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Contribution to investigation of antimicrobial activity of styrylquinolines

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ABSTRACT

Series of new ring-substituted styrylquinolines and two oxorhenium complexes were prepared and characterized. The compounds were analyzed using RP-HPLC to determine lipophilicity. Primary in vitro screening of the synthesized compounds was performed against fungal and bacterial strains. Some compounds were active against bacteria at micromolar level and against fungi at submicromolar level. Compounds 5,7-dichloro-2-[2-(2-ethoxyphenyl)vinyl]quinolin-8-ol expressed excellent antifungal activity comparable with or higher than the standard fluconazole as well as antibacterial activity against *Staphylococcus* strains comparable with or higher than the standards bacitracin, penicillin and ciprofloxacin. The structure–activity relationships are discussed.

1. Introduction

Over the past several decades, the considerable growth of fungal infections can be noticed. This correlates in part with the increasing number of patients being admitted to intensive care units, HIV-positive individuals/AIDS patients, organ transplant recipients as well as individuals who are hospitalised for long periods and subjected to antibiotic therapy, application of corticosteroids, parenteral nutrition and invasive medical procedures as intensive care.[1–3] Fungal infections caused by common and unusual fungi pathogens are occurring with increasing frequency and result in both significant morbidity and mortality. The majority of these infections are caused by *Candida* spp., *Aspergillus fumigatus* and *Cryptococcus neoformans*. Less common (but emerging) pathogens include *Zygomycetes*, *Fusarium* spp., *Trichosporon beigelii*, *Blastoschizomyces*, *Scedosporium*, *Acremonium* and some dematiaceous fungi.[4] Our armament of antifungal drugs is wider than ever; however, it is not sufficient to cure many of infections. The main reason for this is the resistance emerging among the pathogenic fungi.[5,6] Older drugs are considerably toxic and suitable only for topical use, while the newer are susceptible for resistance. An example may be azoles, an important class of antimycotic drugs of choice for many infections and occasionally used off label in treatment of various malignancies.[7] Unfortunately resistance to this group is especially common, and the resistance emerged to one drug usually means the resistance to the whole class. The discovery of new drugs for the treatment of systemic mycoses is a major challenge in infectious disease research.[8] Recently we have proposed the used quinoline scaffold for design and synthesis of novel antifungals.[9] Quinoline moiety is present in many bioeffectors and has been claimed as a privileged structure.[10–12] Simple derivatives of quinoline have a long history as antifungal agents and some of them are still in use.[13] In our approach styrylquinolines were used for their similarity with allylamines, known antifungals[14–18], see Figure 1. Further studies on the topic has led to the conclusion that an aromatic quinoline moiety is required as quinazoline or 2,4-dioxoquinolines appeared less or completely inactive when compared to 8-hydroxyquinoline derivatives.[19–22] In this paper we wish to report our last findings on ring substituted styrylquinolines designed on the basis of dichloroquinoline.

Dichloroquinoline (chloroxine) is used for long time as an antibacterial agent, *i.e.* in antibacterial antidiarrheic pharmaceutics or antidandruff shampoos. Other mono or dihalogenated quinolines were studied for antifungal activity by Gershon et al.[23–27] The mechanism of action of antibacterial or antifungal quinolines remains unclear which hamper the more rational design of new compounds. Thus structure derived functionalization and fragment based design are still the best in this regards. This paper deals with synthesis and antimicrobial activities of new ring-substituted styrylquinolines as innovative antibacterial and antifungal compounds based on fragments of chloroxine-like compounds and allylamines. The structure–activity relationships between the

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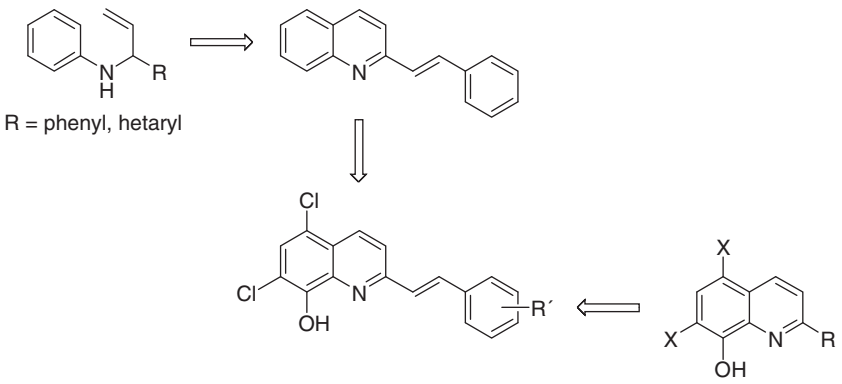


Figure 1. Design of the halogenated styrylquinolines, their relationship to chloroxine-like compounds and their structural analogy/similarity with allylamines.

chemical structure, physical properties and in vitro biological activities of the evaluated compounds are discussed.

