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Syntheses, in vitro antibacterial and cytotoxic activities of a series of 3-substituted succinimides

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Abstract

We have synthesized a series of 3-substituted succinimides and their in vitro antibacterial activities have been tested towards Gram-positive and Gram-negative bacteria from the ATCC collection. Some of them possess significant antibacterial activity against Gram-positive organisms (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212) but all are poorly active or inactive against Gram-negative organisms (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). The compounds with the lowest minimal inhibitory concentrations (esters of 3-hydroxy succinimides) are also the most cytotoxic against green monkey Vero cell line (ATCC CCL-81) and could explain that perhaps apoptosis should be implicated in eukaryotic cell cytotoxicity of succinimides.

1. Introduction

We have recently reported on the synthesis and antimicrobial activities of *N*-substituted maleimides [1]. We described herein the antibacterial and the cytotoxicity of a series of 3-substituted succinimides which biological properties were poorly documented. It appeared that only a patent in date of 1982 described a synthesis of 3-substituted succinimides and their use as fungicides [2] and a paper in date of 1997 reported their analgesic actions [3].

2. Materials and methods

2.1. Bacteriological assays

In vitro antimicrobial activities were determined by the twofold broth dilution method in Mueller Hinton nutrient broth. The concentration of mother solutions was 1024 μg/ml (in 50/50 water–dimethyl sulfoxide solution). Minimal inhibitory concentration (MIC) was defined as the lowest drug concentration resulting in complete inhibition of growth after 18 h of incubation at 37 °C. Dimethyl sulfoxide (DMSO) had no antibacterial activity at a concentration up to 20% in water. The tested organisms were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

2.2. Cytotoxicity assays

2.2.1. Cells

African green monkey (*Cercopithecus aethiops*) kidney cells (Vero cell line n°. ATCC CCL-81) was grown in RPMI 1640 (BioWest, Nuaille France) containing 100 UI/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine (BioWest, Nuaille France), supplemented with 10% fetal calf serum (FCS). Cells were grown at 37 °C under 5% CO_2 and routinely passed before they reached confluence every 3 days. Cells were harvested using trypsin EDTA solution (Bio-Whittaker Europe, Belgium) and washed. Cellular suspension was incubated (4500 cells per well in 150 μl of media) with various concentrations of imides (0–2000 μg/ml, four wells per concentration) in 96-wells plates for 2 days.

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Daily cell examinations were performed under a phase-contrast microscope to determine the minimum concentration that induced cell morphology alterations such as swelling, shrinkage, granularity and floating. Cytotoxicity of imides was assessed using determination of cell viability performed by neutral red dye method as previously described by McLaren et al. [4] and Langlois et al. [5]. Briefly, 50 µl of media containing neutral red dye (0.02%) was added by well. After 4 h incubation at 37 °C, the media was removed and the cells were washed four times. Lysis buffer (50 µl 1% acetate 50% ethanol per well) before reading DO absorbance at 540 nm. A second method was the reduction of tetrazolium salt by viable cells. Cell titer 96 (Promega, France, Charbonnière) (20 µl per well) was added and the plates incubated 4 h incubation at 37 °C before reading DO absorbance at 492 nm.

3. Results

3.1. Chemistry

The syntheses of maleimides **1–10** and succinimide **11** have been previously reported by our group [1]. The syntheses of 3-acetoxysuccinimides **12–26** have been synthesized, starting with malic acid, using the method that we described in reference [6].

3.1.1. Physicochemical properties of derivatives *12–26*

12: ^{1}H RMN: 1.62 (d, 3H, J = 7.3, CH_3); 2.20 (s, 3H, CH_3); 2.74 (dd, 1H, J = 18.4, J = 4.9, CH); 3.23 (m, 1H, CH); 3.77 (s, 3H, CH_3); 4.87 (m, 1H, CH); 5.5 (m, 1H, CH). IR: 1750, 1719 cm^{-1}.

13: ^{1}H RMN: 2.2 (s, 3H, CH_3); 2.78 (dd, 1H, J = 18.4, J = 4.9, CH); 3.29 (dd, 1H, J = 18.4, J = 8.7, CH); 3.79 (s, 3H, CH_3); 4.3 (s, 2H, CH_2); 5.57 (dd, 1H, J = 8.7, J = 4.9, CH). IR: 1746, 1720 cm^{-1}.

14: ^{1}H RMN: (two diastereoisomers) 2.12–2.16 (2s, 3H, CH_3); 2.45–2.58 (2m, 1H, CH); 2.97–3.10 (2m, H, CH); 3.43–3.51 (2m, 2H, CH_2); 3.80 (2s, 3H, CH_3); 5.05 (2m, 1H, CH); 5.25–5.36 (2m, 1H, CH_2); 7.14–7.31 (2m, 5H, Ar). IR: 1749, 1721 cm^{-1}.

15: ^{1}H RMN: 2.19 (s, 3H, CH_3); 2.82 (dd, 1H, J = 18.4, J = 5.1, CH); 3.31 (dd, 1H, J = 18.4, J = 9.0, CH); 3.83 (s, 3H, CH_3); 5.59 (m, 1H, J = 9.0, J = 5.1, CH); 5.98 (s, 1H, CH); 6.70 (s, 1 H, CH). IR: 1729 cm^{-1} (broad).

16: ^{1}H RMN: 1.22–2.17 (m, 10H, CH_2); 2.18 (s, 3H, CH_3); 2.64 (dd, 1H, J = 18.3, J = 4.9, CH); 3.12 (dd, 1H, J = 18.3, J = 8.8, CH); 4.02 (m, 1H, CH); 5.38 (dd, 1H, J = 8.8, J = 4.9, CH). IR: 1747, 1708 cm^{-1}.

