Full Paper

Synthesis, Antibacterial and Antifungal Activities of Bifonazole Derivatives

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Two series of chlorinated benzhydryl imidazole and triazole derivatives were synthesized and tested *in vitro* against representative strains of potent pathogenic bacteria (*Staphylococcus aureus* CIP 4.83, *Escherichia hirae* CIP 5855, *Pseudomonas aeruginosa* CIP 82118, *Escherichia coli* CIP 53126) and fungi (*Aspergillus niger* IP 1431.83, *Candida albicans* IP 48.72, *Candida krusei* IP 208.52, *Trichophython rubrum* IP 1657.86). Most of these compounds were devoid of any antimicrobial activity, but several of them inhibited *T. rubrum* with MIC values in the range of 0.125 to 32 μg/mL, similar or superior to those of bifonazole and clotrimazole, used as standard controls. The replacement of the imidazole ring with a triazole moiety in these compounds led to derivatives with less antifungal activity. A preliminary SAR was undertaken on the effect of the number and the position of chlorine atoms on the distribution of negative charge on the surface of some compounds on antifungal activity.

Keywords: Antibacterial activity / Antifungal activity / Imidazole derivatives / Triazole derivatives

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Introduction

In the recent years, the widespread use of antifungal and antibacterial agents has resulted in the development of multi-drug resistant pathogens causing an increase in morbidity and mortality, particularly in immuno-compromised hosts [1]. The class of azoles (imidazole and triazole derivatives) has given rise to many effective antifungal drugs that are currently in clinical use. The mechanism of their antifungal action includes the inhibition of cytochrome $P450_{14DM}$ which is essential for ergosterol biosynthesis at the step of lanosterol 14-demethylation [2, 3]. The understanding of their therapeutic target and the identification of the pharmacophore responsible for their activity has led to the development of new promising antifungal molecules. Since the discovery of bifonazole, used in the therapy of various dermatomycoses, many modifications to the structure of azoles have been carried out, with the goal of increasing the antifungal potency and selectivity and improving the bio-availability.

Bifonazole is derived from the structure of clotrimazole but contains no halogen atoms (Fig. 1). Fluorine or chlorine atoms are present as common substituents on the aromatic rings of the next generation of the most potent antifungal azoles (imidazole or triazole derivatives) such as clotrimazole, fluconazole, voriconazole, ketoconazole. Different studies [3, 4] have shown that the introduction of halosubstituents on the phenyl rings linked to the pyrazole nucleus of bifonazole analogs produced modifications in the microbiological profile. In particular, derivatives bearing 2 or 3 chlorine atoms emerged as the most interesting antifungal or antibacterial compounds. In parallel, it was found that triazole compounds showed better antimicrobial activity and lower toxicity than imidazole derivatives [5]. In order to improve the antifungal and antibacterial activities of bifonazole, we have carried out various structural modifications such as the introduction of chlorine atoms on the aromatic rings and the replacement of the imidazole ring by a triazole nucleus.

In this paper, we present the synthesis and the *in-vitro* biological evaluation of some halogenated bifonazole derivatives in order to investigate their preliminary structure–activity relationships (SARs).

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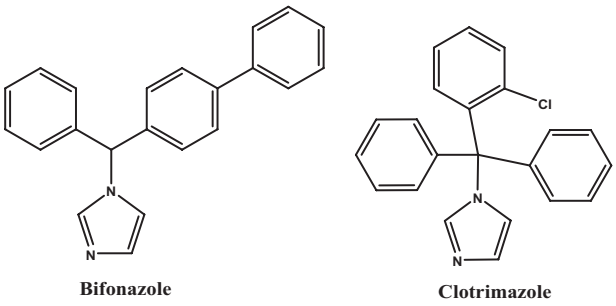


Figure 1. Structures of bifonazole and clotrimazole.

Results and discussion

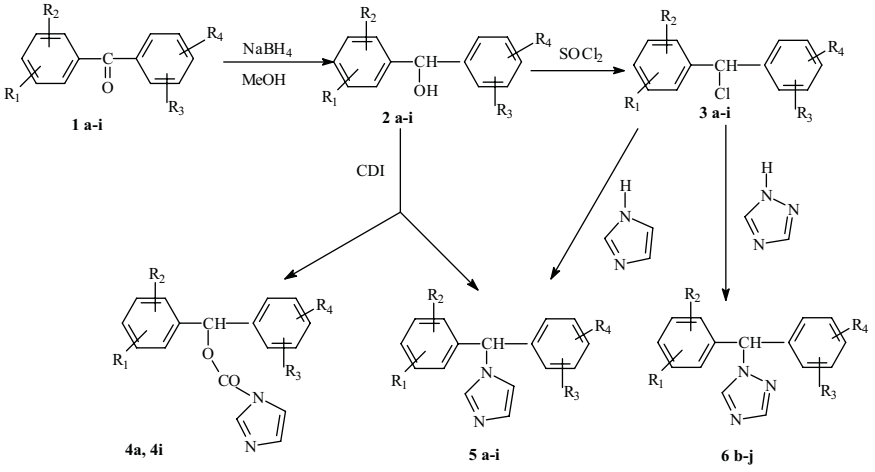
Chemistry

The synthesis of carbamates **4a**, **4i**, imidazoles **5a–i** and triazoles **6b–j** derivatives is depicted in Scheme 1.

Non-commercially available carbinols **2b**, **2c**, **2e**, **2f**, **2h–j** were obtained by sodium borohydride reduction of commercially accessible ketones (**1b**, **1c**, **1e**, **1f**, **1j**), or ketones synthesized in our laboratory (**1h**, **1i**) by the reaction of benzoyl chloride with halogenobenzene in the presence of aluminium trichloride. Usually imidazole and triazole derivatives were synthesized directly from alcohols with 1,1′-carbonyldiimidazole (CDI) under mild conditions [6]. However, as it has been reported in the literature [7, 8], the treatment of compounds **2a** and **2i** led to the corresponding carbamates **4a** and **4i**, but the subsequent decarboxylation by heating in refluxing toluene, gave *N*-imidazole derivatives **5a** and **5i** in very low yields. Therefore, in order to obtain the required imidazole and triazole derivatives, we followed another route: treatment of carbinols with $SOCl_2$ and successive reactions of crude chloro derivatives with 1,3-imidazole or 1,2,4-triazole in the presence of triethylamine.

