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Salicylanilide *N*-monosubstituted carbamates: Synthesis and in vitro antimicrobial activity

Martin Krátký *, Jarmila Vinšová

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

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ABSTRACT

The research of innovative antimicrobial agents represents a cutting edge topic. Hence, we synthesized and characterised novel salicylanilide *N*-monosubstituted carbamates. Twenty compounds were evaluated in vitro against eight bacterial strains and eight fungal species. The lowest minimum inhibitory concentrations (MICs) were found to be ⩽0.49 μM. Genus *Staphylococcus*, including methicillin-resistant *Staphylococcus aureus*, and fungus *Trichophyton mentagrophytes* showed uniformly the highest rate of susceptibility, whilst Gram-negative bacteria and most of the fungi were less susceptible. A wide range of carbamates provided comparable or superior in vitro antimicrobial activity in comparison to established drugs. Interestingly, extended-spectrum β-lactamase producing strain of *Klebsiella pneumoniae* was inhibited with MICs starting from 31.25 μM. With respect to *Staphylococci*, 2-[(4-bromophenyl) carbamoyl]-4-chlorophenyl phenylcarbamate exhibited the lowest MIC values (⩽0.98 μM). 2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl benzylcarbamate showed the widest spectrum of antifungal action. The results indicate that some salicylanilide carbamates can be considered to be promising candidates for future investigation.

1. Introduction

Problems related to drug-resistance have been reported for many human pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, and the family of *Enterobacteriaceae* that produce extended-spectrum β-lactamases (ESBL) or genus *Candida*. Mycoses have become an important public health issue because of the increasing number of immunocompromised patients and nosocomial infections.[1,2] Some of these infections are difficult to treat with established antimicrobial drugs. The search for antibacterial and antifungal agents, especially those with innovative mechanisms of action, should bring novel compounds to overcome the microbial resistance to clinically used drugs.

Phenolic compounds have been investigated widely as potential antimicrobial compounds, especially those obtained from natural sources. Some of them have displayed in vitro activity even against drug-resistant bacterial and fungal strains,[2–6] or they were synergic with antimycotics.[7] *O*-Aromatic carbamates have been reported as potential antimicrobial agents including their involvement in the prodrug design strategy.[8–10]

Salicylanilide derivatives have exhibited significant antimicrobial activity against various pathogenic species. Salicylanilides,[11,12] salicylanilide benzoates,[13] 4-substituted benzoates,[11,14,15] *N*,*N*-disubstituted methyl/phenyl salicylanilide carbamates[8] and *N*-alkyl salicylanilide carbamates[16,17] were investigated as potential antibacterial and antifungal agents. Although lipophilicity seems to be one of the factors modulating positively the antimicrobial activity, an escalated lipophilicity could hamper in vitro evaluation due to solubility problems in testing media.[11,14,15]

In this study, we synthesized and evaluated twenty novel salicylanilide *N*-cycloalkyl/aryl/arylalkyl carbamates as potential antimicrobial agents. In contrast to the Zadrazilova's paper,[17] which presents only MRSA inhibition activity of our earlier antimycobacterial active *N*-monoalkylated carbamates (Fig. 1),[16] this study includes expansion of new compounds evaluated against panel of eight bacterial and eight fungal strains as our ongoing research. Here presented carbamates cover varied substitution patterns in both salicylanilide and carbamate parts to identify possible structure–activity relationship. The structures of previously published salicylanilide carbamates in comparison with novel derivatives is depicted in Figure 1.

* Corresponding author. Tel.: +420 495067343; fax: +420 495067166.
E-mail address: martin.kratky@faf.cuni.cz (M. Krátký).

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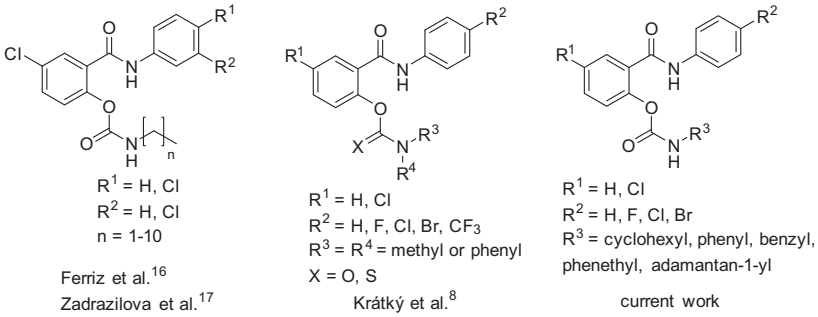


Figure 1. Comparison of the general formulas of antimicrobial active salicylanilide carbamates.

2. Material and methods

2.1. Chemistry

2.1.1. General methods

All of the reagents and solvents were purchased from Sigma–Aldrich (Darmstadt, Germany) or Penta Chemicals (Prague, Czech Republic) and used as received. The reactions and the purity of the products were monitored by thin-layer chromatography using a mixture with a ratio of toluene to ethyl acetate of 4:1 or a ratio of toluene to methanol of 9:1 as the eluent. The plates were coated with 0.2-mm Merck 60 F254 silica gel and were visualised by UV irradiation (254 nm). The melting points were determined on a Büchi Melting Point B-540 apparatus (BÜCHI, Flawil, Switzerland) using open capillaries, and the reported values are uncorrected.

Elemental analysis (C, H, and N) was performed on an automatic microanalyser CHNS-O CE instrument (FISONS EA 1110, Milano, Italy). Infrared spectra (ATR) were recorded on a FT-IR spectrometer (Nicolet 6700 FT-IR) in the range of 400–4000 cm^{-1}. The NMR spectra were measured in DMSO at ambient temperature using a Varian V NMR S500 instrument (500 MHz for ^1H and 125 MHz for ^{13}C; Varian Comp., Palo Alto, CA, USA) or a Varian Mercury-Vxbb 300 (300 MHz for ^1H and 75.5 MHz for ^{13}C; Varian, Inc., Palo Alto, CA, USA). The chemical shifts, δ, are given in ppm with respect to tetramethylsilane, which was used as an internal standard. The coupling constants (J) are reported in Hz.

The calculated log$P$ values (C log$P$), which are the logarithms of the partition coefficients for octan-1-ol/water, were determined using the CS ChemOffice Ultra program (version 13.0, CambridgeSoft, Cambridge, MA, USA).

2.1.2. Synthesis

The parent salicylanilides **SAL** were synthesised according to our previously described microwave-assisted synthetic method.[11]

For the synthesis of the carbamates **1**–**4**, we used slightly modified method according to Férriz et al.[16] An equivalent of appropriate salicylanilide (1 mmol) was suspended under vigorous stirring in dry acetonitrile (MeCN; 8 mL), and then 1 mmol of tertiary amine (triethylamine or *N*,*N*-diisopropylethylamine) was added in one portion. The mixture was stirred for 5 min to allow complete dissolution of the reaction mixture. Then, appropriate isocyanate (1.1 of equivalents) was added in two portions, portion after 15 min, and the mixture was stirred at room temperature for additional 1–2 h (cyclohexyl, phenyl, benzyl and phenethyl isocyanates) or 12 h (1-adamantyl isocyanate). The reaction was monitored using TLC. Then, the resulting precipitate was collected by filtration, washed with a small volume of cold methanol and dried. If necessary, products were recrystallised from ethyl acetate.

The syntheses of carbamates from phenyl isocyanate and 1-adamantyl isocyanate were performed under nitrogen atmosphere and various solvents were tried. Dry acetone or tetrahydrofuran served as a convenient solvent for phenyl derivatives **2b** and **3e**, respectively. The synthesis of carbamates **1b** and **3b** was carried out in a mixture of MeCN and dry acetone (a ratio of 1:1) with addition of a catalytic amount of triethylamine (20 μL).