17: ^{1}H RMN: 0.91 (t, 3H, J = 7.34, CH_3); 1.3 (m, 2H, CH_2); 1.56 (m, 2H, CH_2); 2.15 (s, 3H, CH_3); 2.64 (dd, 1H, J = 18.3, J = 4.8, CH); 3.14 (dd, 1H, J = 18.3, J = 8.7, CH); 3.52 (t, 2H, J = 7.41, CH_2); 5.41 (dd, 1H, J = 8.7, J = 4.8, CH). IR: 1751, 1714 cm^{-1}.

18: ^{1}H RMN: 1.42 (d, 6H, J = 18.3, CH_3); 2.18 (s, 3H, CH_3); 2.64 (dd, 1H, J = 18.3, J = 4.9, CH); 3.12 (dd, 1H, J = 18.3, J = J = 8.9, CH); 4.42 (m, 1H, CH); 5.37 (dd, 1H, J = 8.9, J = 4.9, CH). IR: 1750, 1709 cm^{-1}.

19: ^{1}H RMN: 0.93 (m, 3H, CH_3); 1.64 (m, 2H, CH_2); 2.18 (s, 3H, CH_3); 2.69 (dd, 1H, J = 18.3, J = 4.8, CH); 3.16 (dd, 1H, J = 18.3, J = 8.7, CH); 3.53 (t, 2H, J = 7.46, CH_2); 5.43 (dd, 1H, J = 8.7, J = 4.8, CH). IR: 1750, 1712 cm^{-1}.

20: ^{1}H RMN: 2.18 (s, 3H, CH_3); 2.69 (dd, 1H, J = 18.4, J = 4.9, CH); 3.19 (dd, 1H, J = 18.4, J = 8.7, CH); 4.72 (m, 2H, CH_2); 5.47 (dd, 1H, J = 4.9, J = 8.7, CH); 7.37 (m, 5H, Ar). IR: 1749, 1706 cm^{-1}.

21: ^{1}H RMN: 2.04 (s, 3H, CH_3); 2.19 (s, 3H, CH_3); 2.71 (dd, 1H, J = 4.9, J =18.3, CH); 3.2(dd, 1H, J = 18.3, J = 8.8, CH); 3.84 (t, 2H, J = 5.2, CH_2); 4.27 (m, 2H, CH_2); 5.46 (dd, 1H, J = 4.9, J = 8.8, CH). IR: 1789, 1744, 1717 cm^{-1}.

22: ^{1}H RMN: 2.07(s, 6H, CH_3); 2.18 (s, 3H, CH_3); 2.73 (dd, 1H, J = 5.1, J = 18.3, CH); 3.18 (dd, 1H, J = 18.3, J = 8.9, CH); 4.48 (m, 4H, CH_2); 4.60 (m, 1H, CH); 5.41(dd, 1H, J = 5.1, J = 8.9, CH). IR: 1790, 1744, 1718 cm^{-1}

23: ^{1}H RMN: 1.62 (s, 6H, CH_3); 2.06 (s, 3H, CH_3); 2.18 (s, 3H, CH_3); 2.64 (dd, 1H, J = 5.6, J = 18.1, CH); 3.08 (dd, 1H, J = 18.1, J = 9.1, CH); 4.44 (m, 2H, CH_2); 5.36 (dd, 1H, J = 5.6, J = 9.1, CH). IR: 1790, 1745, 1715 cm^{-1}.

24: ^{1}H RMN: 1.61 (s, 9H, CH_3); 2.19 (s, 3H, CH_3); 2.61 (dd, 1H, J = 5.5, J = 18.1); 3.06 (dd, 1H, J = 18.1, J = 8.1, CH); 5.35 (dd, 1H, J = 5.5, J = 8.1, CH). IR: 1750, 1713 cm^{-1}.

25: ^{1}H RMN: 2.23 (s, 3H, CH_3); 2.34 (s, 3H, CH_3); 2.90 (dd, 1H, J = 5.1, J = 18.5, CH); 3.36(dd, 1H, J = 18.5, J = 9.0, CH); 5.58 (dd, 1H, J = 5.1, J = 9.0, CH); 7.25 (m, 2H, Ar); 7.38 (m, 2H, Ar). IR: 1789, 1747, 1714 cm^{-1}.

26: ^{1}H RMN: 1.98 (m, 2H, CH_2); 2.09 (s, 3H, CH_3); 2.19 (s, 3H, CH_3); 2.7 (dd, 1H, J = 4.8, J = 18.4, CH); 3.18(dd, 1H, J = 18.4, J = 8.8, CH); 3.68(t, 2H, J = 7.0, CH_2); 4.1(t, 2H, J = 6.1, CH_2); 5.4(dd, 1H, J = 4.8, J = 8.8, CH). IR: 1789, 1745, 1717 cm^{-1}.

The 3-methylsuccinimides **27–29** were obtained by condensation of 2-methylsuccinic acid with the appropriate amine and further cyclisation, according to the method described in reference [6], Fig. 1.

3.1.2. Physicochemical properties of derivatives *27–29*

27: ^{1}H NMR (MeOD) 1.26 (d, 3H, CH_3, J = 6.0); 2.36 (m, 1H, CH); 2.93 (m, 2H, CH_2); 4.6 (s, 2H, CH_2); 7.29 (m, 5H, Ar). IR: 1701 cm^{-1}.