Biological activities

The prepared compounds **5–6** were evaluated *in vitro* for their antimicrobial activities against representative Gram-positive and Gram-negative bacterial strains (*S. aureus* CIP 4.83, *E. hirae* CIP 5855, *P. aeruginosa* CIP 82118, *E. coli* CIP 53126) and fungal species (*C. albicans* IP 48.72, *A. niger* IP 1431.83, *C. krusei* IP 208.52, *T. rubrum* IP 1657.86). The determined minimal



	R_1	R_2	R_3	R_4
a	H	H	H	H
b	2-Cl	H	H	H
c	3-Cl	H	H	H
d	4-Cl	H	H	H
e	2-Cl	4-Cl	H	H
f	3-Cl	4-Cl	H	H
g	4-Cl	H	4-Cl	H
h	2-Cl	4-Cl	4-Cl	H
i	2-Cl	4-Cl	2-Cl	4-Cl
j	H	H	H	C_6H_5

Scheme 1. Schematic diagram showing the synthesis of compounds **4**, **5** and **6**.

Table 1. *In-vitro* antibacterial activity of compounds **5a–i** and **6b–j** (MIC and MBC in μg/mL).

Compounds	Microorganisms							
	***S. aureus* CIP 4.83**		***E. hirae* CIP 5855**		***P. aeruginosa* CIP 82118**		***E. coli* CIP 53126**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
5a	32	64	256	512	256	512	512	512
5b	256	512	256	512	256	512	512	512
5c	128	512	256	512	256	512	512	512
5d	256	512	256	512	256	512	512	512
5e	256	512	256	512	256	512	512	512
5f	16	256	256	512	256	512	256	512
5g	256	512	256	512	512	512	512	512
5h	16	64	16	16	256	512	256	512
5i	256	512	512	512	256	512	512	512
6b	512	512	256	256	128	256	256	256
6c	512	512	512	512	256	512	512	512
6d	512	512	512	512	256	512	512	512
6e	512	512	512	512	256	512	512	512
6f	512	512	512	512	256	512	512	512
6g	256	256	256	256	128	256	256	256
6h	512	512	512	512	256	512	512	512
6i	256	256	256	256	128	256	256	256
6j	128	128	128	128	64	128	128	128
Ciprofloxacin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

Table 2. *In-vitro* antifungal activity of compounds **5a–i** and **6b–j** (MIC and MFC in μg/mL).

Compounds	Microorganisms							
	***A. niger* IP 1431.83**		***C. albicans* IP 208.52**		***C. krusei* IP 1657.86**		***T. rubrum***	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
5a	16	–	32	32	64	128	2	32
5b	4	–	32	64	32	256	0.5	8
5c	32	–	16	64	32	64	2	8
5d	128	–	32	128	64	256	8	32
5e	16	–	128	256	32	64	0.125	16
5f	128	–	128	256	128	256	4	16
5g	512	–	16	128	256	256	16	128
5h	128	–	256	256	16	32	4	16
5i	512	–	256	256	128	256	32	128
6b	128	–	128	128	128	256	8	64
6c	512	–	128	256	256	512	128	256
6d	256	–	128	256	256	512	64	256
6e	512	–	256	256	256	256	16	128
6f	512	–	256	256	256	256	256	512
6g	256	–	128	128	128	256	128	256
6h	256	–	256	256	256	256	256	256
6i	256	–	128	256	128	256	128	128
6j	128	–	64	64	64	64	64	128
Bifonazole	4	–	128	256	128	128	4	8
Clotrimazole	1	–	4	8	0.125	0.5	0.25	8

inhibitory concentrations (MIC) and minimal germicidal concentrations (MBC or MFC) are reported in Tables 1 and 2. Bifonazole, clotrimazole and ciprofloxacin were used as reference drugs in the antifungal and antibacterial assays.

Antibacterial activity

Most of the tested compounds were devoid of any antibacterial activity. Only compounds **5a**, **5f** and **5h** showed weak inhibition against *S. aureus* (MIC/MBC: 32/64 μg/mL,

16/256 μg/mL and 16/64 μg/mL, respectively) and compound **5h** against *E. hirae* (MIC/MBC: 16/16 μg/mL).

Antifungal activity

All synthesized imidazole compounds **5** showed inhibitory effects against *T. rubrum*, with MIC in the range of 0.125 to 32 μg/mL, comparable to those of bifonazole and clotrimazole. Compound **5b**, containing one chlorine atom at the 2-position of the phenyl ring, and compound **5e**, bearing two chorine atoms on the same benzyl ring at the 2- and 4-positions, emerged as the most potent derivatives with an MIC/MFC of 0.5/8 μg/mL and 0.125/16 μg/mL, respectively. Other compounds turned out to be weakly effective against the tested yeast species and only compound **5b** showed similar activity to that of bifonazole against *A. niger* (MIC 4 μg/mL). The MIC values (MIC/MFC: 16/64 μg/mL and 16/128 μg/mL respectively) for compounds **5c** and **5g** against *C. albicans*, and the MIC/MFC: 16/32 μg/mL of compound **5h** against *C. krusei*, demonstrated their antifungal activity superior to that of bifonazole towards these fungi.

Imidazole derivatives **5** had higher antifungal activity than the triazoles **6** towards the different fungal strains tested. Only compounds **6b** and **6e** showed weak inhibitor activity against *T. rubrum* (MIC/MFC: 8/64 μg/mL and 16/128 μg/mL, respectively).

The replacement of the imidazole moiety of bifonazole with a triazole ring (compound **6j**) led to a significant decrease in the antifungal activity against *T. rubrum*, the MIC/MFC was 4/8 μg/mL for bifonazole and 64/128 μg/mL for compound **6j**.