2.1.2.1. 4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl cyclohexylcarbamate (1a). White solid; yield 83%; mp 192.5–194 °C. IR (ATR): 3261 (N–H), 2944, 2929, 2854, 1720 (C═O carbamate), 1684 (amide I), 1591, 1551, 1528 (amide II), 1490, 1478, 1400, 1316, 1266, 1221, 1104, 1090, 1013, 883, 838, 824, 734, 668 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.40 (1H, bs, amide NH), 7.78–7.68 (3H, m, carbamate NH, H2′, H6′), 7.65 (1H, d, J = 2.7 Hz, H3), 7.56 (1H, dd, J = 2.7 Hz, J = 8.8 Hz, H5), 7.38 (2H, d, J = 8.9 Hz, H3′, H5′), 7.24 (1H, d, J = 8.7 Hz, H6), 3.25–3.13 (1H, m, CH), 1.72–1.43 (5H, m, cyclohexyl), 1.27–0.98 (5H, m, cyclohexyl). ^{13}C NMR (75 MHz, DMSO): δ 163.23, 152.89, 147.43, 138.17, 132.00, 131.02, 129.04, 128.70, 127.44, 125.40, 122.43, 121.41, 50.06, 32.48, 25.27, 24.65. Anal. Calcd for $C_{20}H_{20}Cl_2N_2O_3$ (407.29): C, 58.98; H, 4.95; N, 6.88. Found: C, 59.03; H, 4.84; N, 7.00.

2.1.2.2. 4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl phenylcarbamate (1b). White solid; yield 50%; mp 210–212 °C. IR (ATR): 3314 (N–H), 3268 (N–H), 3072, 1722 (C═O carbamate), 1650 (amide I), 1595, 1554, 1524 (amide II), 1491, 1447, 1406, 1315, 1233, 1215, 1200, 1179, 1103, 1006, 828, 754, 727, 697, 670 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.58 (1H, bs, amide NH), 10.25 (1H, s, carbamate NH), 7.76 (1H, d, J = 2.7 Hz, H3), 7.70 (2H, d, J = 8.8 Hz, H2′, H6′), 7.64 (1H, dd, J = 2.7 Hz, J = 8.8 Hz, H5), 7.47–7.34 (5H, m, H6, H3′, H5′, H2″, H6″), 7.27 (2H, t, J = 7.9 Hz, H3″, H5″), 7.01 (1H, t, J = 7.4 Hz, H4″). ^{13}C NMR (75 MHz, DMSO): δ 163.16, 151.15, 146.91, 138.02, 131.83, 131.27, 129.68, 129.02, 128.86, 128.78, 128.66, 127.59, 125.86, 122.43, 121.57, 118.57. Anal. Calcd for $C_{20}H_{14}Cl_2N_2O_3$ (401.24): C, 59.87; H, 3.52; N, 6.98. Found: C, 59.63; H, 3.70; N, 7.10.

2.1.2.3. 4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl benzylcarbamate (1c). White solid; yield 93%; mp 187–188.5 °C. IR (ATR): 3338 (N–H), 3282 (N–H), 3032, 1720 (C═O carbamate), 1653 (amide I), 1595, 1533, 1520 (amide II), 1493, 1476, 1400, 1317, 1287, 1261, 1219, 1098, 1015, 921, 895, 833 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.50 (1H, bs, amide NH), 8.35 (1H, t, J = 6.1 Hz, carbamate NH), 7.73 (2H, d, J = 8.8 Hz, H2′, H6′), 7.69 (1H, d, J = 2.7 Hz, H3), 7.59 (1H, dd, J = 2.7 Hz, J = 8.7 Hz, H5), 7.40 (2H, d, J = 8.8 Hz, H3′, H5′), 7.29 (1H, d, J = 8.7 Hz, H6), 7.25–7.18 (5H, m, H2″, H3″, H4″, H5″, H6″), 4.21 (2H, d, J = 6.1 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO): δ 163.24, 154.17, 147.34, 139.18, 138.17, 132.08, 131.11, 129.31, 128.74, 128.55, 128.33, 127.48, 126.97, 126.95, 125.53, 121.48, 44.04. Anal. Calcd for $C_{21}H_{16}Cl_2N_2O_3$ (415.27): C, 60.74; H, 3.88; N, 6.75. Found: C, 60.61; H, 3.79; N, 7.00.

2.1.2.4. 4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl phenethylcarbamate (1d). White solid; yield 95%; mp 159–161 °C. IR (ATR): 3330 (N–H), 3275 (N–H), 3029, 2945, 1714 (C=O carbamate), 1655 (amide I), 1593, 1536, 1515 (amide II), 1493, 1475, 1397, 1316, 1288, 1260, 1220, 1101, 1015, 957, 923, 863, 821, 775, 735, 698, 669 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.46 (1H, bs, amide NH), 7.91 (1H, t, J = 5.7 Hz, carbamate NH), 7.74 (2H, d, J = 8.8 Hz, H2′, H6′), 7.67 (1H, d, J = 2.6 Hz, H3), 7.57 (1H, dd, J = 2.6 Hz, J = 8.6 Hz, H5), 7.39 (2H, d, J = 8.8 Hz, H3′, H5′), 7.27–7.10 (6H, m, H6, H2″, H3″, H4″, H5″, H6″), 3.19 (2H, dt, J = 6.2 Hz, J = 7.9 Hz, N-CH_2), 2.67 (2H, t, J = 7.3 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO): δ 163.21, 153.68, 147.26, 139.29, 138.16, 131.99, 130.97, 129.15, 128.83, 128.75, 128.45, 127.50, 126.25, 125.48, 121.48, 42.28, 35.26. Anal. Calcd for $C_{22}H_{18}Cl_2N_2O_3$ (429.30): C, 61.55; H, 4.23; N, 6.53. Found: C, 61.65; H, 4.31; N, 6.45.

2.1.2.5. 4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl adamantan-1-ylcarbamate (1e). White solid; yield 69%; mp 234–236.5 °C. IR (ATR): 3299 (N–H), 2927, 2907, 2855, 1716 (C=O carbamate), 1663 (amide I), 1589, 1534, 1519 (amide II), 1489, 1476, 1397, 1359, 1310, 1297, 1283, 1252, 1214, 1108, 1087, 1023, 1011, 920, 882, 823, 756, 674 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.34 (1H, bs, amide NH), 7.72 (2H, d, J = 8.4 Hz, H2′, H6′), 7.65 (1H, d, J = 2.6 Hz, H3), 7.59–7.52 (2H, m, carbamate NH, H5), 7.38 (2H, d, J = 8.4 Hz, H3′, H5′), 7.20 (1H, d, J = 8.9 Hz, H6), 1.98–1.89 (3H, m, CH), 1.84–1.74 (6H, m, C-CH_2), 1.63–1.47 (6H, m, CH-CH_2). ^{13}C NMR (75 MHz, DMSO): δ 165.12, 157.47, 147.33, 138.18, 137.40, 133.12, 128.85, 128.60, 127.90, 122.34, 120.01, 119.45, 55.94, 44.63, 35.20, 29.26. Anal. Calcd for $C_{24}H_{24}Cl_2N_2O_3$ (459.36): C, 62.75; H, 5.27; N, 6.10. Found: C, 62.74; H, 5.50; N, 6.22.

2.1.2.6. 2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl cyclohexylcarbamate (2a). White solid; yield 83%; mp 196.5–198.5 °C. IR (ATR): 3262 (N–H), 2941, 2929, 2854, 1710 (C=O carbamate), 1655 (amide I), 1587, 1551, 1527 (amide II), 1487, 1477, 1394, 1315, 1278, 1265, 1219, 1105, 1068, 1009, 882, 838, 820, 782, 734, 700, 668 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.40 (1H, bs, amide NH), 7.75 (1H, d, J = 8.0 Hz, carbamate NH), 7.71–7.46 (6H, m, H3, H5, H2′, H3′, H5′, H6′), 7.24 (1H, d, J = 8.8 Hz, H6), 3.26–3.12 (1H, m, CH), 1.72–1.43 (5H, m, cyclohexyl), 1.27–0.98 (5H, m, cyclohexyl). ^{13}C NMR (75 MHz, DMSO): δ 163.21, 152.86, 147.41, 138.56, 131.97, 131.74, 131.00, 129.01, 128.46, 125.37, 121.78, 115.48, 50.04, 32.45, 25.24, 24.62. Anal. Calcd for $C_{20}H_{20}BrClN_2O_3$ (451.74): C, 53.18; H, 4.46; N, 6.20. Found: C, 52.97; H, 4.43; N, 6.25.