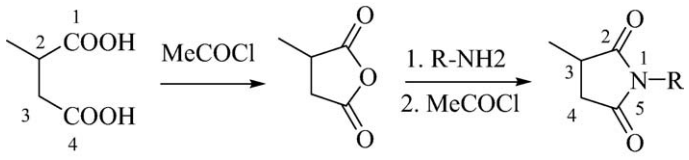

Fig. 1. Syntheses of 3-methylsuccinimides **27–29**.

28: ^{1}H NMR: 1.30 (d, 3H, J = 7.0, CH_3); 1.36 (dd, 6H, CH_3, J = 7.0, J = 1.1); 2.24 (dd, 1H, CH, J = 4.2, J = 17.6); 2.7 (m, 1H, CH); 2.8 (dd, H, J = 9; J = 17.6, CH); 4.35 (d, 1H, J = 6.9, CH). IR: 1713 cm^{-1}.

29: ^{1}H NMR: 1.30 (d, 3H, J = 7.2, CH_3); 2.08 (m, 2H, CH_2); 2.34 (m, 1H, CH); 2.91 (m, 2H, CH_2); 3.55 (t, 2H, CH_2, J = 6.9) 1.3 (t, 2H, J = 5.96, CH_2). IR: 1700 cm^{-1}.

The corresponding 3-hydroxysuccinimides **30–34** were acquired by transesterification of the above described acetoxysuccinimides, using acetyl chloride in hot ethanol.

In a typical example, the synthesis of the compound **34** was realized according to the following protocol: Under argon, 2.2 ml (3 eq.) of acetyl chloride was added to a stirred solution of 2.01 g (10.1 mmol, 1 eq.) of the compound **19** in 60 ml of anhydrous ethanol. The resulting mixture was heated to 50 °C for 3 h. The mixture was cooled and distilled to dryness under reduced pressure. The crude product was purified by column chromatography on silica gel. The product 34 was obtained with a good yield (81%, 1.28 g) and crystallizes spontaneously in pentane (m.p. 68 °C).

*3.1.3. Physicochemical properties of derivatives **30–34***

30: ^{1}H NMR: 2.70 (dd, 1H, J = 4.8, J = 18.2, CH); 3.08 (dd, 1H, J = 18.2, J = 8.4, CH); 4.63 (dd, 1H, J = 4.8, J = 8.4, CH); 4.68 (d, 2H, J = 3.0, CH_2); 7.35 (m, 5H, Ar). IR: 1704 cm^{-1}.

31: ^{1}H NMR (CH_3COCH_3) 2.51(dd, 1H, J = 4.6, J = 17.8); 3.05(dd, 1H, J = 17.8, J = 8.4); 3.57(m, 2H, CH_2); 3.64(m, 2H, CH_2); 3.87(s, 1H, OH); 4.66(dd, 1H, J = 4.6, J = 8.4, CH); 5.21(s, 1H, OH). IR: 1703 cm^{-1}.

32: ^{1}H NMR: 2.04 (s, 3H, CH_3); 2.72(dd, 1H, J = 4.8, J = 18.2, CH); 3.11(dd, 1H, J = 18.2, J = 8.5, CH); 3.2(s, 1H, OH); 3.82(t, 2H, J = 5.2, CH_2); 4.27(m, 2H, CH_2); 4.67(m, 1H, CH). IR: 1707 cm^{-1}.

33: ^{1}H NMR (DMSO): 2.6(dd, 1H, J = 4.7, J = 17.6, CH); 3.09(dd, 1H, J = 17.6, J = 8.5, CH); 4.6(dd, 1H, J = 4.7, J = 8.5, CH); 6.2(m, 1H, OH); 6.8(m, 2H, Ar); 7.1(m, 2H, Ar); 9.8(s, 1H, OH). IR: 1705 cm^{-1}.

34: ^{1}H NMR 0.93(t, 3H, J = 7.5, CH_3); 1.64(m, 2H, CH_2); 2.7(dd, 1H, J = 4.8, J = 18.2, CH); 2.94(s, 1H, OH); 3.09(dd, 1H, J = 18.2, J = 8.4, CH); 3.51 (t, 2H, CH_2); 4.65(dd, 1H, J = 4.8, J = 8.4, CH). IR: 1701 cm^{-1}.

The esters of 3-hydroxy succinimides were produced by esterification of 3-hydroxysuccinimides, using phenylacetyl chloride (**35–37**), cinnamoyl chloride (**38**) or decanoyl chloride (**39**).

In a typical example, the synthesis of the compound **36** was realized according to the following protocol: under argon, at 5–10 °C, 0.5 ml (1.5 eq.) of benzoyl chloride in 10 ml of dry toluene was added drop wise to a stirred solution of 0.5 g (2.44 mmol, 1 eq.)) of the compound **30** in 10 ml of dry toluene. The resulting solution was heated to 75 °C for 4 h and then cooled at room temperature. The mixture was distilled to dryness under reduced pressure. The crude product was purified by column chromatography on silica gel, using diethyl ether–pentane (50–50%) as eluant, to give 0.56 g of the product 36 (71%).

*3.1.4. Physicochemical properties of derivatives **35–39***

35: ^{1}H NMR: 0.93(t, 3H, J = 7.5, CH_3); 1.64(m, 2H, CH_2); 2.64(dd, 1H, J = 4.8, J = 18.3, CH); 3.15(dd, 1H, J = 18.3, J = 8.7, CH); 3.53(t, 2H, J = 7.5, CH_2); 3.75(s, 2H, CH_2); 5.46(dd, 1H, J = 4.8, J = 8.7, CH); 7.33(m, 5H, Ar). IR: 1750, 1701 cm^{-1}.