2. Results and discussion

2.1. Chemistry

All studied compounds were prepared according to Scheme 1. Compounds **12**, **13**, **14**, **16** and **19** were obtained according to a previously described procedure.[13,28,29] Microwave-assisted organic synthesis was used to obtain the group of styrylquinoline-like compounds **1**, **3**, **4** and **11**, **17**, **18**. The appropriate quinaldine (1.0 equiv) was mixed with aldehyde (4.0 equiv) and irradiated in the microwave reactor for 10 min. Condensation of the appropriate quinaldine (1.0 equiv) and the appropriate aldehyde (2.0 equiv) yielded compounds **2**, **5**, and **6**–**10** in a two-step reaction in acetic anhydride followed by pyridine/water.

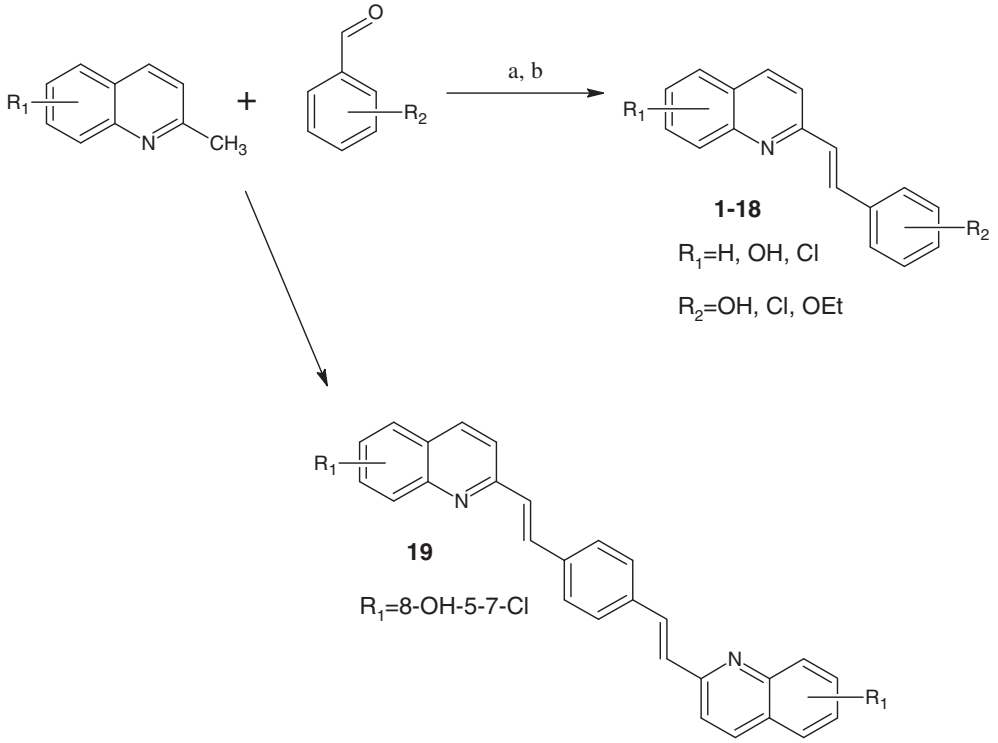
The oxorhenium(V) complexes **20** and **21** of the type *cis*-X, X-[$ReOX_2(N–O)(PPh_3)$] have been prepared by refluxing of [$ReOX_3(PPh_3)_2$] and 5,7-dichloro-2-[2-(2-chlorophenyl)vinyl]quinolin-8-ol (**11**) in acetonitrile. It was isolated as an air-stable, microcrystalline solid, soluble in common organic solvents, as described previously.[30] (see Scheme 2)

Recently some interesting results on transition metal complexes with hetereocyclic compounds as indoles[31] and quinolines[32] has been reported. Especially quinoline based complexes appeared to be effective against several pathogenic bacteria and parasites.[32] On the other hand we are involved in search of ne oxorhenium complexes with quinoline related ligands as potential radiopharmaceuticals in cancer treatment.[30,33–35] Thus we decided to measure antimicrobial activity spectrum of these compounds.

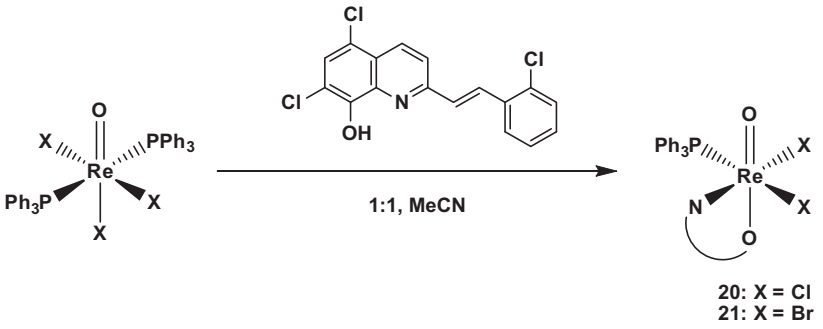
2.2. In vitro antifungal and antibacterial activity

The studied compounds were tested for their activity against eight fungi strains and some bacterial strains. All the results are presented in Tables 1 and 2, respectively. The antifungal and antibacterial activities are expressed as minimal inhibitory concentration (MIC).

