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Note

Synthesis and antimicrobial activities of *N*-substituted imides

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Abstract

In the field of our research programs concerning novel antimicrobial agents, a series of *N*-substituted imides was synthesized. These compounds were obtained by cyclization of amido-acids in acetic anhydride/sodium acetate or hexamethyldisilazane/zinc bromide for the hydroxy-aromatic derivatives. The hydroxy-alkyl maleimides were directly prepared by condensation of the corresponding amino-alcohol with maleic anhydride in boiling toluene. Most of *N*-substituted maleimides showed an interesting antimicrobial activity towards bacteria from the ATCC collection (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) but the MIC values for *P. aeruginosa* were always high (128 μg/ml). The imides with alkyl substituents showed higher activities than aromatic analogues with MIC values in the range of 8–32 μg/ml. Comparatively, succinimides were practically inactive. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Antimicrobial activities; *N*-Substituted imides; Maleimides

1. Introduction

Bacterial resistance to antibiotics is an increasing problem that concerns clinicians, the pharmaceutical industry and chemists. The multidrug-resistant bacteria are the major cause of failure in the treatment of infectious diseases. Thus the need for novel antibiotics is more and more important.

Thiol-reactive compounds are often inhibitors of cysteine proteinases or other proteins with an essential cysteine. Important biological properties concerning bactericidal, fungicidal and anticancer were reported for *N*-substituted imides such as maleimides [1–6] and related compounds: acrylamides such as isohematinic acid [7], a natural antibiotic produced by *Actinoplanes philippinensis*, some vinyl ketones [8] with activities against yeast and fungi (including *Aspergillus* species), vinyl sulfones [9] with antimalarial activities, butenolides [10] and thalidomide, an old drug reinvestigated mostly as anticancer agent and in the therapy of leprosis [11], whose structures are reported in Fig. 1. Early reports [12–14] concerning maleimides concluded that they interact preferably with the hydrophobic domains of enzymes, based on the fact that the inactivation of sulfhydryl groups is greatly affected by the side-chain length of the derivatives.

In the present paper, we studied the antibacterial activities of a series of maleimides and succinimides against few reference bacteria from the ATCC collection. Some of these compounds have been previously described, but only a very few of them were tested for their antibacterial activities [1].

2. Materials and methods

2.1. Chemical preparations

The most suitable starting point for the synthesis of maleimides is an amido-acid, obtained by acylation of the amine by the corresponding anhydride. The subsequent cyclization into *N*-substituted imide is performed by $(CH_3CO)_2O/CH_3COONa$ [15] (path a) or HMDS/

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$ZnBr_2$ for the hydroxy-aromatic derivative [16] (path b). The hydroxy-alkyl maleimides were directly prepared by condensation of the corresponding amino-alcohol with maleic anhydride in boiling toluene (path c). All products were characterized by ^1H NMR (Bruker Avance DPX 400), IR (Bruker IRS 55) and the microanalyses were in agreement with the calculated values: C ± 0.25%, H ± 0.20%, N ± 0.20%.

2.2. Bacteriological assays

In vitro antimicrobial activities of the compounds were determined by the two-fold broth dilution method in Mueller Hinton nutrient broth. The concentration of mother solutions were 1024 μg/ml (50/50 water–dimethyl sulfoxide). Minimal inhibitory concentration (MIC) is defined as the lowest drug concentration re-