36: ^{1}H NMR: 2.64 (dd, 1H, J = 4.8, J = 18.3, CH); 3.16 (dd, 1H, J = 18.3, J = 8.7, CH); 3.75 (dd, 2H, J = 19.9, J = 14.8, CH_2); 4.71 (dd, 2H, J = 19.9, J = 14.8, CH_2); 5.49 (dd, 1H, J = 4.8, J = 8.7, CH); 7.36 (m, 10H, Ar). IR: 1715 cm^{-1}.

37: ^{1}H NMR: 2.58(dd, 1H, J = 4.9, J = 18.3, CH); 3.06(dd, 1H, J = 18.3, J = 8.8, CH); 3.59(s, 2H, CH_2); 3.75(s, 2H, CH_2); 3.85(t, 2H, J = 5.2, CH_2); 4.30(m, 2H, CH_2); 5.34(dd, 1H, J = 4.9, J = 8.8, CH); 7.32(m, 10H, Ar). IR: 1737, 1716 cm^{-1}.

38: ^{1}H NMR: 0.96(t, 3H, J = 7.4, CH_3); 1.68(m, 2H, CH_2); 2.78(dd, 1H, J = 4.8, J = 18.3, CH); 3.25(dd, 1H, J = 18.3, J = 8.7, CH); 3.58(m, 2H, CH_2); 5.56(dd, 1H, J = 4.8, J = 8.7, CH); 6.5(d, 1H, J = 16.0, CH); 7.43(m, 3H, Ar); 7.56(m, 2H, Ar); 7.8(d, 1H, J = 16.0 CH). IR: 1712 cm^{-1}.

39: ^{1}H NMR: 0.9 (t, 3H, J = 7.4, CH_3); 0.94(t, 3H, J = 7.0, CH_3); 1.3(m, 12H, CH_2); 1.66(m, 4H, CH_2); 2.4(m, 2H, CH_2); 2.67(dd, 1H, J = 4.8, J = 18.3, CH); 3.17(dd, 1H, J = 18.3, J = 8.7, CH); 3.54(t, 2H, J = 7.2, CH_2); 5.44(dd, 1H, J = 4.8, J = 8.7, CH). IR: 1708, 1739 cm^{-1}.

Compounds **40–42** were synthesized from itaconic acid, using the method previously reported by Stratford and Curley [7].

Other derivatives: **43** were obtained by reacting benzoyl chloride with the free base of compound **41**. From the free amine of compound **42**, the amino-methyl *N*-benzyl succinimides **44–49** were produced, using the appropriate alkanoyl chloride (**44**: benzoyl; **45**: cinnamoyl; **46**: crotonyl; **47**: acetyl; **48**: hexanoyl; **49**: trimethylacetyl).

In a typical example, the synthesis of the compound **44** was realized according to the following protocol: Under argon, 0.26 ml (1.05 eq.) of triethylamine in 5 ml of tetrahydrofurane (THF) was added to a stirred solution of 0.45 g (1.76 mmol, 1 eq.) of the compound **42** in 15 ml of THF. The resulting suspension was agitated for 18 h at room temperature. Then, 0.26 ml of triethylamine and 0.30 ml (1.25 eq.) of benzoyl chloride was added and the mixture was stirred for 24 h at room temperature. After filtration of the triethylamine hydrochloride, the mixture was distilled to dryness under reduced pressure. The crude oily product crystallizes spontaneously in acetone (m.p. 114 °C). 0.38 g of the product **44** was obtained (64%).

*3.1.5. Physicochemical properties of derivatives **43–49***

43: RMN ^{1}H NMR: 2.49–2.76 (m, 2H, CH_2), 2.98 (m, 1H, CH), 3.49–3.97 (m, 4H, CH_2), 4.61 (s, 2H, CH_2), 4.72 (s, 2H, CH_2), 7.12–7.41 (m, 15H, Ar). IR: 1774, 1703, 1648 cm^{-1}.

44: ^{1}H NMR: 2.47–2.80 (m, 2H, CH_2); 2.91 (m, 1H, CH), 3.44–3.65 (m, 4H, CH_2), 4.58 (s, 2H, CH_2), 6.13(t, 1H, *J* = 5.9, NH), 7.18–7.36(m, 10H, Ar). IR: 1773, 1701, 1650 cm^{-1}.

45: ^{1}H NMR: 2.63–3.0(m, 2H, CH_2), 3.10 (m, 1H, CH), 3.59–3.93 (m, 2H, CH_2), 4.67(s, 2H, CH_2), 6.12(s, 1H, NH), 6.28 (d, 1H, *J* = 15.6, CH), 7.29–7.52 (m, 10H, Ar) 7.63 (d, 1H, CH); IR: 1773, 1701, 1659 cm^{-1}.

46: ^{1}H NMR: 1.86 (dd, 3H, *J* = 6.8, *J* = 1.2, CH), 2.6–2.9 (m, 2H, CH_2), 3.0 (m, 1H, CH), 3.55–3.93 (m, 2, CH_2), 4.64 (s, 2H, CH_2) 5.69 (dd, 1H, *J* = 15.2, *J* = 1.4, CH) 5.9 (s, 1H, NH), 6.81(m, 1H, CH); 7.31–7.39 (m, 5H, Ar). IR: 1771, 1699, 1665 cm^{-1}.