Thus, the results of the microbiological assays showed that the presence of the imidazole ring, and the number of chlorine atoms and their position on the aromatic ring, had a notable effect on the antifungal activity. As already observed [3, 4] the number and the position of chloro substituents on phenyl rings have emerged as determinant factors for antifungal activities, but we did not find the beneficial effect of the triazole ring as described in the literature [5].

Structure–activity relationship

We were interested in assigning the appropriate molecular parameters required for the optimal binding of azole antifungal compounds to the active site of cytochrome P450 and their ability to be efficient antifungals. The introduction of chlorine atoms on the phenyl ring linked to the azole ring led to modifications in the physico-chemical properties and consequently these parameters can affect the affinity of molecules for the iron of the heme binding site.

Therefore in this study, parallel to the microbiological assays, we calculated the following molecular properties to be correlated with the biological activity: log*P*, molecular volume and superficial area, partial atomic charges (electrostatic potential), and the energy of the HOMO and LUMO frontier orbitals (ionization potential, hardness, polarizability). The calculated values are listed in Table 3.

Geometry of compounds

Our molecules show limited flexibility due to the presence of two aromatic rings near to the azole moiety. Moreover, the presence of chlorine atoms on the aromatic rings can decrease their ability to rotate. Consequently, the phenyl rings are disposed in perpendicular planes and only the azole ring, linked by a single bond to the aromatic rings, can rotate around 180°. The difference in the heat of formation for these two conformers is inferior to 1 kJ/mol for compounds **5**, except for compounds **5e** and **5h** with a difference of about 2 kJ/mol. These conformers could instead easily convert one into the other.

logP, molecular volume and surface

These data have become a key parameter in studies of ADMET properties. The lipophilic character is described by log P. The range of the calculated values of log P of the imidazole **5** and triazole **6** derivatives was between 3.08 and 5.94; these values were similar to those of bifonazole and clotrimazole. We observed a slight rise in the lipophilicity with the increasing number of chlorine atoms on the aromatic rings.

Small molecules exhibit faster diffusion rates through the cell membrane than the large ones [9]. The size of molecules may play a key role in their entry into cells. The azole derivatives **5** and **6** are small molecules, with surfaces between 264 and 345 $Å^2$ and volumes between 298 and 387 $Å^3$, inferior to that of bifonazole and clotrimazole, and therefore may interact with the active site of the target without steric hinderance.

Molecular electrostatic potential

The results of molecular modeling showed (Fig. 2) several areas of negative potential situated on the nitrogen atom of the azole ring and on the aromatic ring bearing chlorine atoms. The negative charge on the nitrogen N(3) of the imidazole derivatives **5** dropped from −0.31 to −0.29, increasing with the number of chlorine atoms on the phenyl rings; these values are close to that of bifonazole (−0.303) and clotrimazole (−0.296). For the triazole derivatives **6**, that showed no antifungal activity, the negative charge of the triazole ring is shared between the two nitrogen atoms N(2) and N(4). The negative charge of N(2) is about −0.25 and of N(4) −0.31, greater than that of the nitrogen N(3) of the imidazole ring. It is possible that these electronic characteristics of azole compounds are not suitable for antifungal activity, as demonstrated by the lower efficacy of the triazole derivatives in comparison with the imidazole derivatives.

Table 3. Calculated molecular properties of compounds **5a–i** and **6b–i.**

N°	Number/Position of chlorine		MW	Volume (Å^3) / Area (Å^2)	HOMO / LUMO (eV)	Hardness (η) (LUMO−HOMO)/2	Charge azole*	log*P*
5a	0		234.3	298.4 / 264.4	−9.260 / −0.254	4.503	−0.3037	3.08
5b	1	2	268.8	320.1 / 274.8	−9.336 / −0.465	4.436	−0.3026	3.68
5c	1	3	268.8	324.2 / 283.8	−9.392 / −0.478	4.457	−0.3015	3.68
5d	1	4	268.8	324.9 / 283.7	−9.390 / −0.500	4.445	−0.3014	3.68
5e	2	2,4	303.2	346.9 / 294.1	−9.368 / −0.918	4.225	−0.2965	4.29
5f	2	3,4	303.2	348.4 / 300.2	−9.477 / −0.761	4.358	−0.3000	4.15
5g	2	4,4′	303.2	349.5 / 302.7	−9.501 / −0.744	4.379	−0.2976	4.27
5h	3	2,4,4′	337.6	372.5 / 313.6	−9.492 / −1.030	4.231	−0.2942	4.88
5i	4	2,2′,4,4′	372.1	386.7 / 319.1	−9.559 / −1.019	4.270	−0.2933	5.94
6b	1	2	269.7	315.2 / 270.9	−9.801 / −0.480	4.661	−0.2403 / −0.3250	3.64
6c	1	3	269.7	317.4 / 285.7	−9.699 / −0.560	4.555	−0.2494 / −0.3133	3.64
6d	1	4	269.7	318.3 / 285.4	−9.642 / −0.563	4.540	−0.2483 / −0.3137	3.64
6e	2	2,4	304.2	341.4 / 298.4	−9.814 / −0.848	4.483	−0.2522 / −0.3148	4.24
6f	2	3,4	304.2	341.0 / 302.1	−9.628 / −0.798	4.415	−0.2497 / −0.3123	4.11
6g	2	4,4′	304.2	341.5 / 302.5	−9.697 / −0.766	4.466	−0.2521 / −0.3116	4.23
6h	3	2,4,4′	338.6	364.1 / 315.1	−9.736 / −0.968	4.384	−0.2546 / −0.3113	4.84
6i	4	2,2′,4,4′	373.1	382.5 / 316.4	−9.883 / −1.105	4.389	−0.2419 / −0.3217	5.45
6j	0		311.4	386.5 / 345.2	−9.279 / −0.395	4.442	−0.2475 / −0.3155	4.80
Bifonazole		0	311.4	393.1 / 343.2	−9.263 / −0.373	4.445	−0.3034	4.80
Clotrimazole		1	345.8	406.1 / 320.9	−9.227 / −0.395	4.416	−0.2958	5.44