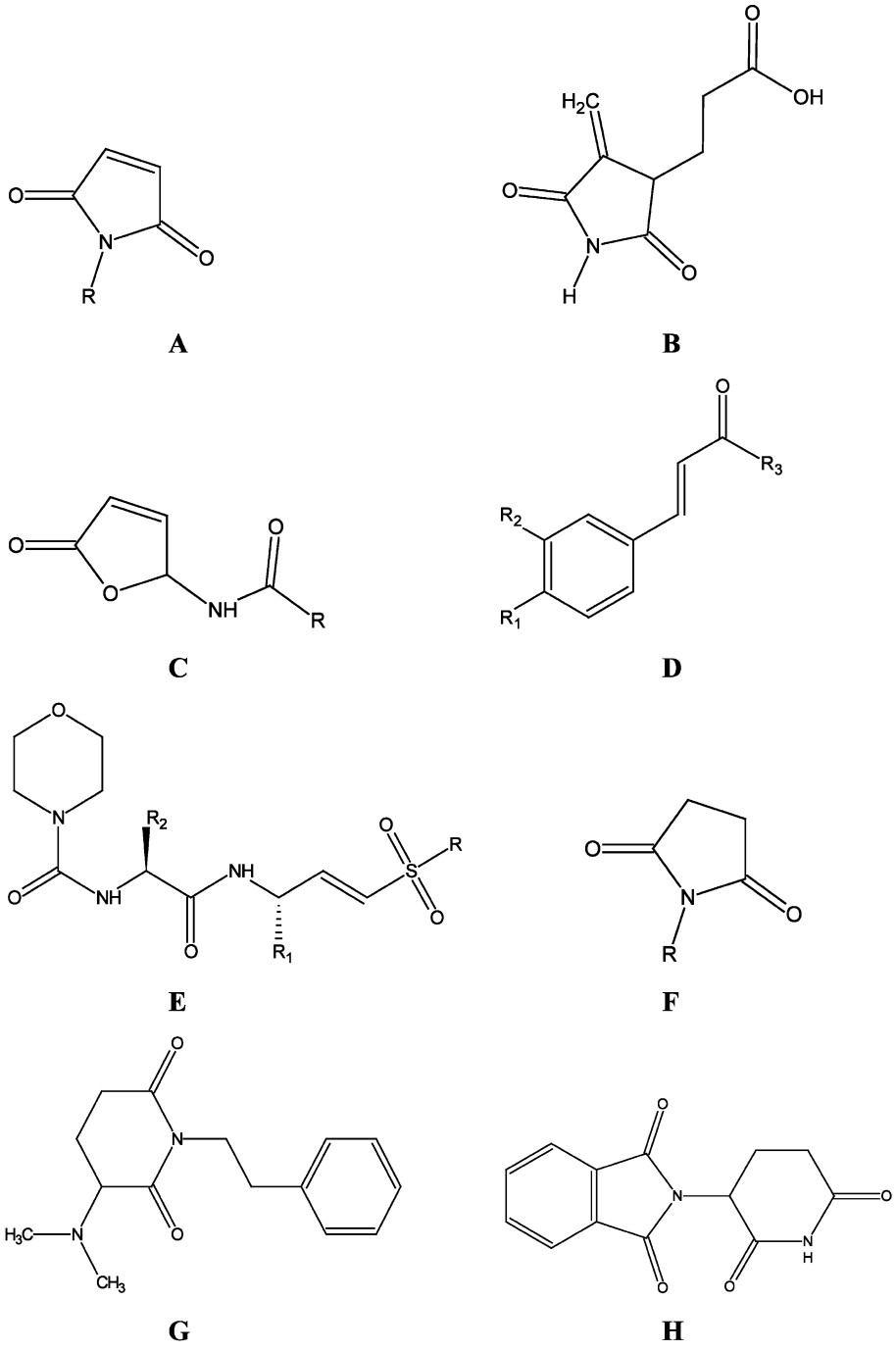

Fig. 1. Chemical structures of maleimides **A** (1–6), isohematinic acid **B** (7), butenolides **C** (10), vinyl ketones **D** (8) and vinyl sulfones **E** (9), succinimides **F**, phyllanthimide **G** (1), thalidomide **H** (11).

sulting in complete inhibition of growth after 18 h of incubation at 37 °C. Dimethylsulfoxide has 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.

3. Results

3.1. Chemistry

N-Substituted maleimides and succinimides were prepared by one of three available procedures (paths a, b and c) and identified by the usual methods (IR, NMR and microanalysis).

3.1.1. General procedure (path a) for the synthesis of imide derivatives **1**–**10**, **12**–**14** and **18**–**31**

First, under argon, to a stirred solution of maleic or succinic anhydride (100 mmol) in ethyl ether or CH_2Cl_2 (100 ml) was added dropwise at 5–10 °C, 105 mmol of amine or amino acid, methyl ester, in ethyl ether or CH_2Cl_2 (20 ml). The resulting suspension was refluxed for 2 h and then cooled at room temperature. The crystallized acid was filtered off and washed with dry ethyl ether. Yields are close to 95%.

Then, under argon, 50 mmol of the maleamic or succinamic acid was added to a mixture of 20 ml of acetic anhydride and 2 g of anhydrous sodium acetate. The resulting suspension was heating on a steam bath for 8 h. The mixture was cooled and evaporated to dryness under reduced pressure. The residue was dissolved in ethyl ether or CH_2Cl_2, washed with 2×30 ml of cold saturated $NaHCO_3$ and dried ($MgSO_4$). The solvent, distillated under reduced pressure, gave a brown oil. This crude product was purified by column chromatography on silica gel. Most of the products were obtained with good yields ($> 80\%$) and some of them crystallize spontaneously.

Note that compounds **1**, **2**, **3**, **4**, **5**, **6**, have been described by Takatori [4], **7**, **8**, **10**, **13**, **14**, **24** by Trujillo-Ferrara [16], **9** by Tome [17], **12** by Razna [19], **19** by Biagini [22], **20** by Yoon [23], **25** by Allen [24], **26** by Gesson [25], **27** by Puertas [26], **28** by Houlihan [27], **29** by Schreiber [28], **30** by Kraus [29] and **31** by Neuberger [30].

