

## Preliminary communication

# Synthesis and antimicrobial activity of heterocyclic ionone-like derivatives

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**Abstract** – A number of heterocyclic ionone-like derivatives **5** were prepared with appropriate bifunctional reagents by one-pot cyclisation of 3-dimethylamino-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2,4-pentadienal **3a**, which was, in turn, obtained from  $\alpha$ -ionone with N, N-dimethylformamide/phosphorus oxychloride. All compounds **5** possess remarkable activity against the selected Gram-positive, Gram-negative bacterial strains and against *Candida albicans*. Derivatives **5a2** and **5a6**, acting at a high level especially against both *Propionibacterium acnes* and *Staphylococcus aureus*, could play a potential role in the treatment of acne and related skin disorders. © 1999 Éditions scientifiques et médicales Elsevier SAS

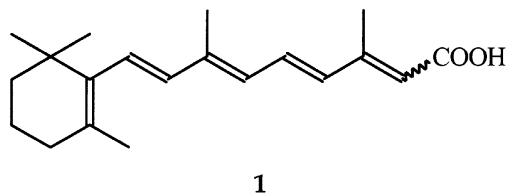
ionone-like derivatives / short retinoids / antimicrobial activity

## 1. Introduction

Since their introduction nearly two decades ago [1], the use of retinoids for topical and systemic treatment of psoriasis and other hyperkeratotic and parakeratotic skin disorders has increased. They are also used for the treatment of severe acne and acne-related dermatoses. Nonetheless, various side effects, the most serious of which being the teratogenicity, coupled with their long elimination half life, have limited their use [2]. Furthermore, while the recovery of *Propionibacterium acnes* and other anaerobic bacteria in the skin is markedly reduced, there is an increase in the colonisation of *Staphylococcus aureus* on the skin and, in general, a significant rise in the incidence of cutaneous staphylococcal infection in patients treated with oral retinoids [3].

While searching for more biologically active and less toxic compounds, the structure of retinoic acid **1** (figure 1) has been modified both in the cyclohexenyl ring and in the polyene side chain.

Major transformations have led to retinoids which barely resemble the original retinoic acid [4, 5]. All these modifications, which lead to the three generations of

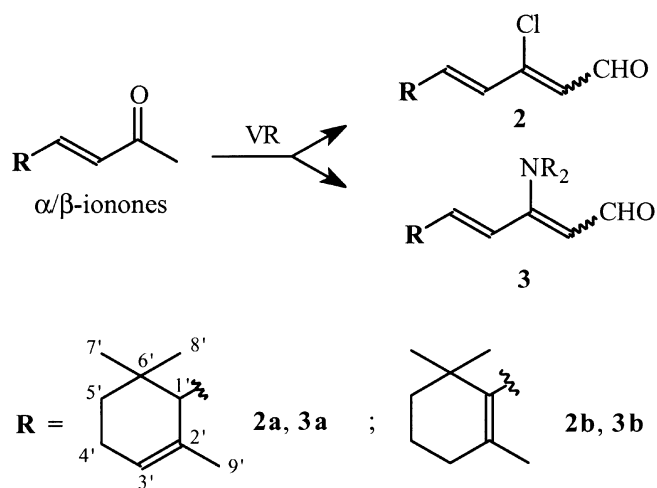


**Figure 1.** The structure of retinoic acid **1**.

retinoids known today (non-aromatic, mono-aromatic and poly-aromatic), are often associated with reduced toxicity while biological activity is maintained or even enhanced. Among these, Etretinate and Acitretin are currently prescribed in systemic use, while Adapalene [4, 5] and Tazarotene [4, 6] have recently been introduced on the market for topical use.

Having a method to synthesise new heterocyclic ionone-like derivatives on hand [7] and because of the similarity of these compounds to the so-called “short heteroretinoids” [8–11], we focused our biological attention both on the reduction of the *P. acnes* and the decrease of staphylococcal infections. In fact, retinoids would be of interest for topical use if they were found to possess

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**Figure 2.** The formation of the classical  $\beta$ -chlorovinyl-aldehydes **2** and the unexpected products **3**.

the following two properties: counteracting both the proliferation of *P. acnes* and of other bacteria (including *S. aureus*) which are responsible for the majority of infections observed.

## 2. Chemistry

Our laboratory recently began to investigate the synthesis of new short retinoid-like derivatives [7]. In fact, while studying the Vilsmeier reaction (VR) on  $\alpha$ - and  $\beta$ -ionones, we outlined both the formation of the classical  $\beta$ -chlorovinylaldehydes **2** and the unexpected products **3** (figure 2), which is unprecedented in a Vilsmeier reaction.