* Charge on: N(3) for compounds **5**; N(2)/N(4) for compounds **6.**

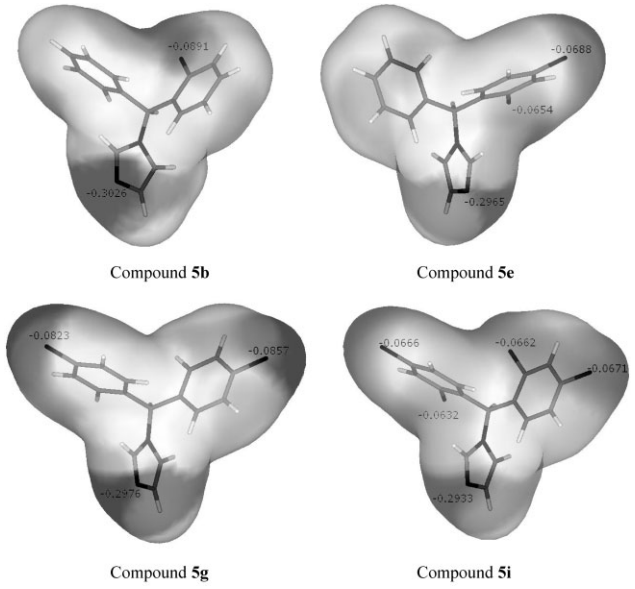


Figure 2. Molecular electrostatic potential of compounds **5b**, **5e**, **5g** and **5i.** Imidazole derivatives shown as sticks colored by element (carbon: grey ; hydrogen: white ; nitrogen and chlorine: black) with the molecular surface colored according to the distribution of the electrostatic potential (black: negative potential, white: positive potential). Images were generated using Accelrys DS visualizer 2.0, with Mopac 2009 molecular modeling data.

The negative charge of aromatic rings arises at the site of the chlorine atoms. The chlorine atoms at position 2 of the most active compounds **5b** and **5e** induce a zone of negative potential in the central part of the molecule (Fig. 2), while the chlorine at the 4-position causes the dispersion of negative charge at the extremity of the phenyl ring, as in the less active compounds **5g** and **5i** (Fig. 2). We observed a similar distribution of electrostatic potential on the surface of triazole **6**. Among the triazole derivatives, only compounds **6b** and **6e** (2- and 2,4-chloro derivatives) showed some inhibitory effect. Therefore, an appropriate electrostatic potential distribution seems indispensable for antifungal activity.

Energies of the frontier molecular orbitals (E_{LUMO} and E_{HOMO})

Chemical hardness is closely related to the polarizability that can measure the ability of a ligand to participate in covalent bond formation with iron at the enzyme binding site. This parameter can be expressed using the energies of the frontier molecular orbitals:

$$\eta = (E_{\mathrm{LUMO}} - E_{\mathrm{HOMO}})/2 \quad (1)$$

The easily polarizable molecules (soft molecules) have a small HOMO–LUMO gap, that corresponds to small excitation energies to the manifold of excited states. Consequently, the hard molecules have a large HOMO–LUMO gap.

We observed that the hardness of azole derivatives decreased slightly with the increasing number of chlorine atoms linked to the aromatic rings. These values are very

close for all the compounds but the most active compound **5e** had the lowest hardness (4.225) whereas the triazole derivatives had the highest. Consequently, this parameter is not a discriminating factor for biological activity.

Conclusion

Our goal was the synthesis and evaluation of the antibacterial and antifungal activities of relative small and structurally simple compounds that were derivatives of bifonazole. We describe the synthesis of eighteen imidazole and triazole derivatives with the majority of them being new compounds. These compounds had no antibacterial activity, like bifonazole and clotrimazole, against strains representative of potent pathogenic Gram negative and Gram positive bacteria. However, several of these new compounds (particularly **5b** and **5e**) may be considered as highly potential antifungal agents against *T. rubrum*.

With the aim of investigating the effect of physico-chemical properties on antifungal activity, the correlation of antifungal activity against *T. rubrum* (log MIC) and different calculated descriptors (electronic, physico-chemical, topologic) was evaluated by using the multiple linear regression (MLR) method (using XLStatistics software [10]) but no statistically significant relationship between the experimental activity and any of the calculated properties was found (supplementary material S1). However there was a correlation found between the distribution of negative charge on the molecules and antifungal activity.

Experimental

Chemistry

Melting points were determined on a DSC-50 Shimadzu apparatus (Kyoto, Japan). Infra-red spectra were recorded on a Perkin-Elmer Spectrum One FT IR spectrometer (Perkin-Elmer, USA). ^{1}H- and ^{13}C-NMR spectra were obtained in $CDCl_3$ on a DPX 300 spectrometer (Brüker Biosciences, USA), and peak positions are given as s (singlet), d (doublet), t (triplet), q (quadruplet) or m (multiplet). Chemical shift (δ) values are given in parts per million. Reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica gel plates 60 F-254 and product mixtures were purified by column chromatography using silica gel 70–200 mesh (SDS, France). All yields are calculated for analytical pure materials. The microanalyses were performed in the Microanalytical Laboratory of ENSIACET in Toulouse, France, and the results obtained were within ±0.4% of the theoretical values. Commercially available compounds **1b**, **1c**, **1e**, **1f**, 1**j**, **2a**, **3a**, **3d**, **3g** were purchased from Acros Organics (Halluin, France).

*General procedure for the preparation of compounds **1h** and **1i***

Compounds **1h** [11] and **1i** [12] were prepared as previously described and their physico-chemical data are in agreement with the literature data.

*General procedure for the preparation of compounds **2b**, **2c**, **2e**, **2f**, **2h–j***

A solution of ketone (20 mmol) in methanol (200 mL) was treated with sodium borohydride (5.5 mmol) and stirred at room temperature, until the disappearance of the ketone, and then evaporated under reduced pressure. The crude pale yellow oil was dissolved in water (100 mL), extracted with ether (3 × 50 mL), dried over Na_2SO_4 and evaporated. The resulting colorless oil solidified to give a crystalline solid.

The physico-chemical data of compounds **2b–c** [13], **2e** [14], **2f** [15], **2h** [14], **2i** [16], **2j** [17] are in agreement with the literature data.