2.1.2.7. 2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl phenylcarbamate (2b). White solid; yield 55%; mp 221–223.5 °C. IR (ATR): 3304 (N–H), 3272 (N–H), 1716 (C=O carbamate), 1652 (amide I), 1604, 1536 (amide II), 1516, 1489, 1476, 1445, 1394, 1318, 1228, 1205, 1101, 1070, 1016, 1001, 837, 818, 758, 743, 718, 690, 665 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.58 (1H, bs, amide NH), 10.26 (1H, s, carbamate NH), 7.76 (1H, d, J = 2.6 Hz, H3), 7.68–7.61 (3H, m, H5, H2′, H6′), 7.52–7.37 (5H, m, H6, H3′, H5′, H2″, H6″), 7.27 (2H, t, J = 7.9 Hz, H3″, H5″), 7.02 (1H, t, J = 7.4 Hz, H4″). ^{13}C NMR (75 MHz, DMSO): δ 163.17, 151.14, 146.91, 138.44, 131.82, 131.77, 131.68, 131.28, 129.68, 129.01, 128.96, 128.66, 125.85, 122.77, 121.94, 115.68. Anal. Calcd for $C_{20}H_{14}BrClN_2O_3$ (445.69): C, 53.90; H, 3.17; N, 6.29. Found: C, 53.97; H, 3.34; N, 6.33.

2.1.2.8. 2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl benzylcarbamate (2c). White solid; yield 65%; mp 179.5–181.5 °C. IR (ATR): 3338 (N–H), 3278 (N–H), 3066, 1713 (C=O carbamate), 1655 (amide I), 1591, 1534, 1511 (amide II), 1489, 1478, 1394, 1314, 1290, 1264, 1216, 1102, 1072, 1025, 1013, 921, 890, 823, 746, 729, 698 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.50 (1H, bs, amide NH), 8.36 (1H, t, J = 6.1 Hz, carbamate NH), 7.71–7.76 (3H, m, H3, H2′, H6′), 7.59 (1H, dd, J = 2.6 Hz, J = 8.7 Hz, H5), 7.56–7.50 (2H, m, H3′, H5′), 7.29 (1H, d, J = 8.7 Hz, H6), 7.25–7.17 (5H, m, H2″, H3″, H4″, H5″, H6″), 4.21 (2H, d, J = 6.1 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO): δ 163.25, 154.17, 147.33, 139.17, 138.58, 132.08, 131.12, 129.31, 128.54, 128.34, 126.98, 126.95, 125.53, 121.86, 115.55, 44.05. Anal. Calcd for $C_{21}H_{16}BrClN_2O_3$ (459.72): C, 54.86; H, 3.51; N, 6.09. Found: C, 54.90; H, 3.39; N, 6.16.

2.1.2.9. 2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl phenethylcarbamate (2d). White solid; yield 56%; mp 164–166 °C. IR (ATR): 3337 (N–H), 3281 (N–H), 3029, 2945, 1716 (C=O carbamate), 1655 (amide I), 1589, 1535, 1513 (amide II), 1490, 1476, 1393, 1315, 1288, 1258, 1215, 1103, 1071, 1012, 958, 922, 836, 817, 774, 735, 698, 664 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.46 (1H, bs, amide NH), 7.91 (1H, t, J = 5.7 Hz, carbamate NH), 7.74–7.63 (3H, m, H3, H2′, H6′), 7.57 (1H, dd, J = 2.7 Hz, J = 8.8 Hz, H5), 7.54–7.47 (2H, m, H3′, H5′), 7.27–7.09 (6H, m, H6, H2″, H3″, H4″, H5″, H6″), 3.19 (2H, dt, J = 6.2 Hz, J = 7.9 Hz, N-CH_2), 2.67 (2H, t, J = 7.3 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO): δ 163.25, 153.70, 147.27, 139.30, 138.59, 132.01, 131.67, 131.00, 129.15, 128.85, 128.47, 126.27, 125.48, 121.86, 115.57, 42.28, 35.27. Anal. Calcd for $C_{22}H_{18}BrClN_2O_3$ (473.75): C, 55.78; H, 3.83; N, 5.91. Found: C, 55.64; H, 3.88; N, 6.03.

2.1.2.10. 2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl adamantan-1-ylcarbamate (2e). White solid; yield 63%; mp 212–213.5 °C. IR (ATR): 3295 (N–H), 2924, 2855, 1715 (C=O carbamate), 1665 (amide I), 1585, 1534, 1518 (amide II), 1486, 1476, 1393, 1359, 1310, 1298, 1283, 1252, 1215, 1107, 1066, 1022, 1007, 978, 921, 882, 837, 821, 756, 669 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.34 (1H, bs, amide NH), 7.71–7.62 (3H, m, H3, H2′, H6′), 7.60–7.46 (4H, m, carbamate NH, H5, H3′, H5′), 7.20 (1H, d, J = 8.7 Hz, H6), 1.97–1.89 (3H, m, CH), 1.85–1.74 (6H, m, C-CH_2), 1.62–1.47 (6H, m, CH-CH_2). ^{13}C NMR (75 MHz, DMSO): δ 163.27, 151.41, 143.44, 138.61, 132.08, 131.56, 131.03, 128.91, 128.44, 125.37, 121.89, 115.48, 50.47, 40.97, 36.04, 28.92. Anal. Calcd for $C_{24}H_{24}BrClN_2O_3$ (503.82): C, 57.21; H, 4.80; N, 5.56. Found: C, 57.40; H, 4.90; N, 5.38.

2.1.2.11. 4-Chloro-2-[(4-fluorophenyl)carbamoyl]phenyl cyclohexylcarbamate (3a). White solid; yield 81%; mp 216–218.5 °C. IR (ATR): 3321 (N–H), 3276 (N–H), 2937, 2855, 1703 (C=O carbamate), 1650 (amide I), 1570, 1531, 1512 (amide II), 1477, 1409, 1317, 1281, 1246, 1215, 1100, 1020, 893, 831, 802, 759, 691, 667, 652 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 10.31 (1H, bs, amide NH), 7.78–7.62 (4H, m, carbamate NH, H3, H2′, H6′), 7.56 (1H, dd, J = 2.7 Hz, J = 8.5 Hz, H5), 7.27–7.12 (3H, m, H6, H3′, H5′), 3.27–3.14 (1H, m, CH), 1.81–1.43 (5H, m, cyclohexyl), 1.30–0.95 (5H, m, cyclohexyl). ^{13}C NMR (125 MHz, DMSO): δ 163.27, 158.40 (d, J = 240.3 Hz), 152.91, 147.42, 135.58 (d, J = 2.5 Hz), 132.11, 130.92, 129.04, 128.48, 125.43, 121.69 (d, J = 8.1 Hz), 115.36 (d, J = 22.3 Hz), 50.06, 33.54, 32.49, 24.65. Anal. Calcd for $C_{20}H_{20}ClFN_2O_3$ (390.84): C, 61.46; H, 5.16; N, 7.17. Found: C, 61.55; H, 4.99; N, 7.20.

2.1.2.12. 4-Chloro-2-[(4-fluorophenyl)carbamoyl]phenyl phenylcarbamate (3b). White solid; yield 79%; mp 204–206.5 °C. IR (ATR): 3330 (N–H), 3082, 1748 (C=O carbamate), 1641 (amide I), 1599, 1538, 1509 (amide II), 1476, 1442, 1415, 1316, 1238, 1210, 1192, 1179, 1106, 1001, 994, 834, 822, 772, 759, 691 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.49 (1H, bs, amide NH), 10.25 (1H, s, carbamate NH), 7.75 (1H, d, J = 2.6 Hz, H3), 7.71–7.66 (2H, m, H2′, H6′), 7.63 (1H, dd, J = 2.6 Hz, J = 8.7 Hz, H5), 7.47–7.42 (2H, m, H3′, H5′), 7.39 (1H, d, J = 8.7 Hz, H6), 7.28 (2H, t, J = 7.9 Hz, H2″, H6″), 7.28 (2H, t, J = 7.9 Hz, H3″, H5″), 7.02 (1H, t, J = 7.5 Hz, H4″). ^{13}C NMR (75 MHz, DMSO): δ

162.97, 158.49 (d, J = 240.3 Hz), 151.15, 146.90, 135.41 (d, J = 2.6 Hz), 131.95, 131.13, 129.66, 128.98, 128.93, 128.61, 125.83, 121.88 (d, J = 7.9 Hz), 118.34, 115.42 (d, J = 22.3 Hz). Anal. Calcd for $C_{20}H_{14}ClFN_2O_3$ (384.79): C, 62.43; H, 3.67; N, 7.28. Found: C, 62.29; H, 3.64; N, 7.29.