Surprisingly, the chlorine of compounds **2** had difficulty in reacting, while the dialkylamino group of **3** acted as an outstanding leaving group, allowing for the formation of new heterocyclic retinoids. In fact, all attempts to cyclise compounds **2** with bifunctional reagents failed. We were only able to isolate the classical formyl derivatives as the semicarbazones **4a** and **4b**. On the other hand, compound **3a** reacted rapidly with bifunctional reagents such as hydrazine and guanidine. The reaction, which involved both the formyl and the dimethylamino groups gave a new series of heterocyclic ionone-like derivatives.

To complete both the previously reported study and for biological purposes, we have re-synthesised compounds **5a1**, **5a3–a5**, thus improving the yield, and have extended the ring closure on key intermediates **3a** and **3b** by other bifunctional reagents, therefore obtaining new “short heteroretinoids” (figure 3). All reagents used reacted quickly, giving the expected compounds in high

yield and without side products, confirming the particular behaviour of the enamines **3**.

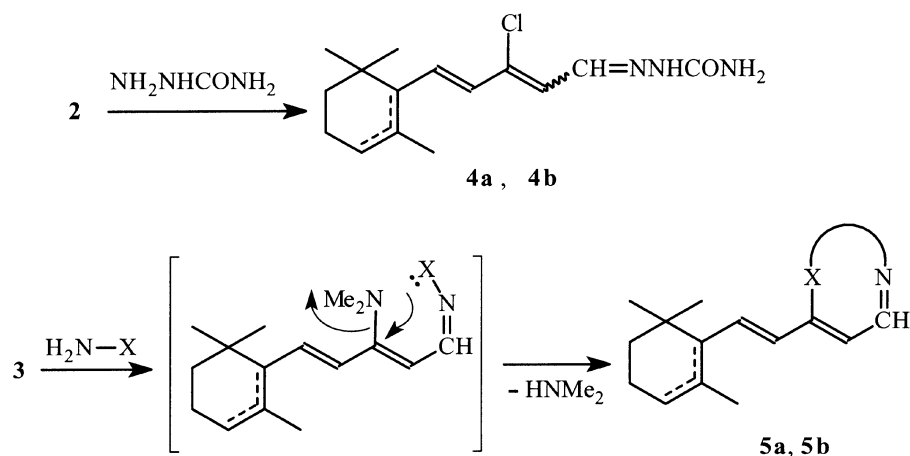
## 3. Results and discussion

Table I summarises the germicidal (GE log values) and killing percentage effects of compounds **5a1–a6**, **5b** and the synthon **3a** against two Gram-positive, two Gram-negative bacterial strains and *Candida albicans* (see Experimental for biological methods). Pyrazole derivatives **5a1–a3** exhibited a good to excellent activity against both *C. albicans* and *P. acnes*. Introduction of a phenyl ring in the position-1 of the pyrazole led to considerable enhancement of the antibacterial activity versus *S. aureus*, while the presence of the 2,4-dinitrophenyl group in the same position decreased the activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *S. aureus* compared to the other two, although the activity against *P. acnes* and *C. albicans* remained almost unchanged. The substitution of one nitrogen with oxygen, which led to the isoxazole derivative **5a4**, decreased almost all the activity against all the microbes but for *P. acnes*. Moreover, in this case the activity was only second to phenyl-pyrazole derivative **5a2**. Introduction of a pyrimidine in place of a pyrazole or an isoxazole ring did not improve the activity against *C. albicans*, *P. aeruginosa* and *E. coli*. On the contrary, they showed a good to excellent level of potency against *S. aureus* and *P. acnes*.

From a general point of view it could be stated that the already notable activity of the key intermediate **3a** versus *C. albicans*, *S. aureus* and *P. acnes*, is enhanced and specifically directed towards one of the three above bacterial strains when a particular heterocyclic moiety is introduced in synthon **3a**: namely pyrazole versus *C. albicans*, phenylpyrazole versus *P. acnes*, methyltiopyrimidine versus *S. aureus*. Moreover, lipophilicity, while not increasing selectivity, enhances activity towards all the species. In fact, the more lipophilic **5a2** and **5a6** present the highest activity towards *P. acnes* and *S. aureus*, respectively, and also possess a good to excellent activity versus the other species.