*General procedure for the preparation of compounds **3b**, **3c**, **3e**, **3f**, **3h–j***

A mixture of benzhydrol (20 mmol) in an excess of thionyl chloride (10 mL) was stirred at r.t. for about 24 h. The transformation of benzhydrol to chloride was monitored by IR, until the disappearance of the absorption bands of the hydroxyl group at 3330 cm^{-1}. The mixture was then evaporated under reduced pressure. The brownish oily residue was dissolved in acetonitrile (30 mL) and evaporated to remove the rest of the $SOCl_2$. The crude product was used without further purification.

*General procedure for the preparation of compounds **4a** and **4i***

A solution of carbinol **2a** or **2i** (10 mmol) and 1,1′-carbonyldiimidazole (20 mmol) in dry toluene (150 mL) was refluxed for 4 h. After being cooled, the solvent was removed and the residue was dissolved in ethyl acetate (300 mL). The organic solution was washed with brine (3 × 200 mL) and dried over $MgSO_4$. The removal of the solvent afforded the crude product, which was purified by recrystallization from ethanol or by column chromatography on silica gel using DCM as eluent.

*Benzhydryl-oxycarbonyl-1H-imidazole **4a***

Yield: 73%; m.p.: 126.5°C; IR (KBr) ν cm^{-1} : 3150–3000 (CH), 1760 (C=O), 1600, 1480 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 8.21 (s, 1H, imidazole-H_2), 7.48 (d, 1H, imidazole-H_5, J = 1.5 Hz), 7.39–7.35 (m, 10H, Ar–H), 7.07 (d, 1H, imidazole-H_4, J = 1.5 Hz), 7.03 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR ($CDCl_3$): 139.5 (Ar–C_1 and $C_{1'}$), 135.6, 132.5 (imidazole-C_2 and C_4), 128.6, 128.1, 126.9 (Ar) and (C=O), 117.5 (imidazole-C_5), 81.1 (Ph–*C*H–Ph); Analysis calculated for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.29; H, 5.19; N, 9.98.

*2,2′,4,4′-Tetrachloro-benzhydryl-oxycarbonyl-1H-imidazole **4i***

Yield: 92%; m.p.: 81°C; IR (KBr) ν cm^{-1} : 3200–3000 (CH), 1760 (C=O), 1600, 1480 (C=C); ^{1}H-NMR ($CDCl_3$) δ: 8.14 (s, 1H, imidazole-H_2), 7.52 (s, 1H, Ph–C*H*–Ph), 7.47 (d, 2H, Ar–H, J = 1.9 Hz), 7.42 (d, 1H, imidazole-H_5, J = 1.5 Hz), 7.28 (d, 2H, Ar–H, J = 8.0 Hz), 7.17 (d, 2H, Ar–H, J = 8.0 Hz), 7.08 (d, 1H, imidazole-H_4, J = 1.5 Hz); ^{13}C-NMR ($CDCl_3$): 139.2 (Ar–C_1 and $C_{1'}$), 135.6 (imidazole-C_2 or C_4), 134.6, 134.1 (Ar–C_2–Cl, Ar–$C_{2'}$–Cl, Ar–C_4–Cl and Ar–$C_{4'}$–Cl), 132.5 (imidazole-C_2 or C_4), 130.0, 129.6, 128.4 (Ar) and (C=O), 117.3 (imidazole-C_5), 80.2 (Ph–*C*H–Ph); Analysis calculated for $C_{17}H_{10}N_2O_2Cl_4$: C, 49.07; H, 2.42; N, 6.73. Found: C, 49.01; H, 2.54; N, 6.65.

*General procedure for the preparation of compounds **5a–i** and **6b–j***

Benzhydryl chloride derivative **3** (10 mmol) was dissolved in dioxane (30 mL) and added dropwise to a solution of imidazole (or 1*H*-1,2,4-triazole) (30 mmol) in the same solvent (20 mL). The mixture was refluxed for several days, and the progress of the reaction was monitored by TLC. Then the mixture was evaporated to give a yellow oil which was dissolved in ethyl acetate (70 mL), washed with water (3 × 50 mL), dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on silica gel using DCM/EA as eluents.

All imidazole **5a–i** and triazole **6b–j** compounds gave the same IR absorption bands towards ν 3000 cm^{-1} (CH), 1600 cm^{-1} (C=C) and 1270–1240 cm^{-1} (C=N).

The physico-chemical data of compounds **5a**, **5g**, and **6g** [18] are in agreement with the literature data.

*2-Chlorobenzhydryl-1H-imidazole **5b***

Yield: 65%; n_D^{22}: 1.5672; ^{1}H-NMR ($CDCl_3$) δ: 7.49 (dd, 1H, Ar–H, J = 7.8 Hz, 1.5 Hz), 7.44–7.38 (m, 4H, Ar–H), 7.36–7.27 (m, 2H, imidazole-H_2 and Ar–H), 7.16–7.09 (m, 3H, imidazole-H_5 and Ar–H), 6.95 (s, 1H, imidazole-H_4), 6.90 (dd, 1H, Ar–H, J = 7.8 Hz, 1.5 Hz), 6.87 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR ($CDCl_3$): 137.6, 137.5 (Ar–C_1 and $C_{1'}$), 137.0 (imidazole-C_2), 133.7 (Ar–C_2–Cl), 130.1, 129.8, 129.3, 129.2, 128.9 (Ar), 128.9 (imidazole-C_4), 128.6, 128.2, 127.3 (Ar), 119.5 (imidazole-C_5), 61.9 (Ph–*C*H–Ph); Analysis calculated for $C_{16}H_{13}N_2Cl$: C, 71.51; H, 4.88; N, 10.42. Found: C, 71.45; H, 4.96; N, 10.49.