2.1.2.13. 4-Chloro-2-[(4-fluorophenyl)carbamoyl]phenyl benzylcarbamate (3c). White solid; yield 82%; mp 227–229.5 °C. IR (ATR): 3315 (N–H), 3233 (N–H), 3033, 1711 (C═O carbamate), 1652 (amide I), 1627, 1572, 1537, 1511 (amide II), 1471, 1454, 1415, 1315, 1251, 1215, 1110, 1096, 1030, 827, 816, 788, 751, 730, 697, 669 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 10.41 (1H, bs, amide NH), 8.35 (1H, t, J = 6.2 Hz, carbamate NH), 7.74–7.70 (2H, m, H2′, H6′), 7.68 (1H, d, J = 2.6 Hz, H3), 7.58 (1H, dd, J = 2.7 Hz, J = 8.6 Hz, H5), 7.29 (1H, d, J = 8.7 Hz, H6), 7.26–7.15 (7H, m, H3′, H5′, H2″, H3″, H4″, H5″, H6″), 4.22 (2H, d, J = 6.1 Hz, CH_2). ^{13}C NMR (125 MHz, DMSO): δ 163.03, 158.45 (d, J = 240.0 Hz), 154.18, 147.33, 139.18, 135.57 (d, J = 2.6 Hz), 132.18, 130.98, 129.29, 128.51, 128.32, 126.98, 126.94, 125.54, 121.76 (d, J = 7.8 Hz), 115.38 (d, J = 22.1 Hz), 44.05. Anal. Calcd for $C_{21}H_{16}ClFN_2O_3$ (398.81): C, 63.24; H, 4.04; N, 7.02. Found: C, 62.99; H, 4.30; N, 7.11.

2.1.2.14. 4-Chloro-2-[(4-fluorophenyl)carbamoyl]phenyl phenethylcarbamate (3d). White solid; yield 67%; mp 231–232.5 °C. IR (ATR): 3340 (N–H), 3258 (N–H), 3066, 1714 (C═O carbamate), 1658 (amide I), 1538, 1511 (amide II), 1483, 1464, 1414, 1313, 1286, 1268, 1213, 1157, 1115, 1098, 1022, 968, 902, 833, 824, 786, 749, 699, 682, 661 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 10.37 (1H, bs, amide NH), 7.90 (1H, t, J = 5.7 Hz, carbamate NH), 7.74–7.70 (2H, m, H2′, H6′), 7.66 (1H, d, J = 2.6 Hz, H3), 7.56 (1H, dd, J = 2.7 Hz, J = 8.7 Hz, H5), 7.26–7.11 (8H, m, H6, H3′, H5′, H2″, H3″, H4″, H5″, H6″), 3.20 (2H, dt, J = 5.9 Hz, J = 7.7 Hz, N-CH_2), 2.68 (2H, t, J = 7.3 Hz, CH_2). ^{13}C NMR (125 MHz, DMSO): δ 163.00, 158.46 (d, J = 240.3 Hz), 153.69, 147.25, 139.29, 135.55 (d, J = 2.5 Hz), 132.07, 130.86, 129.13, 128.83, 128.44, 128.38, 126.24, 125.47, 121.76 (d, J = 7.9 Hz), 115.39 (d, J = 22.2 Hz), 42.29, 35.27. Anal. Calcd for $C_{22}H_{18}ClFN_2O_3$ (412.84): C, 64.00; H, 4.39; N, 6.79. Found: C, 64.13; H, 4.30; N, 6.77.

2.1.2.15. 2-(Phenylcarbamoyl)phenyl cyclohexylcarbamate (4a). White solid; yield 80%; mp 154–155 °C. IR (ATR): 3322 (N–H), 3262 (N–H), 3057, 2932, 2852, 1704 (C═O carbamate), 1655 (amide I), 1597, 1541 (amide II), 1499, 1454, 1438, 1329, 1315, 1275, 1232, 1215, 1096, 1019, 895, 779, 745, 705, 688, 667 cm^{-1}. ^1H NMR (300 MHz, DMSO): 10.10 (1H, bs, amide NH), 7.74–7.66 (3H, m, H3, H2′, H6′), 7.63–7.46 (2H, m, carbamate NH, H4), 7.35–7.26 (3H, m, H5, H3′, H5′), 7.19 (1H, d, J = 8.0 Hz, H6), 7.06 (1H, t, J = 7.3 Hz, H4′), 3.28–3.14 (1H, m, CH), 1.80–1.42 (5H, m, cyclohexyl), 1.27–0.96 (5H, m, cyclohexyl). ^{13}C NMR (75 MHz, DMSO): δ 164.48, 153.25, 148.54, 139.41, 131.24, 130.68, 128.98, 128.73, 125.09, 123.64, 123.47, 119.87, 50.00, 32.55, 25.29, 24.69. Anal. Calcd for $C_{20}H_{22}N_2O_3$ (338.40): C, 70.99; H, 6.55; N, 8.28. Found: C, 71.06; H, 6.80; N, 8.37.

2.1.2.16. 2-(Phenylcarbamoyl)phenyl phenylcarbamate (4b). White solid; yield 74%; mp 160.5–163 °C. IR (ATR): 3349 (N–H), 3313 (N–H), 1720 (C═O carbamate), 1655 (amide I), 1598, 1530 (amide II), 1498, 1482, 1443, 1323, 1219, 1195, 1098, 1011, 919, 793, 751, 705, 689, 671 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.33 (1H, bs, amide NH), 10.21 (1H, s, carbamate NH), 7.76–7.64 (3H, m, H3, H2′, H6′), 7.61–7.53 (1H, m, H4), 7.50–7.23 (8H, m, H5, H3′, H5′, H2″, H3″, H4″, H5″, H6″), 7.09–6.95 (2H, m, H6, H4′). ^{13}C NMR (75 MHz, DMSO): δ 164.53, 151.49, 148.05, 139.32, 131.41, 130.65, 128.98, 128.92, 128.79, 125.67, 123.86, 123.78, 121.15, 120.02, 118.35. Anal. Calcd for $C_{20}H_{16}N_2O_3$ (332.35): C, 72.28; H, 4.85; N, 8.43. Found: C, 72.06; H, 4.99; N, 8.31.

2.1.2.17. 2-(Phenylcarbamoyl)phenyl benzylcarbamate (4c) White solid; yield 92%; mp 154.5–155.5 °C. IR (ATR): 3350 (N–H), 3292 (N–H), 3058, 2924, 1708 (C═O carbamate), 1651 (amide I), 1598, 1533 (amide II), 1518, 1498, 1486, 1441, 1326, 1273, 1220, 1087, 1025, 921, 899, 873, 763, 749, 698, 691, 669 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.24 (1H, bs, amide NH), 8.31 (1H, t, J = 6.1 Hz, carbamate NH), 7.75 (2H, d, J = 7.9 Hz, H2′, H6′), 7.63 (1H, dd, J = 1.8 Hz, J = 7.6 Hz, H3), 7.53 (1H, td, J = 1.7 Hz, J = 7.8 Hz, H4), 7.39–7.16 (9H, m, H5, H6, H3′, H5′, H2″, H3″, H4″, H5″, H6″), 7.09 (1H, t, J = 7.4 Hz, H4′), 4.23 (2H, d, J = 6.1 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO): δ 164.57, 154.55, 148.47, 139.46, 139.32, 131.29, 130.83, 128.98, 128.79, 128.33, 126.99, 126.89, 125.33, 123.67, 123.58, 119.94, 44.04. Anal. Calcd for $C_{21}H_{18}N_2O_3$ (346.38): C, 72.82; H, 5.24; N, 8.09. Found: C, 72.88; H, 5.14; N, 8.21.