## 4. Conclusion

From a structural-activity point of view, certain features can be noted. Compounds **5** could be classified as “short-heteroretinoids” [8–11], related to the natural ones through the presence of the cyclohexenyl ring which is a feature of  $\alpha$ - and  $\beta$ -ionones. On the other hand, the potential therapeutic use of these compounds (in partic-



Comp.	5a1	5a2	5a3	5a4	5a5	5a6	5b
Yield (%)	88	85	90	88	85	85	75

<sup>a</sup>DNB = 2,4-dinitrophenyl

**Figure 3.** Synthesis of compounds **4a**, **4b**, **5a** and **5b**.

ular **5a2** and **5a6**) is closer to the new Adapalene and Tazarotene [4–6] than classical retinoids. The latter, in fact, may not only cause *S. aureus* infections when administered orally, but at the same time do not have a significant effect on aerobic and anaerobic bacteria in vitro [3].

Compounds **5a2** and **5a6**, acting both on *S. aureus* and on *P. acnes*, could be considered as candidates for topical treatment of acne and/or a coadjuvant in the oral administration of classical retinoids.

## 5. Experimental protocols

### 5.1. Chemistry

Melting points were determined with Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded in chloroform or in potassium bromide disks on

a Perkin-Elmer 398 spectrometer. The <sup>1</sup>H and <sup>13</sup>C-NMR spectra were recorded on a Bruker AC 300 (300 MHz, <sup>1</sup>H; 75 MHz, <sup>13</sup>C) or a Varian Gemini 200 (200 MHz, <sup>1</sup>H; 50 MHz, <sup>13</sup>C) spectrometers in deuteriochloroform solutions with tetramethylsilane as the internal standard (δ = 0). The purity of all compounds was checked by thin-layer chromatography on silica gel 60-F-254 pre-coated plates and the spots were located in UV light or by vanillin in sulphuric acid. Elemental analyses were performed on a Carlo Erba 1106 Elemental Analyser in the Microanalysis Laboratory in our Institute and the results were within ± 0.4% of theoretical values. For elemental analysis and spectral data of **5a1**, **5a3–a5** see [7].

#### 5.1.1. 5-(2, 6, 6-Trimethyl-2-cyclohexen-1-yl)ethenyl-1H-pyrazole **5a1**

A solution of 0.25 g (1 mmol) of **3a** and 1 mL of hydrazine hydrate (20 mmol) in 10 mL of ethanol was

**Table I.** Germicidal effect (GE) and killing percentage effect (KE %) of “short-retinoids” **5** and **3a** against the five species mentioned.

Compound		<i>C. albicans</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. acnes</i>	mean GE $\pm$ DS
<b>3a</b>	GE	2.447	0.286	0.456	2.058	2.041	1.46 $\pm$ 1.01
	KE %	99.64	48.18	65.00	99.13	99.09	
<b>5a1</b>	GE	3.56	0.26	0.56	0.98	2.35	1.54 $\pm$ 1.38
	KE %	99.97	45.46	72.50	89.41	99.55	
<b>5a2</b>	GE	2.05	1.16	1.50	1.94	4.91	2.31 $\pm$ 1.19
	KE %	99.11	93.13	96.87	98.85	99.99	
<b>5a3</b>	GE	2.263	0.176	0.301	0.503	2.981	1.25 $\pm$ 1.28
	KE %	99.46	33.33	50.00	68.57	99.90	
<b>5a4</b>	GE	1.21	0.36	0.60	1.38	3.01	1.31 $\pm$ 1.04
	KE %	93.90	53.67	75.00	95.79	99.90	
<b>5a5</b>	GE	0.75	0.30	0.58	1.93	2.01	1.12 $\pm$ 0.80
	KE %	82.31	50.00	73.91	98.82	99.03	
<b>5a6</b>	GE	1.83	1.15	1.29	3.02	2.17	1.89 $\pm$ 0.75
	KE %	98.52	92.94	94.82	99.91	99.33	
<b>5b</b>	GE	0.75	0.27	0.58	1.90	2.06	1.11 $\pm$ 0.81
	KE %	82.50	45.45	74.40	97.42	99.13	
	mean %	94.43	57.77	75.31	93.49	99.49	
	DS	7.67	22.54	15.13	10.61	0.4	

allowed to stand at  $-10^{\circ}\text{C}$  for 30 min and 1 h at room temperature. The resulting oil, purified by chromatography on silica gel (toluene/ethyl acetate 1:1), gave **5a1** in 88% yield as a thick oil.