Antifungal activities are expressed as MIC/IC_{80} [μmol/L] for yeasts and MIC/IC_{50} [μmol/L] for moulds. Antibacterial activity is expressed as MIC/IC_{90} [μmol/L].



Scheme 1. Synthesis of studied compounds **1**–**19**: (a) aldehyde, microwave irradiation; (b) aldehyde, Ac_2O, Py/H_2O.



Scheme 2. Synthesis of studied compounds **20** and **21**.

Fluconazole was selected as standard in the antifungal assay and bacitracin (BAC), penicillin V (PEN) and ciprofloxacin (CPX) were used as antibacterial standards. The antifungal and antibacterial activity of compounds **12**, **13**, **14**, **16** and **19** was discussed recently by Musiol et al.[22], but in Tables 1 and 2 these derivatives can be also found for overall summary.

Generally, Group 2 (compounds **11**–**19**) showed higher activity than their parent counterparts, see **3**/**9** and **17** or **10** versus **18**, which indicates the necessity of the hydroxyl moiety presence in the position $C_{(8)}$ of the quinoline ring B. Subsequent substitution by chlorine of $C_{(5)}$ and $C_{(7)}$ positions of the quinoline ring B significantly increased antimicrobial activities along with lipophilicity, which is in general an important parameter for antimicrobial activity.[13]

In the case of antifungal activity, most of compounds showed only moderate activity; therefore only general structure–activity relationships can be mentioned. Compounds 5,7-dichloro-2-[2-(2-ethoxyphenyl)vinyl]quinolin-8-ol (**17**) and 5,7-dichloro-2-(2-{4-[2-(5,7-dichloro-8-hydroxyquinolin-2-yl)vinyl]phenyl}vinyl) quinolin-8-ol (**19**) expressed the highest antifungal activity against almost all tested fungal strains. Both compounds showed activity comparable with or higher than fluconazole. Styrylquinolines substituted with the hydroxyl group in styryl are moderately active against moulds *Aspergillus fumigatus*, *Absidia corymbifera* and *Trichophyton mentagrophytes* and inactive against yeast. Compounds **12**, **13** and **16** demonstrated lower level of activity than fluconazole in case of *A. corymbifera*. This pattern of activity cannot be observed for non-chlorinated compounds.

Table 1
In vitro antifungal activity (IC_{80}/IC_{50}) of compounds in comparison with fluconazole (FLU) standard

Comp.	R_1	R_2	[1,2]MIC/[a]IC_{80}/[b]IC_{50} [μmol/L]							
			CA[a] 24 h 48 h	CT[a] 24 h 48 h	CK[a] 24 h 48 h	CG[a] 24 h 48 h	TB[a] 24 h 48 h	AF[b] 24 h 48 h	AC[b] 24 h 48 h	TM[b] 72 h 120 h
1	H	2-Cl	>500	>500	>500	>500	>500	>500	>500	>500
2	H	2-OH	>125	>125	>125	>125	>125	>125	>125	>125
3	H	4-OEt	>500	>500	>500	>500	>500	>500	>500	>500
4	8-Cl	2-Cl	>500	>500	>500	>500	>500	>500	>500	>500
5	8-OH	2-Cl	>500	>500	>500	>500	>500	>500	>500	>500
6	8-OH	2-OH	31.25	125	31.25	31.25	31.25	500	62.50	31.25
			62.50	500	62.50	31.25	62.50	>500	>500	62.50
7	8-OH	3-OH	>125	>125	>125	>125	>125	>125	>125	>125
			>125	>125	>125	>125	>125	>125	>125	>125
8	8-OH	2,4-OH	125	>125	62.50	62.50	62.50	125	>125	31.25
			>125	>125	125	125	125	125	>125	62.50
9	8-OH	2-OEt	>500	>500	>500	>500	>500	>500	>500	>500
10	8-OH	4-OEt	>500	>500	>500	>250	>250	>500	>500	>500
11	8-OH-5,7-Cl	2-Cl	31.25	>125	31.25	7.81	31.25	31.25	31.25	15.62
			62.50	>125	62.50	31.25	62.50	62.50	62.50	15.62
12[3]	8-OH-5,7-Cl	2-OH	>125	>125	>125	>125	>125	125	62.50	62.50
			>125	>125	>125	>125	>125	125	125	62.50
13[3]	8-OH-5,7-Cl	4-OH	125	125	125	125	125	125	31.25	15.62
			>125	>125	>125	>125	>125	125	62.50	62.50
16[3]	8-OH-5,7-Cl	3,5-OH	>125	>125	>125	>125	>125	125	62.50	15.62
			>125	>125	>125	>125	>125	125	62.50	15.62
17	8-OH-5,7-Cl	2-OEt	0.49	0.98	0.98	0.98	0.49	3.90	15.62	1.39
			0.98	0.98	3.90	1.95	1.95	3.90	15.62	1.52
18	8-OH-5,7-Cl	4-OEt	125	125	125	>125	125	>125	>125	125
			125	125	125	>125	125	>125	>125	125
19[3]	Scheme 1		0.98	1.95	0.98	0.49	3.90	3.90	15.62	3.90
			3.90	7.81	1.95	1.95	15.62	15.62	31.25	7.81
20	Scheme 2		>125	>125	>125	>125	>125	>125	>125	>125
			>125	>125	>125	>125	>125	>125	>125	>125
21	Scheme 2		>125	>125	>125	>125	>125	>125	>125	>125
FLU	–		0.06	0.12	3.91	0.98	0.24	>125	>125	1.95
			0.12	>125	15.62	3.91	0.48	>125	>125	3.91