Products **18**, **21**, **22** and **23** are new ones:

18: R = Ser(OAc) CO_2Me: Colorless oil. IR: 1749, 1714 cm^{-1}. ^1H NMR ($CDCl_3$): 2.0 (s, 3H, CH_3COO); 3.8 (s, 3H, OMe); 4.5 (dd, ABX, 1H, $J = 11.6$ Hz; $J' = 10.0$ Hz); 4.8 (dd, ABX, 1H, $J = 11.7$ Hz, $J' = 4.3$ Hz); 5.0 (m, ABX, 1H, $J = 10.0$ Hz, $J' = 4.3$ Hz); 6.8 (s, 2H, CH=).

21: R = Phe CO_2Me: Colorless oil. IR: 1747; 1713 cm^{-1}. ^1H NMR ($CDCl_3$): 3.48 (m, *AB*X, 2H, CH, $J = 11.7$ Hz, $J' = 5.1$ Hz); 3.8 (s, 3H, OMe); 5.0 (m, AB*X*, 1H, CH, $J = 11.7$ Hz, $J' = 5.1$ Hz); 6.61 (s, 2H, CH=); 7.2 (m, 5H, Ar).

22: R = Lys CO_2Me: Yellow oil. IR: 1744, 1706 cm^{-1}. ^1H NMR ($CDCl_3$): 1.27 (m, 2H, CH_2); 1.63 (m, 2H, CH_2); 2,18 (m, 2H, CH_2); 3.5 (m, 2H, CH_2); 3.7 (s, 3H, OMe); 4.63 (t, 1H, CH); 6.7 (s, 2H, CH=); 6.77 (s, 2H, CH=).

23: R = C=CH_2 CO_2Me: Oil. IR: 1722, 1640 cm^{-1}. ^1H NMR ($CDCl_3$): 3.83 (s, 3H, OMe); 5.93 (s, 1H, =CH); 6.63 (s, 1H, =CH); 6,84 (s, 2H, CH=).

3.1.2. General procedure (path b) for the synthesis of imide derivatives **11** and **15**

Maleamic acids are synthesized as described previously (path a).

The maleamic acid (10 mmol) in dry toluene(50 ml) and $ZnBr_2$ (1 equiv.) was heated to 80 °C then a solution of HMDS (1.5 equiv.) in dry toluene (10 ml) was slowly added, in 30 min. The reaction mixture was refluxed for an additional 6 h and concentrated under reduced pressure to led a crude product, which was dissolved in EtOAc (60 ml), washed twice 15 min with 0.1 M HCl (2×60 ml). The organic phase was then washed with 2×30 ml of saturated $NaHCO_3$, dried ($MgSO_4$) and concentrated under reduced pressure. The oily product was purified by column chromatography on silica gel. The yields are close to 85%.

Products **11** and **15** have been described, respectively, by Beyer [18] and Choi [20].

3.1.3. General procedure (path c) for the synthesis of imide derivatives **16** and **17**

The hydroxy-alkyl maleimides were synthesized directly by condensation of the maleic anhydride (50 mmol) with corresponding amino-alcohol (1.05 equiv.) in toluene (60 ml). The resulting suspension was heated 2 h at 80 °C. The imide was removed under reduced pressure from the organic layer, to give a oily product, which was purified by crystallization (yields 25%).

Note that compounds **16** and **17** have been described respectively by Beyer [18] and Miyadera [21].

3.2. Microbiological evaluation

The results of the screening tests of *N*-maleimides and *N*-succinimides derivatives are reported in Tables 1 and 2. These tables show the MIC values of imides 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). Dimethyl sulfoxide has no antibacterial effect at a concentration up to 512 μg/ml.

Table 1
In vitro antimicrobial activities of maleimide compounds (MIC values, μg/ml)

N-(*R*) maleimides	No.	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
C_6H_5	1	64	128	32	256
$C_6H_5CH_2$	2	8	8	32	256
Cyclohexyl	3	16	32	64	128
n-C_4H_9	4	8	16	16	256
n-C_3H_7	5	8	16	16	256
iso-C_3H_7	6	8	8	16	128
o-C_6H_4OMe	7	≥512	≥512	≥512	≥512
o-C_6H_4OAc	8	64	64	128	256
m-C_6H_4OMe	9	32	32	64	128
m-C_6H_4OAc	10	64	64	128	256
m-C_6H_4OH	11	32	64	32	256
p-$C_6H_4NO_2$	12	≥512	≥512	≥512	≥512
p-C_6H_4OMe	13	32	128	64	256
p-C_6H_4OAc	14	32	64	64	256
p-C_6H_4OH	15	32	64	32	256
$(CH_2)_2OH$	16	16	16	16	128
$(CH_2)_3OH$	17	16	16	8	128
Ser(OAc) CO_2Me	18	128	128	64	256
Ala CO_2Me	19	32	64	32	256
Gly CO_2Me	20	64	64	32	256
Phe CO_2Me	21	16	16	256	256
Lys CO_2Me	22	64	64	256	256
$C{=}CH_2$ CO_2Me	23	128	256	64	256