2.1.2.18. 2-(Phenylcarbamoyl)phenyl phenethylcarbamate (4d). White solid; yield 72%; mp 154.5–157 °C. IR (ATR): 3352 (N–H), 3316 (N–H), 3026, 1710 (C═O carbamate), 1655 (amide I), 1599, 1533 (amide II), 1517, 1499, 1485, 1440, 1325, 1265, 1241, 1217, 1074, 946, 892, 794, 778, 753, 698, 668 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.18 (1H, bs, amide NH), 7.85 (1H, t, J = 5.7 Hz, carbamate NH), 7.73 (2H, d, J = 7.9 Hz, H2′, H6′), 7.61 (1H, dd, J = 1.9 Hz, J = 7.6 Hz, H3), 7.50 (1H, td, J = 1.7 Hz, J = 7.8 Hz, H4), 7.37–7.13 (9H, m, H5, H6, H3′, H5′, H2″, H3″, H4″, H5″, H6″), 7.07 (1H, t, J = 7.3 Hz, H4′), 3.19 (2H, dt, J = 6.2 Hz, J = 7.9 Hz, N-CH_2), 2.68 (2H, t, J = 7.2 Hz, CH_2). ^{13}C NMR (75 MHz, DMSO): δ 164.52, 154.04, 148.39, 139.42, 139.37, 131.16, 130.69, 128.93, 128.85, 128.78, 128.45, 126.23, 125.18, 123.68, 123.52, 119.92, 42.30, 35.33. Anal. Calcd for $C_{22}H_{20}N_2O_3$ (360.41): C, 73.32; H, 5.59; N, 7.77. Found: C, 73.33; H, 5.49; N, 8.00.

2.1.2.19. 2-(Phenylcarbamoyl)phenyl adamantan-1-ylcarbamate (4e). White solid; yield 89%; mp 172–174.5 °C. IR (ATR): 3272 (N–H), 3065, 2908, 2859, 1712 (C═O carbamate), 1657 (amide I), 1598, 1551, 1537 (amide II), 1499, 1484, 1444, 1327, 1298, 1279, 1212, 1088, 1016, 921, 775, 752, 711, 689, 671 cm^{-1}. ^1H NMR (300 MHz, DMSO): δ 10.00 (1H, bs, amide NH), 7.71 (2H, d, J = 8.0 Hz, H2′, H6′), 7.61 (1H, dd, J = 1.8 Hz, J = 7.5 Hz, H3), 7.52–7.46 (2H, m, carbamate NH, H4), 7.35–7.27 (3H, m, H5, H3′, H5′), 7.16 (1H, d, J = 8.1 Hz, H6), 7.06 (1H, t, J = 7.3 Hz, H4′), 1.96–1.90 (3H, m, CH), 1.86–1.76 (6H, m, C-CH_2), 1.61–1.48 (6H, m, CH-CH_2). ^{13}C NMR (75 MHz, DMSO): δ 164.39, 151.77, 148.39, 139.36, 131.26, 130.63, 129.00, 128.66, 125.01, 123.63, 123.47, 119.97, 50.38, 41.01, 36.04, 28.92. Anal. Calcd for $C_{24}H_{26}N_2O_3$ (390.47): C, 73.82; H, 6.71; N, 7.17. Found: C, 73.70; H, 6.90; N, 6.98.

2.2. Biology

2.2.1. In vitro antibacterial evaluation[14]

The in vitro antibacterial activities were assayed against eight Gram-positive and Gram-negative strains: *Staphylococcus aureus* CCM 4516/08, methicillin-resistant *Staphylococcus aureus* H 5996/08 (MRSA), *Staphylococcus epidermidis* H 6966/08, *Enterococcus* sp. J 14365/08; *Escherichia coli* CCM 4517, *Klebsiella pneumoniae* D 11750/08, ESBL-positive *Klebsiella pneumoniae* J 14368/08, and *Pseudomonas aeruginosa* CCM 1961.

The microdilution broth method modified according to standard M07-A07 in Mueller-Hinton broth (HiMedia Laboratories, Mumbai, India) adjusted to pH 7.4 (±0.2) was used. The tested compounds were dissolved in DMSO to final concentrations ranging from 500 to 0.49 μM. Benzylpenicillin (penicillin G; PNC) and bacitracin (BAC) were used as comparative drugs. A bacterial inoculum in sterile water was prepared to reach 0.5 on the McFarland scale (1.5×10^8 CFU/mL). The minimum inhibitory

concentrations were assayed as a reduction in growth of at least 90% (IC_{90}) compared with the control. The results were analysed both visually and spectrophotometrically at 540 nm. The MIC values were determined after 24 and 48 h of incubation in the dark at 35 °C (±0.1) in a humid atmosphere.

2.2.2. In vitro antifungal evaluation[11]

The antifungal properties were evaluated in vitro against four *Candida* strains (*Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E28, and *Candida glabrata* 20/I), *Trichosporon asahii* 1188 and three filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, and *Trichophyton mentagrophytes* 445).

The microdilution broth method was used according to the CLSI M27-A3 and M38-A2 guidelines in RPMI 1640 with glutamine (KlinLab, Prague, Czech Republic) buffered to pH 7.0 with 0.165 mol of 3-morpholino-propane-1-sulfonic acid (Sigma–Aldrich, Darmstadt, Germany). DMSO served as a diluent for all of the compounds. In yeast, the final size of the inoculum was $5 \times 10^3 \pm 0.2$ CFU/mL, and in the case of the moulds, the final size of the inoculum was $0.5–5 \times 10^4$ CFU/mL. Fluconazole (FLU) and amphotericin B (AMB) were used as reference drugs. The MIC values for yeasts and filamentous fungi were assayed as a reduction of growth of at least 80% (IC_{80}) or of at least 50% (IC_{50}) compared with the control, respectively. The results were analysed visually and spectrophotometrically at 540 nm. The MIC values were determined after 24 and 48 h of incubation in the dark at 35 °C (±0.1) in a humid atmosphere, but for *T. mentagrophytes*, the final MIC values were determined after 72 and 120 h of incubation.

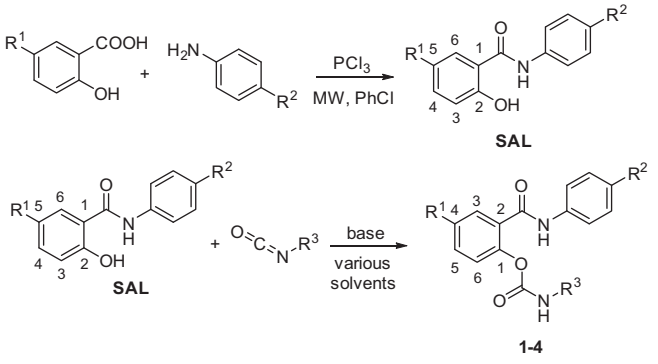
3. Results and discussion

3.1. Chemistry

The synthetic plan is depicted in a Scheme 1. Parent salicylanilides **SAL** were synthesised using microwave irradiation via a previously described method.[11]

Salicylanilide carbamates **1**–**4** were synthesised by a reaction of salicylanilides with isocyanates in the presence of 1 equiv of triethylamine or Hünig's base (*N*,*N*-diisopropylethylamine, DIPEA) at rt. Acetonitrile (MeCN) was used as the solvent for most of the products. In this case, the average reaction time was 12 h for 1-adamantyl isocyanate and 2 hours for remaining isocyanates. In general, yields ranged from 50% to 95%.

When phenyl isocyanate and 1-adamantyl isocyanate served as starting material, the formation of desired carbamates **1**–**4** was diminished or even abolished completely due to formation of urea side products **5b** and **5e**. These unwanted by-products can be the result of the presence of water in the reaction mixture and the extended reaction time promotes this reaction, especially for 1-adamantyl isocyanate. The formation of ureas via carbamic acid unstable intermediate is depicted in Scheme 2. Thus, strictly anhydrous conditions had a beneficial effect on the suppression of this side reaction. The change of the solvent can also improve yields of carbamates **1**–**4** instead of ureas **5**. However, isocyanates are converted to symmetric 1,3-disubstituted ureas directly in the presence of triethylamine or other tertiary bases.[18] This unintentional reaction can be circumvent sufficiently by the difference choice of solvent (dry acetone, toluene, tetrahydrofuran or mixture of solvents), replacement of Et_3N by DIPEA and/or by addition of only a catalytic amount of triethylamine.



