofenil), *N*-(4-fluorofenil) i *N*-(3-bromofenil) kloroacetamidi bili su među najaktivnijima zahvaljujući visokoj lipofilnosti, koja im omogućava da brzo prolaze kroz fosfolipidni dvosloj stanične membrane. Ovi spojevi najviše obećavaju u daljim ispitivanjima, naročito protiv gram-pozitivnih bakterija i patogenih gljivica.

KLJUČNE RIJEČI: *N*-supstituirani amidi; antimikrobni potencijal; kvantitativna analiza kemijske strukture i aktivnosti spojeva