Table 2
In vitro antimicrobial activities of succinimide compounds (MIC values, μg/ml)

N-(*R*) succinimides	No.	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
C_6H_5	24	128	128	128	128
$C_6H_5CH_2$	25	512	256	256	256
Cyclohexyl	26	512	256	256	256
n-C_4H_9	27	128	256	256	256
n-C_3H_7	28	512	256	256	256
iso-C_3H_7	29	512	256	256	256
p-C_6H_4OMe	30	512	256	256	256
Gly CO_2Me	31	512	256	256	256

4. Discussion

A lot of bioactive compounds including many antibiotics, are enzyme inhibitors. In the present work concerning novel antibacterial agents, we looked for thiol-reactive compounds, which are often inhibitors of cysteine proteinases or other proteins with an essential cysteine. It is known that maleimides are inhibitors of cysteine proteinases and *N*-ethylmaleimide (NEM) reacts very rapidly with sulfhydryl groups. NEM is currently used for the titration of cysteines in proteins [31]. This compound, which is too reactive, has a poor specificity but was proposed as antimitotic [32]. In the present work we explored the incidence of *N*-substituents of maleimides on their antibacterial activities.

31 compounds were synthesized as possible antibacterial agents. These compounds belong to two families: maleimides (23 compounds) and succinimides (8 compounds). All succinimides (compounds **24**–**31**) showed practically no antibacterial activity towards the different bacteria strains tested. During a previous work, we observed that some succinimides displayed a noticeable antibacterial activity against bacteria isolated from the marine environment. Maleimides (compounds **1**–**23**) showed more or less interesting antibacterial activities. Globally, within all products, and the organisms used for tests, *P. aeruginosa* is clearly the less susceptible organism and all tested compounds are poorly active with MIC values of 128 μg/ml or higher.

The other organisms are much more susceptible and the most active compounds are: **4**, **5**, **6**, **16** and **17**. They are maleimides substituted by a short alkyl side-chain: propyl **5**, isopropyl **6**, butyl **4**, hydroxy-ethyl **16** or hydroxy-propyl **17**. Related compounds, such as benzyl

2, cyclohexyl **3** have quite comparable activities, but aromatic compound **1** is 8–16 times less active than **2** against Gram-positive organisms.

Ten compounds have an aromatic ring directly linked on the imide nitrogen. Surprisingly, two of them are totally inactive (MICs = 512 μg/ml): maleimides **7** and **12** whereas the majority of the other compounds have MIC values ranging from 32 to 64 μg/ml for *S. aureus*, *E. faecalis* and more or less for *E. coli* (e.g. compounds **1**, **9**, **11**, **13**, **14** and **15**). No clear relationship between the nature and position of the substituents of the phenyl (mostly OH, OMe, OAc) and the antibacterial activity could be given.

Then six maleimides are derived from amino acids (compounds **18**–**23**); all were obtained in the form of racemates. Compound **21**, derived from phenylalanine is the most active of them. Its antibacterial activity shows the importance of an aromatic ring, as observed for example with the closely related benzyl compound **2**. Compound **23** is a by-product of synthesis of **18**. The activity of this dehydro alanine is very similar to that of the parent compound serine **18** but is 2–8 times less active that the alanine imide **19**.

Filho et al. [1] gave MIC values for *N*-benzyl maleimide **2**: 20 μg/ml for an *E. coli* strain and 15 μg/ml for a *S. aureus* as for product **13**, respectively, 35 and 25 μg/ml. These values are in good agreement with our results. Watanabe [6] reported that some maleimides are inactive against *P. aeruginosa*, but **1** had a moderate activity against *B. subtilis*, *S. aureus* and *E. coli*.

In the present study we used four strains from the American Typing Culture Collection (ATCC) in order to obtain results which can be reproduced by others. We observed an interesting antibacterial activity for most of maleimides, the lowest MIC values being 8 μg/ml. *Pseudomonas aeruginosa* is the less susceptible organism as it occurs often with other antibiotics. Maleimides are usually considered as non-specific toxic compounds. Nevertheless some closely related compounds, such as chalcones (compound D, Fig. 1) and vinyl sulphones (compound E) are antimalarial compounds under experimental and clinical research. Moreover, this inexpensive family of products could be interesting candidates for the formulation of new antibacterial agents.

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