[1] The MIC determination was performed according to the CLSI reference protocol: [a]M27-A2 for yeasts (IC_{80} value) and [b]M38-A for moulds (IC_{50} value); CA = *Candida albicans*, CT = *Candida tropicalis*, CK = *Candida krusei*, CG = *Candida glabrata*, TB = *Trichosporon beigelii*, AF = *Aspergillus fumigatus*, AC = *Absidia corymbifera*, TM = *Trichophyton mentagrophytes*.

[2] All compounds were tested for short and long term activity; if inactive then only one value presented. [3]Biological activity was described recently by Musiol et al.[22]

Table 2
In vitro antibacterial activity (IC_{90}) of compounds in comparison with bacitracin (BAC), penicillin V (PEN) and ciprofloxacin (CPX) standards

Comp.	R_1	R_2	[1]MIC/IC_{90} [μmol/L]					
			SA[2]		MRSA[2]		SE[2]	
			24 h	48 h	24 h	48 h	24 h	48 h
1	H	2-Cl	>500	>500	>500	>500	>500	>500
2	H	2-OH	>250	>250	>250	>250	>500	>250
3	H	4-OEt	>500	>500	>500	>500	>500	>500
4	8-Cl	2-Cl	>500	>500	>500	>500	>500	>500
5	8-OH	2-Cl	>500	>500	>500	>500	>500	>500
6	8-OH	2-OH	62.50	62.50	62.50	125	62.50	125
7	8-OH	3-OH	125	125	125	125	250	500
8	8-OH	2,4-OH	15.62	31.25	31.25	62.50	125	250
9	8-OH	2-OEt	>500	>500	>500	>500	>500	>500
10	8-OH	4-OEt	>500	>500	>500	>500	>500	>500
11	8-OH 5,7-Cl	2-Cl	125	125	125	250	250	250
12	8-OH 5,7-Cl	2-OH	7.81	15.62	31.25	62.50	31.25	62.50
13	8-OH 5,7-Cl	4-OH	7.81	15.62	31.25	31.25	62.50	62.50
14	8-OH 5,7-Cl	2,4-OH	15.62	62.50	31.25	62.50	15.62	31.25
15	8-OH 5,7-Cl	3,4-OH	62.50	62.50	31.25	62.50	15.62	31.25
16	8-OH 5,7-Cl	3,5-OH	125	125	125	125	31.25	125
17	8-OH 5,7-Cl	2-OEt	3.9	7.81	3.9	7.81	3.9	7.81
18	8-OH 5,7-Cl	4-OEt	125	125	125	125	125	125
19	Scheme 1		62.50	125	62.50	125	62.50	125
20	Scheme 2		62.50	125	31.25	62.50	15.62	31.25
21	Scheme 2		>125	>125	>125	>125	>125	>125
BAC	–		15.62	31.25	15.62	31.62	15.62	31.62
PEN	–		0.24	0.24	125	125	31.25	125
CPX	–		0.98	0.98	500	500	250	250

[1] All compounds were tested for short and long term activity; if inactive then only one value presented.
[2] SA = *Staphylococcus aureus*, MRSA = methicillin-resistant *S. aureus*, SE = *S. epidermidis*.

5,7-Dichloro-2-[2-(2-chlorophenyl)vinyl]quinolin-8-ol (**11**) was another compound with medium antifungal effect. Based on these facts, it can be assumed that effectivity is connected with substitution in position $\acute{C}_{(2)}$ of styryl by a lipophilic and bulky substituent with minimal electron donor or withdrawing properties (for $2\text{-}OC_2H_5$ Hammett's parameter $\sigma = 0.02$ and bulk parameter expressed as reflecting bulkiness MR = 11.3[36]), as has also been recently described by Otevrel et al.[37]