*3-Chlorobenzhydryl-1H-imidazole **5c***

Yield: 53%; n_D^{22}: 1.6117; ^{1}H-NMR ($CDCl_3$) δ: 7.43 (s, 1H, Ar–H), 7.42–7.31 (m, 5H, imidazole-H_2 and Ar–H), 7.10–7.14 (m, 4H, imidazole-H_5 and Ar–H), 6.97 (m, 1H, Ar–H), 6.86 (s, 1H, imidazole-H_4), 6.50 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR ($CDCl_3$): 141.2 (imidazole-C_2), 138.2, 137.2 (Ar–C_1 and $C_{1'}$), 134.9 (Ar–C_3–Cl), 130.2, 129.3, 129.1 (Ar), 128.8 (imidazole-C_4), 128.8, 128.7, 128.1, 126.1 (Ar), 119.3 (imidazole-C_5), 64.5 (Ph–*C*H–Ph); Analysis calculated for $C_{16}H_{13}N_2Cl$: C, 71.51; H, 4.88; N, 10.42. Found: C, 71.46; H, 4.83; N, 10.55.

*4-Chlorobenzhydryl-1H-imidazole **5d***

Yield: 55%; n_D^{22}: 1.6009; ^{1}H-NMR ($CDCl_3$) δ: 7.42–7.32 (m, 5H, imidazole-H_2 and Ar–H), 7.12–7.02 (m, 6H, imidazole-H_5 and Ar–H), 6.84 (s, 1H, imidazole-H_4), 6.51 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR ($CDCl_3$): 137.5 (Ar–C_1 or $C_{1'}$), 137.0 (imidazole-C_2), 136.6 (Ar–C_1 or $C_{1'}$), 134.6 (Ar–C_4–Cl), 129.5, 129.2, 129.1, 128.9, 128.1 (Ar), 128.9 (imidazole-C_4), 128.3, 128.1, 127.2 (Ar), 119.2 (imidazole-C_5), 65.6 (Ph–*C*H–Ph); Analysis calculated for $C_{16}H_{13}N_2Cl$: C, 71.51; H, 4.88; N, 10.42. Found: C, 71.60; H, 4.95; N, 10.35.

*2,4-Dichlorobenzhydryl-1H-imidazole **5e***

Yield: 45%; n_D^{22}: 1.6093; ^{1}H-NMR ($CDCl_3$) δ: 7.44 (d, 1H, Ar–H, J = 2.1Hz), 7.38–7.34 (m, 4H, imidazole-H_2 and Ar–H), 7.22 (dd, 1H, Ar–H, J = 8.5 Hz, 2.0 Hz), 7.12 (s, 1H, imidazole-H_5), 7.07–7.04 (m, 2H, Ar–H), 6.83 (s, 1H, imidazole-H_4), 6.80 (s, 1H, Ph–C*H*–Ph), 6.77 (d, 1H, Ar–H, J = 8.4 Hz); ^{13}C-NMR ($CDCl_3$): 137.0 (imidazole-C_2), 135.8, 135.3 (Ar–C_1 and $C_{1'}$), 134.4, 134.3 (Ar–C_2–Cl and Ar–C_4–Cl), 130.4, 129.9, 129.2, 129.1 (Ar), 128.8 (imidazole-C_4), 128.2, 127.6 (Ar), 119.3 (imidazole-C_5), 63.1 (Ph–*C*H–Ph); Analysis calculated for $C_{16}H_{12}N_2Cl_2$: C, 63.38; H, 3.99; N, 9.24. Found: C, 63.41; H, 3.88; N, 9.36.

*3,4-Dichlorobenzhydryl-1H-imidazole **5f***

Yield: 55%; n_D^{22}: 1.6170; ^{1}H-NMR ($CDCl_3$) δ: 7.43–7.38 (m, 5H, imidazole-H_2 and Ar–H), 7.19 (d, 1H, Ar–H, J = 2.0 Hz), 7.12–7.10 (m, 3H, imidazole-H_5 and Ar–H), 6.93 (dd, 1H, Ar–H, J = 8.5 Hz, 2.0 Hz), 6.84 (s, 1H, imidazole-H_4), 6.49 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR ($CDCl_3$): 139.4, 137.7 (Ar–C_1 and $C_{1'}$), 137.0 (imidazole-C_2), 136.0, 134.8 (Ar–C_3–Cl and Ar–C_4–Cl), 130.9, 129.8, 129.6, 129.2 (Ar), 128.9 (imidazole-C_4), 128.3, 128.1, 127.2 (Ar), 119.2 (imidazole-C_5), 64.1 (Ph–*C*H–Ph); Analysis calculated for $C_{16}H_{12}N_2Cl_2$: C, 63.38; H, 3.99; N, 9.24. Found: C, 63.32; H, 4.08; N, 9.19.

*2,4,4′-Trichlorobenzhydryl-1H-imidazole **5h***

Yield: 23%; n_D^{23}: 1.5953; ^{1}H-NMR ($CDCl_3$) δ: 7.48 (d, 1H, Ar–H, J = 2.0 Hz), 7.39–7.36 (m, 3H, imidazole-H_2 and Ar–H), 7.28 (dd, 1H, Ar–H, J = 8.2 Hz, 2.0 Hz), 7.14 (s, 1H, imidazole-H_5), 7.03 (d, 2H, Ar–H, J = 8.4 Hz), 6.82 (s, 1H, Ph–C*H*–Ph), 6.80 (s, 1H, imidazole-H_4), 6.77 (d, 1H, Ar–H, J = 8.2 Hz); ^{13}C-NMR ($CDCl_3$): 137.1 (imidazole-C_2), 135.6, 135.2 (Ar–C_1 and $C_{1'}$), 134.9, 134.3 (Ar–C_2–Cl and Ar–C_4–Cl), 130.1, 129.8, 129.4 (Ar), 128.4 (imidazole-C_4), 127.8 (Ar), 119.2 (imidazole-C_5), 60.9 (Ph–*C*H–Ph); Analysis calculated for $C_{16}H_{11}N_2Cl_3$: C, 56.92; H, 3.28; N, 8.30. Found: C, 56.80; H, 3.12; N, 8.39.