#### 5.1.2. 1-Phenyl-5-[(2,6,6-trimethyl-2-cyclohexen-1-yl)ethenyl]-1H-pyrazole **5a2**

A solution of 0.4 g (1.6 mmol) of **3a** and phenylhydrazine hydrochloride [0.5 g (3.2 mmol) in 5 mL of water and 0.8 g of sodium acetate trihydrate] in 10 mL of ethanol was refluxed for 15 min and allowed to stand at room temperature for 1 d. The resulting oil was extracted with chloroform. The combined extracts were dried (sodium sulphate) and evaporated to afford a red oil which was purified by chromatography on silica gel (toluene/ethyl acetate 1:1), giving **5a2** in 85% yield as a thick oil; IR (film):  $\nu$  3 100, 2 920, 1 670, 1 600, 1 500, 1 450, 1 390  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (200 MHz):  $\delta$  0.88 (3H, s,  $\text{CH}_3$ ), 0.92 (3H, s,  $\text{CH}_3$ ), 1.20 (1H, m, H-5'), 1.45 (1H, m, H-5'), 1.60 (3H, s,  $\text{CH}_3$ ), 2.03 (2H, m, H-4'), 2.25 (1H, d, H-1'  $J$  8.98 Hz), 5.45 (1H, m, H-3'), 6.07 (1H, dd, ethene,  $J$  8.98; 15.76 Hz), 6.20 (1H, d, ethene,  $J$  15.76 Hz), 6.47 (1H, d, H-3,  $J$  1.98 Hz), 7.47 (5H, m, arom-H) 7.61 (1H, d, H-4,  $J$  1.98 Hz);  $^{13}\text{C-NMR}$  (50 MHz,  $\text{CDCl}_3$ ) 23.44 ( $\text{CH}_3$ ), 23.53 ( $\text{CH}_2$ ), 27.39 ( $\text{CH}_3$ ), 28.23 ( $\text{CH}_3$ ), 32.00 ( $\text{CH}_2$ ), 33.11 (C), 55.35 (CH), 104.37 (CH), 119.44 (CH), 122.17 (CH), 125.72 (CH), 125.72 (CH), 128.17 (CH), 129.50 (CH), 129.50 (CH), 133.69 (C), 136.62 (CH), 140.17 (C), 140.56 (CH), 141.71 (C). Anal.  $\text{C}_{20}\text{H}_{24}\text{N}_2$  (C, H, N).

#### 5.1.3. 1-(2,4-Dinitrophenyl)-5-[(2,6,6-trimethyl-2-cyclohexen-1-yl)ethenyl]-1H-pyrazole **5a3**

A solution of 0.25 g (1 mmol) of **3a** and 2,4-dinitrophenylhydrazine [0.2 g (1 mmol) in ethanol/ $\text{H}_2\text{SO}_4$ ] in 10 mL of ethanol was allowed to stand at  $-10^{\circ}\text{C}$  for 30 min and 2 h at room temperature. The precipitated solid was collected by filtration and washed with ethanol, yielding **5a3** as already pure yellow crystals (90% yield, m.p. 116–117  $^{\circ}\text{C}$  from ethanol).

#### 5.1.4. 5-(2,6,6-Trimethyl-2-cyclohexen-1-yl)ethenyl-isoxazole **5a4**

A solution of 0.4 g (1.6 mmol) of **3a** and hydroxylamine hydrochloride [0.5 g (7.0 mmol) in 3 mL of water] in 30 mL of ethanol and 2 mL of a 10% water solution of sodium hydroxide was allowed to stand at room temperature for 3 d. The resulting oil, purified by chromatography on silica gel (toluene/ethyl acetate 1:1), gave **5a4** in 88% yield as a thick oil.

#### 5.1.5. 6-(2,6,6-Trimethyl-2-cyclohexen-1-yl)ethenyl-2-aminopyrimidine **5a5**

A solution of 0.5 g (2.0 mmol) of **3a** and guanidine carbonate [0.36 g (2.0 mmol) in 3 mL of water] in 30 mL of ethanol was refluxed for 48 h. The resulting oil was extracted with chloroform. The combined extracts were dried (sodium sulphate) and evaporated to afford an oil which was purified by chromatography on silica gel (toluene/ethyl acetate 1:1), giving **5a5** in 85% yield as a thick oil.