It can be noticed that in case of antibacterial screening Group 2 (dichloroquinolines) was again generally more active than Group 1. Among evaluated bacterial strains also Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were included, but all the discussed compounds were absolutely inactive against G-strains, contrary to chloroxine that is active also against some G-bacteria. 5,7-Dichloro-2-[2-(2-ethoxyphenyl) vinyl]quinolin-8-ol (**17**) was also the most active against all tested Gram-positive bacterial strains and is comparable with all the used standards. This compound was especially highly active against methicillin-resistant *Staphylococcus aureus* and *S. epidermidis*. Though 5,7-dichloro-2-[2-(2-hydroxyphenyl)vinyl]quinolin-8-ol (**12**) and 5,7-dichloro-2-[2-(4-hydroxyphenyl)vinyl]quinolin-8-ol (**13**) demonstrated zero antifungal activity, their antibacterial activity was quite high. The rest of the compounds should be considered as moderately active or inactive. Antibacterial activity seems to be again connected in particular with substitution in position $\acute{C}_{(2)}$ or $\acute{C}_{(4)}$ of styryl. The introduction of another hydroxyl moiety to styryl caused a decrease in activity (compare **12** and **13** with **14**–**16**) irrespective of the substitution pattern on the quinoline core (compare **8**/**14**).

The antibacterial activity was expressed by the negative logarithm of the IC_{90} value (compound concentration in mol/L causing 90% inhibition of bacterial growth). Despite relatively small number of active compounds some remarks on SAR can be drawn. There are apparent correlations between log ($1/IC_{90}$) and physico-chemical parameters as lipophilicity and electron properties of the studied compounds. The dependence of the antibacterial activity of compounds **6**–**8** (Group 1), **11**–**18** (Group 2) against individual G+ strains (SA, MRSA, SE) on lipophilicity (log *k*) was determined. For compounds **6**–**8** it can be stated that their activity against both types of *S. aureus* decreases with increasing lipophilicity, *i.e.* **8** (2,4-OH) > **6** (2-OH) > **7** (3-OH). For *S. epidermidis* optimum lipophilicity can be found: $\log k = 1.40$, *i.e.* **6** (2-OH) > **8** (2,4-OH) > **7** (3-OH). Based on the results obtained for compounds from Group 2, it is possible to declare that lipophilicity (optimum at $\log k = 1.60$) is an important parameter. The dependence of the antibacterial activity of compounds **6**-**8** (Group 1), **11**–**18** (Group 2) against individual G+ strains (SA, MRSA, SE) on electron properties of styryl substituents expressed as Hammett's parameter (σ)[38] was also studied. The active compounds shown a similar trend as in lipophilicity. The activity against both types of *S. aureus* increases with increasing electron donor properties. In case of anti-*S. epidermidis* effect optimum $\sigma = -0.09$ can be found. For compounds **14** (2,4-OH), **15** (3,4-OH), **16** (3,5-OH) similar relations between activity and σ constants can be also found as for compounds **6**–**8**. However these relationships are weak this conclusions are in good agreement to our former findings.[9,20–22]

2.3. Lipophilicity

Lipophilicity is a property that has a major effect on absorption, distribution, metabolism, excretion and toxicity properties as well as pharmacological activity, because drugs cross biological membranes through the passive transport, which strongly depends on their lipophilicity. Lipophilicity has been studied and applied as an important drug property for decades. Different lipophilicity descriptors such as $\log k$, $\log P$, $\log D$, etc. are used for structure–activity relations description and prediction. It has long been recognised that the retention of a compound in reversed-phase liquid chromatography is governed by its lipophilicity/hydrophobicity, and thus shows correlation with an octanol–water partition coefficient. In reversed phase chromatography hydrophobic forces govern the retention, and it has been long recognised as a potential

method for lipophilicity determination. High performance liquid chromatography (HPLC) provides an excellent platform for computer controlled automated measurements with computerised data acquisition for a large number of research compounds. The other advantages in the use of the HPLC retention data for lipophilicity determination are, that there is no need for concentration determination and method validation, small impurities are separated from the main component, small amounts of material are needed for the measurements and they can be completely automated. Therefore the investigation of the true potential of this method is of great importance.

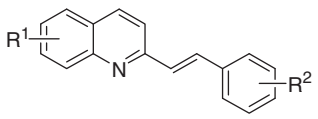
Reversed phase high performance liquid chromatography (RP-HPLC) methods have become popular and widely used for lipophilicity measurement. The general procedure is the measurement of the directly accessible retention time under isocratic conditions with varying amounts of methanol as an organic modifier in the mobile phase using RP columns and calculating the logarithm of the capacity factors ($\log k$). $\log k$ is the logarithm of the capacity factors in chromatographic approaches, which is related to the partitioning of a compound between a mobile and a (pseudo-)stationary phase. Log k is used as the lipophilicity index converted to $\log P$ scale.[39–42] Commercially available chemical software does not resolve various lipophilicity values of individual positional isomers etc. The sowtfare calculates lipophilicity contributions according to different internal databases/libraries and using different approaches.