*2,2′,4,4′-Tetrachlorobenzhydryl-1H-imidazole **5i***

Yield: 36%; m.p.: 134°C; ^{1}H-NMR ($CDCl_3$) δ: 7.51 (d, 2H, Ar–H, J = 2 Hz), 7.37 (s, 1H, imidazole-H_2), 7.28 (dd, 2H, Ar–H, J = 8.4 Hz, 2.0 Hz), 7.16 (s, 1H, imidazole-H_5), 7.07 (s, 1H, Ph–C*H*–Ph), 6.79 (s, 1H, imidazole-H_4), 6.71 (d, 2H, Ar–H, J = 8.4 Hz); ^{13}C-NMR ($CDCl_3$): 137.4 (imidazole-C_2), 135.6, 134.6, 134.1 (Ar–C_1 and $C_{1'}$, Ar–C_2–Cl, Ar-$C_{2'}$-Cl, Ar–C_4–Cl and Ar-$C_{4'}$-Cl), 130.2, 130.0 (Ar), 129.5 (imidazole-C_4), 127.8 (Ar), 119.2 (imidazole-C_5), 58.5 (Ph–*C*H–Ph); Analysis calculated for $C_{16}H_{10}N_2Cl_4$: C, 51.65; H, 2.71; N, 7.53. Found: C, 51.74; H, 2.85; N, 7.46

*2-Chlorobenzhydryl-1H-1,2,4-triazole **6b***

Yield: 35%; m.p.: 72°C; ^{1}H-NMR ($CDCl_3$) δ: 8.05 (s, 1H, triazole-H_5), 7.94 (s, 1H, triazole-H_3), 7.46–7.25 (m, 6H, Ar–H), 7.15 (m, 2H, Ar–H), 6.98 (s, 1H, Ph–C*H*–Ph), 6.92 (d, 1H, Ar–H); ^{13}C-NMR ($CDCl_3$): 152.5 (triazole-C_3 and C_5), 137.6, 137.5 (Ar–C_1 and $C_{1'}$), 133.7 (Ar–C_2–Cl), 130.1, 129.8, 129.3, 129.2, 128.9, 128.6, 128.2, 127.3 (Ar), 67.8 (Ph–*C*H–Ph); Analysis calculated for $C_{15}H_{12}N_3Cl$: C, 66.79; H, 4.48; N, 15.58. Found: C, 66.85; H, 4.41; N, 15.69.

*3-Chlorobenzhydryl-1H-1,2,4-triazole **6c***

Yield: 41%; n_D^{22}: 1.5982; ^{1}H-NMR ($CDCl_3$) δ: 8.06 (s, 1H, triazole-H_5), 7.97 (s, 1H, triazole-H_3), 7.46–7.29 (m, 5H, Ar–H), 7.17 (m, 2H, Ar–H), 7.13 (s, 1H, Ar–H), 7.02 (d, 1H, Ar–H, J = 6.5 Hz); 6.74 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR ($CDCl_3$): 152.5 (triazole-C_3 and C_5), 138.2, 137.2 (Ar–C_1 and $C_{1'}$), 134.9 (Ar–C_3–Cl), 130.2, 129.3, 129.1, 128.8, 128.7, 128.1, 126.1 (Ar), 67.5 (Ph–*C*H–Ph); Analysis calculated for $C_{15}H_{12}N_3Cl$: C, 66.79; H, 4.48; N, 15.58. Found: C, 66.88; H, 4.53; N, 15.43.

4-Chlorobenzhydryl-1H-1,2,4-triazole **6d**

Yield: 31%; m.p.: 88°C; ^{1}H-NMR ($CDCl_3$) δ: 8.05 (s, 1H, triazole-H_5), 7.95 (s, 1H, triazole-H_3), 7.43–7.35 (m, 5H, Ar–H), 7.15 (dd, 2H, Ar–H, J = 7.5 Hz, 2.0 Hz), 7.09 (m, 2H, Ar–H), 6.75 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR ($CDCl_3$): 152.4 (triazole-C_3 and C_5), 137.5, 136.6 (Ar–C_1 and $C_{1'}$), 134.6 (Ar–C_4–Cl), 129.5, 129.2, 129.1, 128.9, 128.1 (Ar), 67.1 (Ph–*C*H–Ph); Analysis calculated for $C_{15}H_{12}N_3Cl$: C, 66.79; H, 4.48; N, 15.58. Found: C, 66.70; H, 4.43; N, 15.66.

2,4-Dichlorobenzhydryl-1H-1,2,4-triazole **6e**

Yield: 42%; n_D^{23} = 1.5450; ^{1}H-NMR ($CDCl_3$) δ: 8.04 (s, 1H, triazole-H_5), 7.95 (s, 1H, triazole-H_3), 7.46 (d, 1H, Ar–H, J = 2.0 Hz), 7.39 (m, 3H, Ar–H), 7.29 (dd, 1H, Ar–H, J = 8.5 Hz, 2.0 Hz), 7.14 (m, 2H, Ar–H), 7.08 (s, 1H, Ph–C*H*–Ph), 6.88 (d, 1H, Ar–H, J = 8.4 Hz); ^{13}C-NMR ($CDCl_3$): 152.0 (triazole-C_3 and C_5), 135.9, 135.3 (Ar–C_1 and $C_{1'}$), 134.4, 134.3 (Ar–C_2–Cl and Ar–C_4–Cl), 130.4, 129.9, 129.2, 129.1, 128.5, 128.2, 127.6 (Ar), 64.1 (Ph–*C*H–Ph); Analysis calculated for $C_{15}H_{11}N_3Cl_2$: C, 59.23; H, 3.65; N, 13.81. Found: C, 59.15; H, 3.56; N, 13.93.

3,4-Dichlorobenzhydryl-1H-1,2,4-triazole **6f**

Yield: 25%; m.p.: 59°C; ^{1}H-NMR ($CDCl_3$) δ: 8.06 (s, 1H, triazole-H_5), 7.98 (s, 1H, triazole-H_3), 7.48–7.40 (m, 4H, Ar–H), 7.23–7.16 (m, 3H, Ar–H), 6.98 (dd, 1H, Ar–H, J = 8.4 Hz, 2.0 Hz), 6.71 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR ($CDCl_3$): 152.3 (triazole-C_3 and C_5), 139.4, 137.7 (Ar–C_1 and $C_{1'}$), 136.1, 134.8 (Ar–C_3–Cl and Ar–C_4–Cl), 130.9, 129.8, 129.6, 129.2, 128.3, 128.1, 127.2 (Ar), 64.0 (Ph–*C*H–Ph); Analysis calculated for $C_{15}H_{11}N_3Cl_2$: C, 59.23; H, 3.65; N 13.81. Found: C, 59.12; H, 3.72; N, 13.75.