47: ^{1}H NMR: 1.93 (s, 3H, CH_3) 2.54–2.9(m, 2H, CH_2), 3.0 (m, 1H, CH), 3.44–3.75 (m, 2H, CH_2), 4.66 (s, 2H, CH_2) 6.0 (s, 1H, NH); 7.3–7.38(m, 5H, C_6H_5). IR: 1773, 1696, 1649 cm^{-1}.

48: ^{1}H NMR: 0.90 (t, 3H, *J* = 7.0, CH_3), 1.29(m, 4H, CH_2); 1.57 (m, 2H, CH_2); 2.10 (m, 2H, CH_2); 2.55–2.90 (m, 2H, CH_2); 2.95 (m, 1H, CH); 3.51–3.72 (m, 2H, CH_2); 4.66 (s, 2H, CH_2); 6.0 (s, 1H, NH); 7.3–7.4 (m, 5H, Ar). IR: 1773, 1702, 1646 cm^{-1}.

49: ^{1}H NMR: 1.1(s, 9H, CH_3); 2.51–2.9(m, 2H, CH_2); 3.0 (m, 1H, CH); 3.48–3.72 (m, 2H, CH_2); 4.65 (s, 2H, CH_2); 6.21 (s, 1H, NH); 7.3–7.38 (m, 5H, Ar). IR: 1773, 1705 cm^{-1}.

From the free amine, derived from salt **42**, the carbamate **50** was produced, by reaction with phenyl chloroformate. From this latter, the urea **51** was acquired with *N*-methyl piperazine and the urea **52** with 4-amino acetophenone [8].

The synthesis compounds **50, 51** and **52** were realized according to the following procedures:

50: Under argon, 0.27 ml (1.05 eq.) of triethylamine in 5 ml of tetrahydrofurane (THF) was added to a stirred solution of 0.47 g (1.85 mmol, 1 eq.) of **42** in 15 ml of THF. The resulting suspension was agitated for 10 h at room temperature. Then, 0.27 ml of triethylamine and 0.25 ml (1.07 eq.) of phenyl chloroformate was added and the mixture was stirred for another 70 h at room temperature. After filtration of the triethylamine hydrochloride, the mixture was distilled to dryness under reduced pressure. The crude oily product crystallizes in acetone after 12 h at –18 °C. 0.38 g of white crystals (m.p. 141–142 °C) were obtained (61%).

51: Under argon, 2.19 ml (6 eq.) of *N*-methylpiperazine in 5 ml of THF were added to a stirred solution of 1.09 g (3.22 mmol, 1 eq.) of **50** in 15 ml of THF. The resulting solution was stirred for 66 h at room temperature. The triethylamine hydrochloride was removed by vacuum filtration and the filtrate was distillated to dryness under reduced pressure. The crude oil was purified by column chromatography on silica gel, using dichloromethane-methanol as eluant (90–10%), to give 0.56 g of yellow oil in a nearly quantitative yield.

52: Under argon, 1.69 g (4 eq.) of 4-aminoacetophenone and 0.9 ml (2 eq.) of triethylamine in 5 ml of THF was added to a stirred solution of 1.06 g (3.13 mmol, 1 eq.) of **50** in 15 ml of THF. The resulting solution was agitated for 70 h at room temperature. The triethylamine hydrochloride was removed by vacuum filtration. The filtrate was distillated under reduced pressure and the crude product was purified by column chromatography on silica gel, using cyclohexane–ethyl acetate as eluant (50–50%), to afford 0.12 g (10%) of **52**, which crystallize spontaneously (m.p. 129 °C).

*3.1.6. Physicochemical properties of derivatives **50–52***

50: ^{1}H NMR: 2.63 (dd, 1H, *J* = 18.3, *J* = 5.2, CH); 2.9 (dd, 1H, *J* = 18.3, *J* = 9.3, CH); 3.05 (m, 1H, CH); 3.54 (m, 1H, CH); 3.72 (m, 1H, CH); 4.68 (s, 2H, CH_2); 5.50 (s, 1H, NH); 7.08–7.39 (m, 10H, Ar). IR: 1778, 1735, 1701 cm^{-1}.

51: ^{1}H NMR: 2.21 (s, 3H, CH_3); 2.25 (m, 4H, CH_2) 2.52 (dd, 1H, *J* = 18.4, *J* = 4.6, CH); 2.74 (dd, 1H, *J* = 18.4, *J* = 9.1, CH); 2.9 (m, 1H, CH); 3.21 (m, 4H, CH_2); 3.40 (m, 1H, CH); 3.59 (m, 1H, CH); 4.55 (s, 2H, CH_2); 5.21 (s, 1H, NH); 7.18–7.29 (m, 5H, Ar). IR: 1779, 1703, 1630 cm^{-1}.

52: ^{1}H NMR: 2.41 (s, 3H, CH_3); 2.65 (dd, 1H, *J* = 18.5, *J* = 5.2, CH); 2.9 (dd, 1H, *J* = 18.5, *J* = 9.2, CH); 3.06 (m, 1H, CH); 3.59 (m, 1H, CH); 3.74 (m, 1H, CH);4.64 (s, 2H, CH_2); 5.70 (s, 1H, NH); 6.97 (s, 1H, NH); 7.18–7.68 (m, 9H, Ar). IR: 1770, 1722, 1702, 1620 cm^{-1}.

From the free amine derived from salt **42**, the oxamide **53** was produced by reaction with oxalyl chloride.