Scheme 1. Synthesis of salicylanilides **SAL** and carbamates **1**–**4**. [R^1 = H, Cl; R^2 = H, Cl, Br, F; R^3 = cyclohexyl, phenyl, benzyl, phenethyl, adamantan-1-yl; MW: microwave irradiation (530 W, 600 rpm, 22 min); PhCl: chlorobenzene].

Compounds were characterised by melting points, IR and NMR spectra; their purity was checked by thin-layer chromatography and elemental analysis. In the IR spectra of carbamates, sharp and strong bands appear at around 1703–1748 cm^{-1} (carbamic C=O) and 1641–1684 (amide I). Amide II bands were observed at around 1509–1541 cm^{-1}. Most of the derivatives displayed also two visible N–H stretch bands (3261–3352 cm^{-1}); in some cases, only one N–H band was well-marked.

Twenty novel carbamates **1**–**4** are summarised in Table 1. In contrast to previously reported salicylanilide carbamates,[8,17] here presented carbamates **1**–**4** have substituted both aromatic rings of salicylanilide core by various and/or different substituents not prepared before. Férriz et al.[16] reported *N*-alkyl (C_2–C_{11}) carbamates of salicylanilides synthesized from 5-chlorosalicylic acid and 3-chloro-, 4-chloro- or 3,4-dichloro-anilines. Previously, we reported three types of salicylanilide *N*,*N*-disubstituted carbamates (dimethyl, methyl(phenyl) and diphenyl carbamates) and *N*,*N*-dimethylthiocarbamates.[8] In this study, 'salicylic part' of compounds **1**–**4** was synthesized from 5-chlorosalicylic or salicylic acids, 'anilide part' from unsubstituted aniline, 4-Cl/Br/F-anilines and *N*-cycloalkyls (derivatives **a**, **e**), *N*-phenyl **b** and *N*-phenylalkyl (**c**, **d**) carbamates are involved.

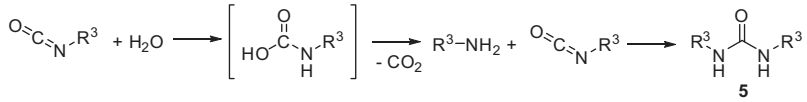
3.2. Biology

3.2.1. Antibacterial activity

Salicylanilide carbamates **1**–**4** were evaluated in vitro for their antibacterial activity against four Gram-positive strains: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Enterococcus* sp., and four Gram-negative strains: *Escherichia coli*, *Klebsiella pneumoniae*, ESBL-positive *Klebsiella pneumoniae* (KP-E) and *Pseudomonas aeruginosa* (Table 1). Benzylpenicillin (G-penicillin; PNC) and bacitracin (BAC) were used as the reference drugs for the comparison. Solubility problems in the testing medium did not allow evaluation of one adamantane derivative (**4e**).

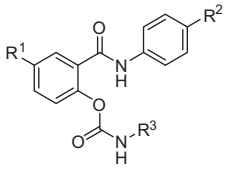
In general, Gram-positive bacteria showed higher susceptibility to investigated compounds (MICs starting from 0.49 μM) than Gram-negative species (MICs ⩾ 31.25 μM). None of the derivatives inhibited the growth of *P. aeruginosa* at a concentration of 125 μM or lower. All carbamates **1**–**4** were active at least against three bacterial strains. Dihalogenated derivatives **1**–**3** showed unequivocally improved antibacterial activity than carbamates **4** derived from unsubstituted salicylanilide (MICs ⩾ 125 μM).

Despite the presence of drug-resistance, the genus *Staphylococcus* (*S. aureus*, *S. epidermidis*) was the most susceptible. They were inhibited by all of the carbamates **1**–**4** with MICs 0.49–500 μM. 2-[(4-Bromophenyl)carbamoyl]-4-chlorophenyl phenylcarbamate **2b** showed the best MIC values (⩽0.49–0.98 μM). When focusing on established antibiotics, five derivatives (**2a**–**e**) were in vitro comparable to PNC and among halogenated carbamates, only the compound **1b** expressed lower activity than BAC. All of the halogen



Scheme 2. Pathway of side-product urea **5** formation in the presence of traces of water (R^3 = phenyl, adamantan-1-yl).

Table 1
Antibacterial activity of carbamates **1**–**4**



Code	R^1	R^2	R^3	MIC/IC_{95} [μM]													
				SA		MRSA		SE		EF		EC		KP		KP-E	
				24 h	48 h	24 h	48 h	24 h	24 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
1a	Cl	Cl	Cyclohexyl	1.95	7.81	3.9	15.62	**0.98**	**0.98**	>125	>125	>125	>125	>125	>125	>125	>125
1b	Cl	Cl	Phenyl	31.25	31.25	31.25	31.25	31.25	31.25	500	500	500	500	500	500	**62.5**	**62.5**
1c	Cl	Cl	Benzyl	3.9	15.62	7.81	15.62	3.9	3.9	>125	>125	>125	>125	**125**	**125**	**62.5**	125
1d	Cl	Cl	Phenethyl	3.9	15.62	3.9	15.62	3.9	15.62	500	500	**125**	500	500	500	**31.25**	**31.25**
1e	Cl	Cl	Adamantan-1-yl	3.9	15.62	3.9	31.25	3.9	3.9	250	250	>250	>250	>250	>250	**62.5**	**62.5**
2a	Cl	Br	Cyclohexyl	⩽**0.49**	**0.98**	⩽**0.49**	1.95	⩽**0.49**	**0.98**	500	500	>500	>500	>500	>500	>500	>500
2b	Cl	Br	Phenyl	⩽**0.49**	**0.98**	⩽**0.49**	**0.98**	⩽**0.49**	**0.98**	500	500	>500	>500	>500	>500	>500	>500
2c	Cl	Br	Benzyl	⩽**0.49**	**0.98**	⩽**0.49**	1.95	⩽**0.49**	**0.98**	>500	>500	>500	>500	>500	>500	>500	>500
2d	Cl	Br	Phenethyl	⩽**0.49**	1.95	**0.98**	1.95	⩽**0.49**	**0.98**	>500	>500	>500	>500	>500	>500	>500	>500
2e	Cl	Br	Adamantan-1-yl	**0.98**	1.95	1.95	3.9	**0.98**	1.95	>500	>500	>250	>250	>250	>250	**31.25**	125
3a	Cl	F	Cyclohexyl	15.62	15.62	31.25	31.25	7.81	7.81	>125	>125	>125	>125	>125	>125	**62.5**	**62.5**
3b	Cl	F	Phenyl	7.81	7.81	7.81	7.81	7.81	7.81	250	250	>500	>500	**250**	**250**	250	250
3c	Cl	F	Benzyl	15.62	15.62	15.62	31.25	15.62	15.62	>500	>500	>500	>500	>500	>500	>500	>500
3d	Cl	F	Phenethyl	7.81	15.62	15.62	62.5	15.62	15.62	>500	>500	>500	>500	>500	>500	>500	>500
3e	Cl	F	Adamantan-1-yl	15.62	15.62	62.5	62.5	250	250	**125**	**125**	>500	>500	>500	>500	>500	>500
4a	H	H	Cyclohexyl	250	250	500	500	500	500	500	500	>500	>500	>500	>500	>500	>500
4b	H	H	Phenyl	500	500	500	500	500	500	>500	>500	>500	>500	>500	>500	>500	>500
4c	H	H	Benzyl	250	250	500	500	500	500	>500	>500	>500	>500	>500	>500	>500	>500
4d	H	H	Phenethyl	125	125	125	125	125	125	>500	>500	>500	>500	>500	>500	>500	>500
4e	H	H	Adamantan-1-yl	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
PNC				0.98	0.98	62.5	125	250	250	7.81	15.62	125	125	500	500	>500	>500
BAC				7.81	15.62	15.62	15.62	15.62	31.25	15.62	62.5	>500	>500	>500	>500	>500	>500

SA: *Staphylococcus aureus* CCM 4516/08; MRSA: methicillin-resistant *Staphylococcus aureus* H 5996/08; SE: *Staphylococcus epidermidis* H 6966/08; EF: *Enterococcus* sp. J 14365/08. *Escherichia coli* CCM 4517, *Klebsiella pneumoniae* D 11750/08; ESBL-positive *Klebsiella pneumoniae* J 14368/08. PNC: benzylpenicillin; BAC: bacitracin.
The best values for each strain are provided in bold.

derivatives **1**–**3** were superior to benzylpenicillin against MRSA and seventeen of them exceeded activity of bacitracin (with exceptions of **1b**, **3a**, and **3e**). For *S. epidermidis*, one carbamate (**3e**) was comparable to PNC but remaining halogenated molecules and one non-halogenated **4d** displayed higher in vitro efficacy. Additionally, most of the carbamates were also superior to BAC (Fig. 2).