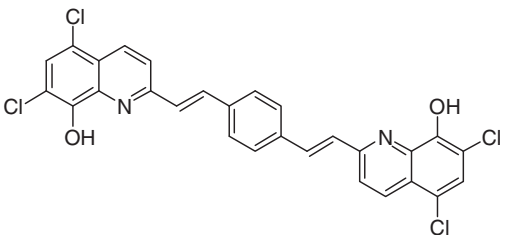
It can be concluded that for small, highly functionalized molecules with a lot of heteroatoms a number of intermolecular forces and intramolecular interactions are typical. The more of these interactions can be expected, the less predictability of common software can be. Therefore experimental lipophilicity determination is of great importance. Therefore, we decided to perform our measurements using water–methanol system as the mobile phase. Log k derived from RP-HPLC retention factors and computational $\log P$ values are given in the manuscript and biological date are related to $\log k$ data, because $\log k$ data specify lipophilicity within the series of the compounds more precisely than available chemical software.

Hydrophobicities ($\log P$/$C\log P$) of compounds **1**–**19** were calculated using two commercially available programs (CS ChemOffice and ACD/Log$P$) and measured by means of RP-HPLC determination of capacity factors k with subsequent calculation of $\log k$. Calculation of $\log P$/$C\log P$ by CS ChemOffice is based on references[43–48], while calculation of $\log P$ by ACD/LogP is based on Hansch and Leo.[36] The program CS ChemOffice did not resolve various lipophilicity values of individual positional isomers, in particular, the same $\log P$/$C\log P$ data were calculated for Group 1 (series without 5,7-Cl, *i.e.* compounds **1**–**10**) and Group 2 (series substituted 5,7-Cl, *i.e.* compounds **11**–**19**), nevertheless all experimentally determined as well as computed data are shown in Table 3.

Generally Group 1 showed lower lipophilicity than Group 2, as expected. Correlation between the computed and determined lipophilicity data within Group 1 is closer than within Group 2. Correlation coefficients for Group 1 are as follows: R^2 ($\log P_{CS}$) = 0.896, R^2($C\log P_{CS}$) = 0.963, R^2($\log P_{ACD}$) = 0.861. Correlation coefficients for Group 2 are as follows: R^2($\log P_{CS}$) = 0.886, R^2($C\log P_{CS}$) = 0.768, R^2($\log P_{ACD}$) = 0.895. The match of calculated data using both programs with experimentally found $\log k$ is illustrated in Figure 2. It can be concluded that the experimentally-determined lipophilicity ($\log k$) values of compounds **1**–**19** are in accordance with the

Table 3
Structure of discussed compounds and comparison of calculated lipophilicities ($\log P$/$C\log P$) with determined $\log k$ values and Hammett's parameter (σ)



Comp.	R^1	R^2	Log k	Log$P$/$C\log P$ CS ChemOffice	Log$P$ ACD/Log$P$	σ[36]
1	H	2-Cl	1.5152	5.25/5.434	5.25 ± 0.30	0.67
2	H	2-OH	1.4260	4.34/4.054	4.61 ± 0.31	−0.09
3	H	4-OC_2H_5	1.4896	4.61/4.64	4.64 ± 0.31	−0.24
4	8-Cl	2-Cl	1.5875	5.85/6.21	5.54 ± 0.33	0.67
5	8-OH	2-Cl	1.5283	4.90/5.483	5.08 ± 0.32	0.67
6	8-OH	2-OH	1.4078	3.95/4.1025	3.75 ± 0.32	−0.09
7	8-OH	3-OH	1.4191	3.95/4.1025	4.40 ± 0.33	0.12
8	8-OH	2,4-OH	1.3912	3.56/3.4355	3.78 ± 0.34	−0.43
9	8-OH	2-OC_2H_5	1.5047	4.55/5.2175	5.02 ± 0.33	0.02
10	8-OH	4-OC_2H_5	1.5011	4.55/5.2175	4.96 ± 0.33	−0.24
11	8-OH-5,7-Cl	2-Cl	1.6412	6.02/6.74425	6.96 ± 0.36	0.67
12	8-OH-5,7-Cl	2-OH	1.5854	5.07/5.36425	5.62 ± 0.35	−0.09
13	8-OH-5,7-Cl	4-OH	1.5866	5.07/5.36425	6.37 ± 0.36	−0.37
14	8-OH-5,7-Cl	2,4-OH	1.5858	4.68/4.69725	5.65 ± 0.37	−0.43
15	8-OH-5,7-Cl	3,4-OH	1.5865	4.68/4.76725	6.42 ± 0.38	−0.25
16	8-OH-5,7-Cl	3,5-OH	1.5867	4.68/4.69725	6.20 ± 0.37	0.24
17	8-OH-5,7-Cl	2-OC_2H_5	1.5957	5.67/6.47925	6.90 ± 0.36	0.02
18	8-OH-5,7-Cl	4-OC_2H_5	1.6146	5.67/6.47925	6.84 ± 0.36	−0.24
19			2.5760	8.88/9.92051	10.50 ± 0.39	