#### 5.1.6. 2-Methylthio-4-[(2,6,6-trimethyl-2-cyclohexen-1-yl)ethenyl]-pyrimidine **5a6**

To the solution of 0.5 g (2.0 mmol) of **3a** in 10 mL of ethanol, 0.55 g (2.0 mmol) of S-methylisourea hydrogen sulphate dissolved in 2 mL of sodium hydroxide 2 N and 10 mL of water was added and the mixture refluxed for 48 h. The resulting oil was extracted with chloroform. The combined extracts were dried (sodium sulphate) and evaporated to afford an oil which was purified by chromatography on silica gel (toluene/ethyl acetate 1:1), giving **5a6** in 85% yield as a thick yellow oil; IR (film):  $\nu$  3 100, 2 900, 2 860, 1 680, 1 560, 1 450, 1 375, 1 200  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (200 MHz):  $\delta$  0.85 (3H, s,  $\text{CH}_3$ ), 0.92 (3H, s,  $\text{CH}_3$ ), 1.24 (1H, m, H-5'), 1.44 (1H, m, H-5'), 1.59 (3H, s,  $\text{CH}_3$ ), 2.04 (2H, m, H-4'), 2.27 (1H, d, H-1'  $J$  8.98 Hz), 2.29 (3H, s,  $\text{SCH}_3$ ), 5.47 (1H, m, H-3'), 6.19 (1H, d, ethene,  $J$  8.65 Hz), 6.59 (1H, d, H-4,  $J$  5.30 Hz), 6.70 (1H, dd, ethene,  $J$  15.15, 8.65 Hz), 8.15 (1H, d, H-5,  $J$  5.30 Hz);  $^{13}\text{C-NMR}$  (50 MHz) 18.95 ( $\text{CH}_3\text{S}$ ), 23.41 ( $\text{CH}_3$ ), 23.56 ( $\text{CH}_2$ ), 27.81 ( $\text{CH}_3$ ), 28.35 ( $\text{CH}_3$ ), 31.78 ( $\text{CH}_2$ ), 33.11 (C), 55.18 (CH), 108.82 (CH), 122.53 (CH), 130.17 (CH), 133.27 (C), 141.89 (CH), 158.34 (CH), 164.408 (C), 164.55 (C). Anal.  $\text{C}_{16}\text{H}_{22}\text{N}_2\text{S}$  (C, H, N, S).

#### 5.1.7. 6-(2,6,6-Trimethyl-1-cyclohexen-1-yl)ethenyl-2-aminopyrimidine **5b**

By use of the procedure in 5.1.5, compound **5b** was prepared from **3b**. The product was finally separated by chromatography on silica gel in 70% yield as a thick oil; IR (film):  $\nu$  3 300, 3 180, 2 900, 1 680, 1 610, 1 550, 1 440  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (200 MHz):  $\delta$  1.02 (6H, s,  $\text{CH}_3$ ), 1.48 (2H, m, H-5'), 1.60 (2H, m, H-4'), 1.74 (3H, s,  $\text{CH}_3$ ), 2.02 (2H, t, H-3'), 5.19 (2H, s,  $\text{NH}_2$ ), 5.92 (1H, d, ethene,  $J$  16.28 Hz), 6.48 (1H, dd, ethene,  $J$  16.28 Hz), 6.58 (1H, d, H-4,  $J$  5.26 Hz), 8.19 (1H, d, H-5,  $J$  5.26). Anal.  $\text{C}_{15}\text{H}_{21}\text{N}_3$  (C, H, N).

#### 5.1.8. Semicarbazone **4a**

An ethanolic solution of 0.5 g of 3-chloro-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2,4-pentadienal **2a**, 0.7 g of sodium acetate trihydrate and 0.5 g of semicarbazide hydrochloride was heated at 90 °C with stirring. After cooling, the precipitate obtained was filtered off and crystallised from ethanol. The yellow crystals formed were pure **4a**, (35% yield; m.p. 209–210 °C from ethanol). IR (KBr):  $\nu$  3 480, 3 140, 2 900, 1 700, 1 580, 1 420, 1 160  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (200 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  0.80 (3H, s,  $\text{CH}_3$ ), 0.90 (3H, s,  $\text{CH}_3$ ), 1.20 (1H, m, H-5'), 1.40 (1H, m, H-5'), 1.55 (3H, s,  $\text{CH}_3$ ), 2.02 (2H, m, H-4'), 2.30 (1H, d, H-1',  $J$  9.06 Hz), 5.48 (1H, m, H-3'), 5.95 (1H, dd, ethene,  $J$  9.06 and  $J$  9.30 Hz), 6.35 (2H, s,  $\text{NH}_2$ ), 6.38 (1H, d, ethene,  $J$  9.30 Hz), 6.50 (1H, d,  $\text{CH-CH=N}$ ,

$J$  10.66 Hz), 7.92 (1H, d,  $\text{CH-CH=N}$ ,  $J$  10.66 Hz), 10.45 (1H, s, NH); Anal.  $\text{C}_{15}\text{H}_{22}\text{N}_3\text{OCl}$  (C, H, N, Cl).