The synthesis of **53** was realized according to the following procedure: Under argon, 0.47 ml (1.05 eq.) of triethylamine in 5 ml of THF was added to a stirred solution of 0.81 g (3.18 mmol, 1 eq.) of **42** in 15 ml of THF. The resulting suspension was stirred for 18 h at room temperature. Then, 0.47 ml of triethylamine and 0.147 ml (0.525 eq.) of oxalyl chloride was added and the mixture was stirred for another 24 h at room temperature. After filtration of the triethylamine hydrochloride, the volatile fractions were removed under reduced pressure. The concentrated solution was dissolved in acetone and the compound **53** (0.48 g, 62%) crystallizes (m.p. 195 °C) with addition of a small amount of diethyl ether.

*3.1.7. Physicochemical properties of derivative **53***

53: ^{1}H NMR: 2.53 (dd, 2H, *J* = 18.1, *J* = 3.85, CH); 2.88 (dd, 2H, *J* = 18.4, *J* = 9.3, CH); 3.06 (m, 2H, CH); 3.63 (m, 2H, CH); 3.76 (m, 2H, CH); 4.65 (s, 4H, CH_2); 7.27–7.39 (m, 10H, Ar); 7.84 (s, 2H, NH); IR: 1701, 1777.

3.2. Microbiological evaluation

The results of the screening tests of succinimides were reported in Tables 1–3. These tables showed the MICs of succinimides against two Gram-positive bacteria (*S. aureus* ATCC 25923, *E. faecalis* ATCC 29212) and two Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853). DMSO had no antibacterial effect at a concentration up to 512 μg/ml. These strains have also been tested with reference antibiotics: ampicillin, kanamycin, erythromycin and ofloxacin. The results are given at the end of Table 1.

Table 1
In vitro antimicrobial activities of *N*-benzyl succinimide, 3-methyl succinimides and 3-hydroxy succinimides (see Fig. 2) and reference compounds (MICs, μg/ml)

No.	R_1	R_2	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
11	$C_6H_5CH_2$–	H–	512	256	256	256
27	$C_6H_5CH_2$–	CH_3–	512	256	256	256
28	*Iso*C_3H_7–	CH_3–	512	256	256	256
29	$HO(CH_2)_3$–	CH_3–	512	256	256	256
30	$C_6H_5CH_2$–	HO–	512	256	256	256
31	$HO(CH_2)_2$–	HO–	512	256	256	256
32	CH_3CO_2 $(CH_2)_2$–	HO–	512	512	512	512
33	*p* OHC_6H_4–	HO–	512	512	512	512
34	*N*C_3H_7–	HO–	512	512	512	512
Reference compounds						
Ampicillin			0.5	1	4	>16
Kanamycin			2	32	2	>16
Erythromycin			0.25	2	>4	>4
Ofloxacin			0.5	2	0.03	2

Table 2
In vitro antimicrobial activities of esters of 3-hydroxy succinimides (MICs, μg/ml) (see Fig. 3). In the presence of cysteine (1 mg/ml) all MICs are >512 μg/ml

No	R_1	R_2	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
12	$CH_3OCOAla$–	CH_3–	32	32	128	256
13	$CH_3OCOGly$–	CH_3–	128	64	128	256
14	$CH_3OCOPhe$–	CH_3–	16	16	256	256
15	$CH_3OCO(CH_2{=}C)$–	CH_3–	128	256	128	256
16	Cyclohexyl–	CH_3–	32	64	256	256
17	*n* C_4H_9–	CH_3–	16	32	256	256
18	*Iso*C_3H_7–	CH_3–	32	64	256	256
19	*n* C_3H_7–	CH_3–	16	32	128	256
35	*n* C_3H_7–	$C_6H_5CH_2$–	64	64	128	256
38	*n* C_3H_7–	$C_6H_5CH{=}CH$–	256	128	256	256
39	*n* C_3H_7–	*n* C_9H_{19}–	128	128	128	256
20	$C_6H_5CH_2$–	CH_3–	64	32	256	256
36	$C_6H_5CH_2$–	$C_6H_5CH_2$–	64	64	256	256
37	$C_6H_5CH_2CO_2(CH_2)_2$–	$C_6H_5CH_2$–	128	64	128	128
21	$CH_3CO_2(CH_2)_2$–	CH_3–	128	128	256	256
22	$(CH_3CO_2CH_2)_2CH$–	CH_3–	128	128	256	256
23	$CH_3CO_2C(CH_3)_2$–	CH_3–	64	128	256	256
24	$C(CH_3)_3$–	CH_3–	128	256	256	256
25	*p*Ac O C_6H_4–	CH_3–	256	256	256	256
26	$CH_3CO_2(CH_2)_3$–	CH_3–	128	128	256	256

Table 3
In vitro antimicrobial activities of 3-amino-methyl *N*-benzyl succinimides (MICs, μg/ml) (see Fig. 4)

No.	R_3	R_4	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
41	H	$C_6H_5CH_2$	256	64	256	*256*
42	H	H	*512*	256	256	*256*
40	$C_6H_5CH_2$–	CH_3CO–	512	256	256	256
44	H–	C_6H_5CO–	512	256	256	256
43	$C_6H_5CH_2$–	C_6H_5CO–	>512	>512	>512	>512
45	H–	$C_6H_5CH{=}CHCO$–	512	256	256	256
46	H–	$CH_3CH{=}CHCO$–	512	256	256	256
47	H–	CH_3CO–	512	256	256	256
48	H–	*n* $C_5H_{11}CO$–	512	256	256	256
49	H–	$(CH_3)_3CCO$–	512	256	256	256
50	H–	C_6H_5OCO–	256	256	256	256
51	H–	See Fig. 5A	256	256	256	256
52	H–	See Fig. 5B	>512	>512	>512	>512
53	H–	See Fig. 5C	>512	256	256	128

3.3. Cytotoxic evaluation (determination of 50% cytotoxic concentration (CC_{50}))

The results of the screening tests of imides are reported in Tables 4–7. Cells were incubated with various concentrations of imide molecules (0–2000 μg/ml, four wells per concentration). The effect of cysteine on imide cytotoxicity was assessed at the concentrate of 1 mg/ml in the media. Correlation curve was obtained between pinocytosis of neutral red (assessed by DO absorbance at 540 nm) and Neperian logarithm of imide concentrations. The CC_{50} of the tested molecules was defined as the concentration that reduced the absorbance to 50% of that of controls. Negatives controls showed that DMSO had no cytotoxic effect at concentration up to 20 mg/ml.