2,4,4′-Trichlorobenzhydryl-1H-1,2,4-triazole **6h**

Yield: 41%; m.p.: 98°C; ^{1}H-NMR ($CDCl_3$) δ: 8.06 (s, 1H, triazole-H_5), 7.99 (s, 1H, triazole-H_3), 7.49 (d,1H, Ar–H, J = 2.0 Hz), 7.41 (d, 2H, Ar–H, J = 8.4 Hz), 7.30 (dd, 1H, Ar–H, J = 8.4 Hz, 2.0 Hz), 7.10 (s, 1H, Ph–C*H*–Ph), 7.07 (s, 2H, Ar–H), 6.92 (d, 1H, Ar–H, J = 8.4 Hz); ^{13}C-NMR ($CDCl_3$): 152.1 (triazole-C_3 and C_5), 135.6, 135.2 (Ar–C_1 and $C_{1'}$), 134.9, 134.3 (Ar–C_2–Cl and Ar–C_4–Cl), 130.1, 129.8, 129.4, 127.8 (Ar), 63.1 (Ph–*C*H–Ph); Analysis calculated for $C_{15}H_{10}N_3Cl_3$: C, 53.20; H, 2.98; N, 12.41. Found: C, 53.12; H, 2.85; N, 12.65.

2,2′,4,4′-Tetrachlorobenzhydryl-1H-1,2,4-triazole **6i**

Yield: 15%; m.p.: 104°C; ^{1}H-NMR ($CDCl_3$) δ: 8.05 (s, 1H, triazole-H_5), 8.00 (s, 1H, triazole-H_3), 7.49 (s, 2H, Ar–H), 7.33 (s, 1H, Ph–C*H*–Ph), 7.29 and 7.26 (2d, 2H, Ar–H, J = 8.4 Hz), 6.89 (d, 2H, Ar–H, J = 8.4 Hz); ^{13}C-NMR ($CDCl_3$): 152.1 (triazole-C_3 and C_5), 135.6 (Ar–C_1 and $C_{1'}$), 134.6, 134.1 (Ar–C_2–Cl, Ar-C_2-Cl, Ar–C_4–Cl and Ar-C4′-Cl), 130.2, 130.0, 129.5, 127.8 (Ar), 61.5 (Ph–*C*H–Ph); Analysis calculated for $C_{15}H_9N_3Cl_4$: C, 48.29; H, 2.43; N, 11.26. Found: C, 48.35; H, 2.55, N, 11.21.

1-[(1,1-biphenyl)-4-yl-phenylmethyl]-1H-1,2,4-triazole **6j**

Yield: 47%; m.p.: 103°C; ^{1}H-NMR ($CDCl_3$) δ: 8.02 (s, 1H, triazole-H_5), 7.96 (s, 1H, triazole-H_3), 7.66–7.58 (m, 5H, Ar–H), 7.46–7.42 (m, 4H, Ar–H), 7.23–7.18 (m, 5H, Ar–H), 6.64 (s, 1H, Ph–C*H*–Ph); ^{13}C-NMR (DMSO-d_6): 152.2 (triazole-C_3 and C_5), 140.7, 139.7, 139.3, 138.5 (Ar–C_1, $C_{1'}$, C_4 and $C_{1''}$), 129.5, 128.8, 128.3, 128.2, 127.7, 127.2 (Ar), 62.2 (Ph–*C*H–Ph); Analysis calculated for $C_{21}H_{17}N_3$: C, 81.00; H, 5.50; N, 13.49. Found: C, 81.13; H, 5.63; N, 13.34.

Microbiological assays

Bifonazole, clotrimazole and ciprofloxacin (Sigma, Saint Quentin Fallavier, France) were used as reference compounds in microbiological assays.

The test compounds were dissolved in a solution consisting of 1/3 DMSO and 2/3 5% aqueous Tween 80 solution to obtain a final concentration of 1000 μg/mL. The resulting solutions were then diluted in microtiter plates in culture medium: trypcase soja (Biomérieux, Craponne, France) for bacteria and Sabouraud (Biomérieux, Craponne, France) for fungi. Gram-positive strains *S. aureus* CIP 4.83, *E. hirae* CIP 5855, and Gram-negative strains *P. aeruginosa* CIP 82118, *E. coli* CIP 53126 were used for the antibacterial assays. For the antifungal assays, cultures of yeasts *C. albicans* IP 48.72, *C. krusei* IP 208.52, *A. niger* IP 1431.83 and a dermatophyte strain *T. rubrum* IP 1657.86 were used. Strains were obtained from the Collection of the Pasteur Institute (Paris, France). Minimal inhibitory concentrations (MICs) and minimal germicidal concentrations (MBCs or MFCs) were determined after incubation of bacterial strains at 37°C and fungal strains at 30 or 22.5°C for 24–48 h (5 days for *T. rubrum*) in the presence of serial dilutions of the test compounds (Tables 1 and 2). The MIC was defined as the compound concentration at which no macroscopic sign of cellular growth was detected in comparison to the control without antimicrobial compound. The MBC/MFC were determined by subculturing on corresponding agar plates after incubation of bacterial strains at 37°C and fungal strains at 30 or 22.5°C for 24–48 h (5 days for *T. rubrum*). The MBC/MFC was defined as the compound concentration at which no macroscopic sign of cellular growth was detected in comparison to the control without antimicrobial compound.

Molecular modeling

The starting geometries of the synthesized compounds were initially created with the ChemDraw and geometric optimizations were carried out with the PM6 method of MOPAC 2009 software [19]. The lowest energy conformation was then selected. The partial atomic charges were calculated with the same method. For the visualization of compounds and the electrostatic potentials Accelrys software was used (Accelrys DS Visualiser 2.0, 2005-2007 Accelrys software Inc.)

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