#### 5.1.9. Semicarbazone **4b**

By use of the above procedure, semicarbazone **4b** was obtained in 38% yield as yellow powder from **2b**, m.p. 196–198 °C from ethanol. IR (KBr):  $\nu$  3 480, 3 120, 2 850, 1 690, 1 580, 1 420, 1 160  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (200 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  1.05 (6H, s,  $\text{CH}_3$ ), 1.45 (2H, m, H-5'), 1.55 (2H, m, H-4'), 1.70 (3H, s,  $\text{CH}_3$ ), 2.05 (2H, m, H-3'), 6.35 (2H, s,  $\text{NH}_2$ ), 6.45 (1H, d, ethene,  $J$  15.78 Hz), 6.60 (1H, d,  $\text{CH-CH=N}$ ,  $J$  10.52 Hz), 6.65 (1H, d, ethene,  $J$  15.78 Hz), 7.98 (1H, d,  $\text{CH-CH=N}$ ,  $J$  10.52 Hz), 10.48 (1H, s, NH); Anal.  $\text{C}_{15}\text{H}_{22}\text{N}_3\text{OCl}$  (C, H, N, Cl).

### 5.2. Biological methods

The micro-organisms used in this study were *Pseudomonas aeruginosa* ATCC 9027 ( $16 \times 10^7$  CFU/mL), *Escherichia coli* ATCC 8739 ( $11 \times 10^7$  CFU/mL), *Staphylococcus aureus* ATCC 6538 ( $40 \times 10^7$  CFU/mL), *Candida albicans* ATCC 10231 ( $77 \times 10^7$  CFU/mL), *Propionibacterium acnes* ATCC 11827 ( $1.54 \times 10^7$  CFU/mL).

The microbicidal activity has been determined by a "Reybrouck in vitro test" [12, 13] which was carried out using 5 min of medication time at  $25 \pm 1$  °C and 30 min of contact time with LPHT inactivator (0.3% lecithin, 3% polysorbate 80, 0.1% histidine, 0.5% sodium thiosulfate).

The bacterial strains were obtained by the 4th subculture at  $37 \pm 1$  °C/48h on TSA slants from freeze-dried stock cultures.

Bacterial suspension was obtained in TSB after an incubation time of 24 h at  $37 \pm 1$  °C followed by centrifugation at 2 000 g for 15 min and resuspension in tryptone dilution water (TDW).

The number of viable organisms in the inoculum were determined by the plating technique, mixing 1.0 mL samples from the dilutions  $10^{-6}$ ,  $10^{-7}$  and  $10^{-8}$  with 20 mL of TSA, melted and tempered to 45 °C. All inoculations were carried out in triplicate.

The test was carried out in a water bath at  $25 \pm 1$  °C. At time zero, 0.1 mL volumes of the different bacterial suspensions were added to 10 mL of the retinoid solution to be tested. After 5 min, 1 mL volumes were transferred from the medication mixtures into 9 mL of inactivator solution (LPHT). After a contact time of 30 min, 0.1 mL portions of the undiluted inactivated mixture and of the  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  dilutions in TDW were spread in triplicate on plates containing 20 mL of TSA. The colony forming units were counted after incubation at  $37 \pm 1$  °C for 24 h.



For the negative controls, which were submitted to the same medication procedure, distilled water was used instead of the retinoid derivatives.

The germicidal activity is numerically expressed using the Germicidal Effect (GE, the decimal reduction time), meaning the ratio, expressed as logarithm, between the number of colony forming units (CFU) per mL in the control mixture without the tested retinoid (Nc) to the number of CFU after the exposure to the retinoids (Ns) or  $GE = \log N_c - \log N_s$ . The corresponding killing effect percentage is obtained by the following mathematical expression:

$$KE \% = \frac{N_c - N_s}{N_c} \cdot 100$$

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