Table 4
In vitro cytotoxic activities of maleimides **1–10** (CC_{50}, μg/ml) (see Fig. 6)

No	R_1	CC_{50} (μg/ml) (with cysteine 1 mg/ml)
1	$C_6H_5CH_2$	2.23 (735)
2	Cyclohexyl	9
3	*n*-C_4H_9–	2.59
5	*p*-C_6H_4OMe	<31.25 [a]
6	$(CH_2)_2OH$–	4.06
7	Ser(OAc) CO_2Me	27
8	Ala CO_2Me	4.95
9	Gly CO_2Me	15.6
10	Phe CO_2Me	<31.25 [a] (493)

[a] For compounds **5** and **10**, the lowest concentration tested was 31.25 μg/ml.

Table 5
In vitro cytotoxic activities of *N*-benzyl succinimide, 3 methyl *N*-benzyl succinimide and 3 hydroxy *N*-benzyl succinimide compounds (CC_{50}, μg/ml) (see Fig. 2)

No	R_1	R_2	CC_{50} (μg/ml)
11	$C_6H_5CH_2$–	H–	898
27	$C_6H_5CH_2$–	CH_3–	735
28	$C_6H_5CH_2$–	HO–	972

Table 6
In vitro cytotoxic activities of esters of 3-hydroxy succinimides (CC_{50}, μg/ml) (see Fig. 3)

No.	R_1	R_2	CC_{50} (μg/ml) (with cysteine 1 mg/ml)
12	$CH_3OCOAla$–	CH_3–	10
13	$CH_3OCOGly$–	CH_3–	40
14	$CH_3OCOPhe$–	CH_3–	<31.25 [a] (>2000)
19	*n*C_3H_7–	CH_3–	4.05 (105)
35	*n*C_3H_7–	$C_6H_5CH_2$–	4.95 (110)
38	*n*C_3H_7–	C_6H_5CH=CH–	89
39	*n*C_3H_7–	*N*C_9H_{19}–	11.2 (181)
20	$C_6H_5CH_2$–	CH_3–	9.97 (1339)
36	$C_6H_5CH_2$–	$C_6H_5CH_2$–	20.9 (>2000)
21	$CH_3CO_2(CH_2)_2$–	CH_3–	31.25
22	$(CH_3CO_2CH_2)_2CH$–	CH_3–	121.5
23	$CH_3CO_2C(CH_3)_2$–	CH_3–	8.58
24	$C(CH_3)_3$–	CH_3–	4.05
25	*p*Ac O C_6H_4–	CH_3–	225
26	$CH_3CO_2(CH_2)_3$–	CH_3–	31.25

[a] For compound **14**, the lowest concentration tested was 31.25 μg/ml.

Table 7
In vitro cytotoxic activities of 3-amino-methyl *N*-benzyl succinimides (CC_{50}, μg/ml) (see Fig. 4)

No.	R_3	R_4	CC_{50} (μg/ml) (with cysteine 1 mg/ml)
41	H–	$C_6H_5CH_2$–	6.05 (67)
42	H–	H–	8.17 (>2000)
43	$C_6H_5CH_2$–	C_6H_5CO–	33
45	H–	C_6H_5CH=CHCO–	110
46	H–	CH_3CH=CHCO–	601
47	H–	CH_3CO–	365
48	H–	*N* $C_5H_{11}CO$–	284
49	H–	$(CH_3)_3CCO$–	572
50	H–	C_6H_5OCO–	299
51	H–	See Fig. 5A	1096
52	H–	See Fig. 5B	<31.25 [a]
53	H–	See Fig. 5C	200

[a] For compound **52**, the lowest concentration tested was 31.25 μg/ml.

4. Discussion

More than 40 succinimides have been synthesized. They were substituted at the nitrogen atom (position 1) and at position 3 (see Figs. 2–5). Ten maleimides (see Fig. 6) were added, in order to compare their cytotoxycities (products **1–10**). Antibacterial activities of these latter have been reported in Ref. [1].

If R_2 is a hydrogen atom, a methyl or a hydroxyl group, the compounds have no noticeable antibacterial activity (see Table 1). Three of them (with R_2 = H: **11**, CH_3: **27** or OH: **28**) were selected for toxicity studies: they demonstrated poor toxicity towards Vero cells, with CC_{50} close to 1 mg/ml.

Twenty molecules with an ester sub-unit display some moderate activities against bacteria, with the lowest MICs in

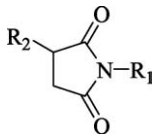

Fig. 2. 3-Methyl succinimides and 3-hydroxy succinimides.

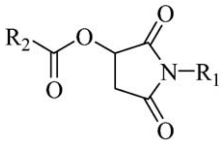

Fig. 3. Esters of 3-hydroxy succinimides.