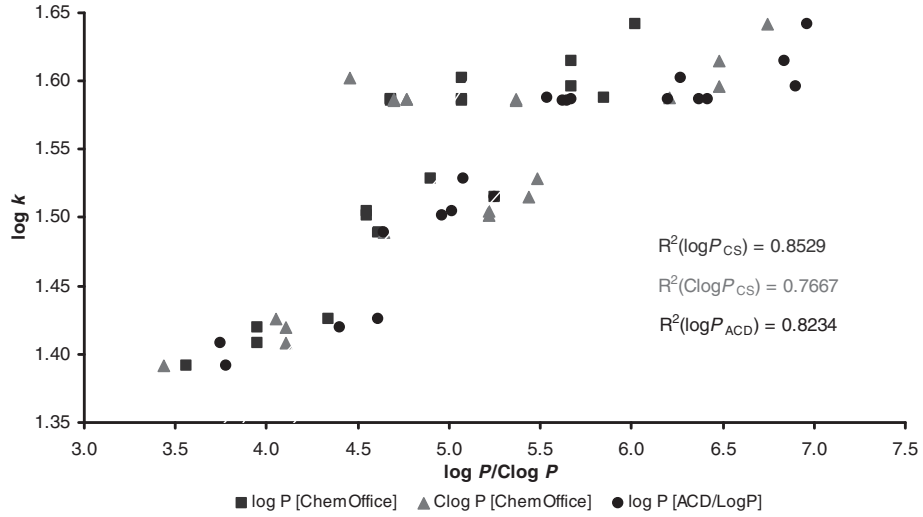


Figure 2. Match of calculated (log*P*/*C*log*P*) data using two programs with experimentally found log*k* values.

calculated values. Nevertheless it is evident that substitution by OH in $C_{(8)}$ or trisubstitution of the quinoline ring B by 8-OH-5,7-Cl causes intramolecular interactions, as it was discussed recently.[22,49] Similarly, lipophilicity prediction by software failed when the styryl moiety was mono- or disubstituted by the OH moiety. It is important to note that inter- or intramolecular interactions of various type based on substituent type and position can significantly influence biological activity, e.g. compare **6**/**7** or **17**/**18**. Therefore it can be assumed that log*k* values specify lipophilicity within individual series of the studied compounds more precisely than calculated log*P*/*C*log*P* data.

3. Conclusions

Eleven new and five known ring-substituted styrylquinolines and two oxorhenium(V) complexes of 5,7-dichloro-2-[2-(2-chlorophenyl)vinyl]quinolin-8-ol (**11**) were successfully prepared according to the recently developed procedure. The compounds were characterized and evaluated for their antibacterial and antifungal activity. Compounds 5,7-dichloro-2-[2-(2-ethoxyphenyl)vinyl]quinolin-8-ol (**17**) and 5,7-dichloro-2-(2-{4-[2-(5,7-dichloro-8-hydroxyquinolin-2-yl)vinyl]phenyl}vinyl)quinolin-8-ol (**19**) expressed antifungal activity comparable with or higher than the standard fluconazole, and the same compound **17** expressed a strong effect against *Staphylococcus* strains. Generally, based on the SAR observation it can be concluded that 5,7-dichloroquinolin-8-ol scaffold is essential for high activity together with *ortho* substitution of the styryl moiety by lipophilic electron-neutral and bulky substituent.

4. Experimental

4.1. General

All reagents were purchased from Aldrich. Kieselgel 60, 0.040–0.063 mm (Merck, Darmstadt, Germany) was used for column chromatography. TLC experiments were performed on alumina-backed silica gel 40 F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and evaluated in iodine vapour. Melting points were determined on Boetius PHMK 05 (VEB Kombinat Nagema, Radebeul, Germany) and are uncorrected. Infrared (IR) spectra were recorded on a Smart MIRacle™ ATR ZnSe for Nicolet™ Impact 6700 FT-IR spectrometer (Thermo Scientific, USA). The spectra were obtained by accumulation of 256 scans with 2 cm^{-1} resolution in the region of 4000–600 cm^{-1}. All ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 (399.95 MHz for ^1H and 100 MHz for ^{13}C, Bruker BioSpin Corp., Karlsruhe, Germany. Chemicals shifts are reported in ppm (δ) to internal $Si(CH_3)_4$ as the reference with diffuse, easily exchangeable signals being omitted. Signals are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; bs, broad singlet.

4.2. Synthesis

4.2.1. Condensation of quinoline derivatives with aromatic aldehydes

4.2.1.1. General Method A. The appropriate quinaldine derivative (1 mmol) was mixed thoroughly with four equiv aldehyde, put in an open vessel and exposed to microwave irradiation for 10 min at 180 °C. Then the reaction mixture was cool down to 0 °C, the precipitate was filtered off. The solid was crystallized from ethanol.

4.2.1.2. General Method B. The appropriate quinoline derivative (2,5 mmol) in acetic anhydride was mixed thoroughly with 2 equiv aldehyde and heated under inert gas atmosphere (N_2) during 16 h at 130 °C. Then the liquid was evaporated in vacuo, pyridine and water in ratio 3:1 were added and the mixture further heated for 3 h at 100 °C. Then mixture was evaporated to dryness and solid was crystallized or chromatographed.