Derivatives of *N*-(4-bromophenyl)-5-chloro-2-hydroxybenzamide (i.e. carbamates **2**) showed improved activity when compared to other substitution patterns, followed by 5-chloro-*N*-(4-chlorophenyl)-2-hydroxybenzamide-based compounds **1**. Focusing on *N*-substitution of carbamates, the compounds were ordered based on decreasing activity against *Staphylococci* as follows: cyclohexyl **a** ⩾ benzyl **c** > phenethyl **d** > phenyl **b** > adamantan-1-yl **e**.

The activity against *Enterococcus* sp. was lower and not uniform. Nine carbamates showed MICs within the range of 125–500 μM (**1b**, **1d**, **1e**, **2a**, **2b**, **3b**, **3d**, **3e**, **4a**), however, all of them were less active than both BAC and PNC.

Among Gram-negative strains, *E. coli* was susceptible to only two molecules, **1b** and especially **1d** (MIC values 125–500 μM). The later carbamate was also the most active compound against ESBL-positive *K. pneumoniae* with MIC of 31.25 μM. In summary, seven carbamates (**1b**–**1e**, **2e**, **3a**, **3b**) affected the growth of KP-E with MICs within the range of 31.25–250 μM. Interestingly, KP-E showed higher susceptibility than ESBL-negative *K. pneumoniae* strain (affected by only four compounds with MICs starting from 125 μM; **1b**–**1d**, **3b**). The evidence that some salicylanilide carbamates are able to inhibit *Klebsiella pneumoniae* including the ESBL-producing strain is unique, whereas previously reported salicylanilide *O*-derivatives avoided any considerable activity (e.g., Refs. 8,13–15). Derivatives **1** synthesized from 5-chloro-*N*-(4-chlorophenyl)-2-hydroxybenzamide and *N*-phenyl carbamates **b** seems to be optimal choice for design of potential agents against Gram-negative bacteria. Nevertheless, their MIC values are comparatively higher than those determined for *Staphylococcus* spp.

Previously, various salicylanilide esters and carbamates have been revealed as potential antibacterial agents.[8,11,13–15,17] The increased lipophilicity in comparison to parent salicylanilides should be beneficial for better crossing through biomembranes or absorption. However, the sharply increased lipophilicity (Table 2) and corresponding low solubility in the testing media hampered in vitro evaluation of a wide range of 4-(trifluoromethyl)benzoates[14] and other highly lipophilic 4-substituted benzoates,[15] whereas no problems with the solubility of benzoic acid esters[13] and *N*-alkyl carbamates[17] were reported. *N*,*N*-Disubstituted carbamates showed sufficient solubility for biological evaluation, even derivatives with an escalated lipophilicity (*N*,*N*-diphenyl

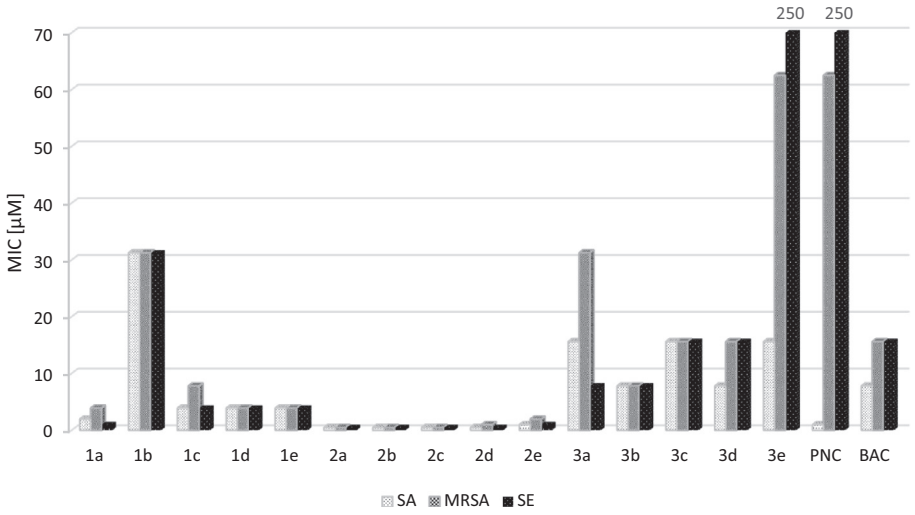


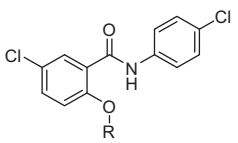
Figure 2. Antibacterial activity: comparison of dihalogenated carbamates **1**–**3** with control drugs against three Gram-positive strains after 24 h incubation (SA: *S. aureus*, MRSA: methicillin-resistant *S. aureus*, SE: *S. epidermidis*, PNC: benzylpenicillin, BAC: bacitracin).

carbamates; Table 2). Presented compounds belong to *N*-monosubstituted derivatives and 'carbamic' hydrogen confers more hydrophilic properties when compared to other salicylanilide *O*-derivatives. For example, *C*log*P* value of *N*-phenyl carbamate **1b** is lower than those calculated for corresponding benzoate (5.11 vs 5.44, respectively; Table 2) and concomitantly, it is sufficiently increased than in the case of parent salicylanilide (3.57).

Presented salicylanilide *N*-monosubstituted carbamates **1**–**4** exhibited more potent antibacterial action than *N*,*N*-disubstituted carbamates,[8] that is, they offer substantially lower MIC values (⩽0.49 μM vs 62.5 μM for the best derivatives, respectively) and also more uniform activity. Among *N*,*N*-disubstituted carbamates, only two derivatives from total fifteen were able to stop the growth of bacteria. Drawing a comparison between *N*-monoalkyl carbamates[17] and derivatives **1**–**4**, the most active compounds provide similar low micromolar or submicromolar MIC values for *Staphylococcus aureus* despite the presence of methicillin-resistance. Among carbamates synthesized from 5-chloro-*N*-(4-chlorophenyl)-2-hydroxybenzamide involved in both papers, *N*-alkyl (C_2, C_4–C_9, C_{11}) derivatives showed mostly indistinguishable or slightly lower MIC values than if carbamic nitrogen is substituted by cycloalkyl/aryl/arylalkyl. In contrast to Zadrazilova et al.[17], novel salicylanilide carbamates **1**–**4** were evaluated against a broader spectrum of bacteria represented by eight Gram-positive and Gram-negative strains. Additional actimicrobial action was identified against *Staphylococcus epidermidis* and *Klebsiella pneumoniae* including an ESBL-producing strain.

A possible additional mechanism of action was proposed for *N*-alkyl carbamates with a longer alkyl chain. The long, unbranched hydrophobic tail can cause membrane damage as non-ionic surfactants. The disruption of biomembranes is followed by leakage of the intracellular content and facilitation of the entry of active agents in the cells where it can interact with specific target(s).[17] However, this 'detergent-like' mechanism of action seems to be an unlikely for carbamates **1**–**4** due to their molecular structures. The antibacterial activity is also independent of the presence 4-chlorophenyl group as postulated by Ref. 17, moreover, its replacement by 4-bromophenyl decreased MIC values substantially.