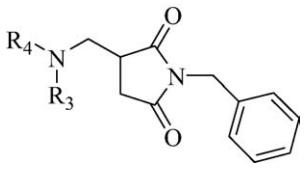

Fig. 4. 3-Amino-methyl *N*-benzyl succinimides.

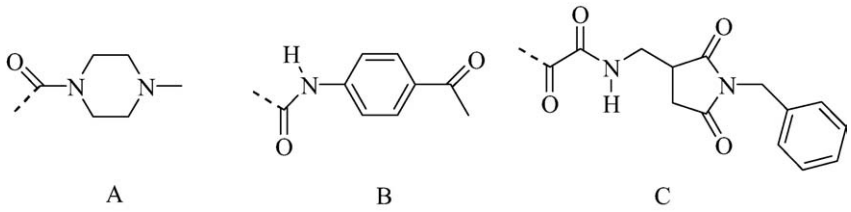

Fig. 5. Nature of substituents R_4.

the range of 16–32 μg/ml towards Gram-positive strains (see Table 2). We could notice that the antibacterial activities against Gram-negative bacteria (*E. coli, P. aeruginosa*) remained very poor. The toxicity against Vero cells was also higher as demonstrated for 15 molecules, but large variations were observed (see Table 6). The most toxic compounds are **19** and **24** with CC_{50} = 4.05 μg/ml and the less toxic is **25** with CC_{50} = 225 μg/ml (55 times less toxic). The most toxic molecules bear a short alkyl chain at position 1. For molecules with R_2 being CH_3, alkyl, benzyl or styryl (see Table 6), the antibacterial activity is generally lower but the toxicity against Vero cells is not changed.

Fourteen amino-methyl succinimides were synthesized (Fig. 4). Only two of them display a moderate antibacterial activity: compound **41** versus *E. faecalis* (MIC: 64 μg/ml) and 53 versus *P. aeruginosa* (128 μg/ml) (see Table 3). With few exceptions, these compounds were weakly toxic (see Table 7).

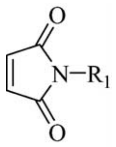

Fig. 6. Maleimides.

Molecules **51** and **52** (ureas) and **53** (oxamide) were also prepared, as they are related to publish substances that possess antibacterial activities [9].

In a first step, we could suspect that the activity of 3-acetoxysuccinimides is due to in vivo elimination of the acetoxy group, leading to maleimide compounds that are known to be inhibitors of SH-enzymes. In a second step, in order to clarify this point, several tests were performed in the presence of 1 mg/ml of cysteine.

Concerning the antibacterial activities and toxicity tests, all combinations were inactive and non-toxic in the presence of cysteine, as it occurs for maleimides (see Table 4).

For molecule **42**, a 3-amino-methyl succinimide taken as an example, formation of a maleimide is strictly impossible in biological conditions. Surprisingly CC_{50} toxicity values shift from 8 to >2000 μg/ml in the presence of cysteine. Thus, the mechanism of action of these molecules remains to be clarified.

Observation of cell morphology alterations during the first part of the tests showed that the cells begin to shrink around the CC_{50} (Fig. 7), although membrane permeability is not yet affected, as described when a cell is undergoing an apoptosis process [10]. Moreover, a previous paper showed that a conjugated vinyl-ketone (ethacrynic acid, a diuretic, inhibitor of some SH-enzymes) which had some similarities with these maleimides induce apoptosis in cell line [11] and it was

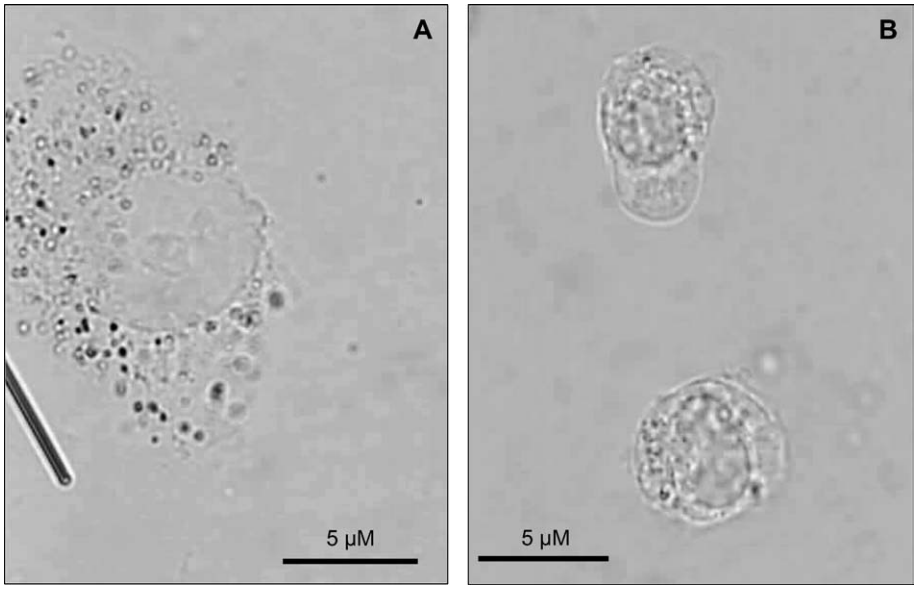

Fig. 7. For an example, cytotoxic effects of succinimide **19** (CC_{50} = 4.05 μg/ml) on Vero cells were grown for 2 days in absence (A) or in presence of succinimide **19** (B) at 31.25 μg/ml, and colored with neutral red dye.

also recently demonstrated that *N*-acetyl-L-cysteine inhibits its cell toxicity [12].

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