Table 2
Calculated lipophilicities (*C*log*P*) of selected salicylanilide derivatives



Group of derivatives	R	*C*log*P*
Parent salicylanilides	H	3.57
Benzoates[13]	Benzoyl	5.44
4-(Trifluoromethyl)benzoates[11,14]	4-(Trifluoromethyl)benzoyl	6.36
N,*N*-Disubstituted carbamates[8]	Methyl(phenyl)carbamoyl	5.35
	Diphenylcarbamoyl	7.01
N-Alkyl carbamates[16,17]	Hexylcarbamoyl	5.53
N-Monosubstituted carbamates	Phenylcarbamoyl **1d**	5.11

3.2.2. Antifungal activity

The antifungal properties of carbamates **1**–**4** were evaluated in vitro against eight species: *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *Trichosporon asahii*, *Aspergillus fumigatus*, *Absidia corymbifera* and *Trichophyton mentagrophytes* (Table 3). Fluconazole (FLU) and amphotericin B (AMB) were involved as reference drugs. As in the case of antibacterial tests, it was not possible to evaluate adamantane derivative **4e** due to limited solubility.

Among all strains, only *Aspergillus fumigatus* showed complete resistance to investigated compounds **1**–**4** and two carbamates (**3e**, **4a**) avoided any antifungal action. On the other side, 2-[(4-bromophenyl)carbamoyl]-4-chlorophenyl benzylcarbamate **2c** was identified as an agent with a broad-spectrum in vitro antifungal activity exhibiting the lowest or second-lowest reached MIC values for seven fungal species.

Candida albicans was inhibited by four derivatives (**2c**, **3c**, **3d**, **4c**) with MICs from 62.5 μM. None of them achieved the activity of fluconazole and amphotericin B. Three carbamates (**1e**, **2c**, **4c**) are able to stop the growth of *C. tropicalis* with MIC values of 125 to 250 μM, thus being superior to FLU. *C. krusei* and *C. glabrata* showed a comparatively higher susceptibility. The first strain was affected by ten carbamates (**1a**–**c**, **1e**, **2b**, **2c**, **3c**, **3d**, **4c**, **4d**; MICs of 62.5 to 250 μM) and the second strain by eight derivatives (**2b**–**d**, **3a**, **3c**, **3d**, **4c**, **4d**) at a concentration of 31.25 μM (**2c**) and higher. With respect to in vitro evaluation results of two established drugs, carbamates are comparable or superior to FLU and significantly less active than AMB.

Among non-*Candida* species, *Absidia corymbifera* was inhibited by five derivatives (**1a**, **2c**, **2d**, **3a**, **3c**), but only **3c** showed identical

Table 3
Antifungal activity of carbamates **1–4**

Code	MIC/IC_{80}/IC_{50} [μM]													
	CA		CT		CK		CG		TA		AC		TM	
	24 h	48 h	24 h	48 h	24 h	24 h	24 h	48 h	24 h	48 h	24 h	48 h	72 h	120 h
1a	>125	>125	>125	>125	**125**	**125**	>125	>125	125	125	125	125	7.81	7.81
1b	>125	>125	>125	>125	**62.5**	**62.5**	>125	>125	62.5	62.5	>125	>125	**1.95**	**1.95**
1c	>125	>125	>125	>125	**62.5**	**62.5**	>125	>125	**15.62**	**15.62**	>125	>125	**1.95**	**1.95**
1d	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	**1.95**	**1.95**
1e	>250	>250	**250**	**250**	**125**	**125**	>250	>250	**31.25**	**31.25**	>250	>250	**3.9**	**3.9**
2a	>125	>125	>125	>125	>125	>125	>125	>125	62.5	62.5	>125	>125	**1.95**	**1.95**
2b	>500	>500	>500	>500	250	250	**125**	**125**	125	125	>500	>500	**3.9**	**3.9**
2c	**62.5**	**62.5**	**125**	**125**	**62.5**	**62.5**	**31.25**	**31.25**	**15.62**	**15.62**	**7.81**	**7.81**	**3.9**	**3.9**
2d	>125	>125	>125	>125	>125	>125	**125**	**125**	62.5	62.5	125	125	**3.9**	**3.9**
2e	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	7.81	7.81
3a	>125	>125	>125	>125	>125	>125	**125**	>125	62.5	>125	125	>125	**1.95**	**1.95**
3b	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	**3.9**	15.62
3c	250	>250	>250	>250	250	>250	**125**	>250	125	>250	250	>250	250	250
3d	250	>500	>500	>500	**125**	>500	**125**	>500	125	>500	>500	>500	>500	>500
3e	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
4a	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
4b	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	125	125
4c	**125**	**125**	**125**	**125**	**125**	**125**	250	250	125	125	>500	>500	15.62	15.62
4d	>500	>500	>500	>500	**125**	**125**	250	250	>500	>500	>500	>500	62.5	62.5
4e	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
FLU	0.24	0.24	>500	>500	125	250	31.25	500	250	500	>500	>500	7.81	125
AMB	0.12	0.49	1.95	1.95	1.95	1.95	0.98	1.95	0.49	0.98	7.81	7.81	1.95	1.95

CA: *Candida albicans* ATCC 44859; CT: *Candida tropicalis*; CK: *Candida krusei* E28; CG: *Candida glabrata* 20/I; TA: *Trichosporon asahii* 1188; AC: *Absidia corymbifera* 272; TM: *Trichophyton mentagrophytes* 445. FLU: fluconazole; AMB: amphotericin B.
The best values for each strain are provided in bold.

MIC as AMB (7.81 μM). Twelve carbamates (**1a–c**, **1e**, **2a–d**, **3a**, **3c**, **3d**, **4c**) abolished the growth of *Trichosporon asahii* at lower concentrations (⩾15.62 μM for **1c** and **2c**) than FLU (250–500 μM). *Trichophyton mentagrophytes* showed the highest rate of susceptibility from all of the investigated fungi. MIC values of sixteen carbamates (**1a–3d**, **4b–d**) ranged of 1.95–250 μM. Most of the compounds were superior to FLU. Interestingly, under our conditions, five derivatives (**1b–d**, **2a**, **3a**) exhibited an identical minimum inhibitory concentration as amphotericin B, a highly efficient antimycotic drug.

The analysis of the results revealed some relationships between substitution pattern(s) and biological response. In general, *O*-carbamoylation of both *N*-(4-bromophenyl)-5-chloro-2-hydroxybenzamide and especially 5-chloro-*N*-(4-chlorophenyl)-2-hydroxybenzamide led to the carbamates **2** and **1** with enhanced in vitro antifungal potency. Unequivocally, *N*-benzyl carbamates exhibited the lowest MIC values in comparison to other *N*-substituents. In most cases, longer bridge connecting nitrogen with phenyl ring (phenethyl derivatives **d**) or its removal to form phenyl carbamates **b** led to the decrease of activity. The effect of the phenyl ring hydrogenation (**a** vs **b**) is ambiguous.

Previously reported salicylanilide *N*,*N*-dialkyl(thio)carbamates[8] showed a narrow-spectrum only against *T. mentagrophytes*. This strain was inhibited solely by six *N*,*N*-dimethyl (thio)carbamates from total twenty with MIC values within the range of 1.95–500 μM with superiority of the thiocarbamates. The introduction of bulkier substituents (one or two phenyls) led to the complete loss of the antifungal properties. Here presented carbamates **1–4** exhibited more uniform activity and the most active derivatives (**1b–d**, **2a**, **3a**) share identical MICs with these previously reported thiocarbamates. Other reports about antifungal activity of salicylanilide carbamates have not been published so far.

4. Conclusions

In this study, we present antimicrobial activity of novel salicylanilide *N*-aryl/arylalkyl/cycloalkyl carbamates obtained from salicylanilides and isocyanates in the presence of tertiary base. The carbamoylation of salicylanilide phenol group led to derivatives with significant antimicrobial properties and sufficient solubility for biological evaluation. Dihalogenated salicylanilide carbamates exhibit potent uniform antibacterial activity against Gram-positive bacteria including MRSA and a fungal strain of *T. mentagrophytes* with low micromolar or even submicromolar MICs for the majority of the derivatives. Their moderate activity against ESBL-producing strain of *K. pneumoniae* is also noteworthy.

In general, derivatives of bromosalicylanilide were superior to other groups of halogenated as well as non-halogenated salicylanilide derivatives. *N*-Monosubstituted carbamates exhibited higher and more uniform in vitro antimicrobial activity than salicylanilide *N*,*N*-disubstituted carbamates. Based on these results, reported salicylanilide derivatives seem to be a promising starting compounds for further research and development of novel antimicrobial agents.

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