4.2.1.2.1. 2-[2-(2-Chlorophenyl)vinyl]quinoline (1). Method A. An beige crystalline compound; yield 7%, Mp. 72–74 °C (lit., 78 °C[50]); HPLC purity 99.58%; UV (nm), λ_{max}/logε: 325.9/3.55; ^1H NMR (400 MHz, DMSO-d_6), δ: 8.40 (d, J = 8.39 Hz, 1H, aromatic), 8.13 (d, J = 16.20 Hz, 1H, vinyl), 8.05–7.95 (m, 3H, aromatic), 7.85 (d, J = 8.54 Hz, 1H, aromatic), 7.78 (ddd, J = 8.43 Hz, J = 6.87 Hz, J = 1.49 Hz, 1H, aromatic), 7.62–7.53 (m, 3H, aromatic), 7.47–7.37 (m, 2H, aromatic); ^{13}C NMR (101 MHz, DMSO-d_6), δ: 155.39, 148.10, 137.28, 134.47, 133.43, 132.11, 130.62, 130.46, 130.36, 129.44, 129.33, 128.34, 128.16, 127.90, 127.74, 126.96, 121.12; EI-HRMS: found 265.06571 (calc. for $C_{17}H_{12}ClN$: 265.06583).

4.2.1.2.2. 2-[2-(2-Hydroxyphenyl)vinyl]quinoline (2). Method B, an yellow solid, yield 6.1%, mp 205 °C[51], HPLC purity 96.13%; UV (nm), λ_{max}/logε: 346.2/3.61; ^1H NMR (400 MHz, DMSO) δ 9.99 (s, 1H, OH), 8.33 (d, J = 8.6 Hz, 1H, aromatic), 8.05 (d, J = 16.5 Hz, 1H, vinyl), 7.99 (d, J = 8.6 Hz, 1H, aromatic), 7.93 (t, J = 7.4 Hz, 1H,

aromatic), 7.80 (d, J = 8.6 Hz, 1H, aromatic), 7.74 (ddd, J = 6.9, 5.9, 1.4 Hz, 1H, aromatic), 7.70 (dd, J = 7.7, 1.4 Hz, 1H, aromatic), 7.57–7.52 (m, 1H, aromatic), 7.48 (d, J = 16.5 Hz, 1H, vinyl), 7.22–7.15 (m, 1H, aromatic), 6.96–6.91 (m, 1H, aromatic), 6.87 (t, J = 7.5 Hz, 1H, aromatic); ^{13}C NMR (101 MHz, DMSO) δ 156.63, 156.30, 148.19, 136.89, 131.22, 130.24, 130.04, 129.09, 128.47, 128.24, 127.87, 127.40, 126.41, 123.48, 120.51, 119.90, 116.52; EI-HRMS: found 247.09938 (calc. for $C_{17}H_{13}NO$: 247.09971).

4.2.1.2.3. 2-[2-(4-Ethoxyphenyl)vinyl]quinoline (3). Method A, an yellow solid, yield 17%, mp 143–145 °C, HPLC purity 98.94%; UV (nm), λ_{max}/log ε: 346.2/3.62; ^{1}H NMR (400 MHz, DMSO) δ 8.32 (d, J = 8.6 Hz, 1H, aromatic), 7.98 (d, J = 8.5 Hz, 1H, aromatic), 7.93 (d, J = 8.0 Hz, 1H, aromatic), 7.84 (d, J = 8.6 Hz, 1H, aromatic), 7.79 (d, J = 16.3 Hz, 1H, vinyl), 7.73 (dd, J = 11.2, 4.2 Hz, 1H, aromatic), 7.68 (d, J = 8.7 Hz, 2H, aromatic), 7.58 – 7.51 (m, 1H, aromatic), 7.33 (d, J = 16.4 Hz, 1H, vinyl), 6.99 (d, J = 8.7 Hz, 2H, aromatic), 4.08 (q, J = 6.9 Hz, 2H, CH_2-ethoxy), 1.35 (t, J = 7.0 Hz, 3H, CH_3-ethoxy); ^{13}C NMR (101 MHz, DMSO) δ 159.60, 158.92, 157.11, 156.46, 148.17, 136.06, 134.35, 131.16, 130.22, 129.24, 128.25, 127.35, 126.81, 122.51, 120.25, 115.27, 114.59, 64.20, 15.10; EI-HRMS: found 275.13145 (calc. for $C_{19}H_{17}NO$: 275.13101).

4.2.1.2.4. 8-Chloro-2-[2-(2-chlorophenyl)vinyl]quinoline (4). Method A, an white crystals, yield 37%, mp 120 °C, HPLC purity 99.72%; UV (nm), λ_{max}/log ε: 335.4/3.54; ^{1}H NMR (400 MHz, DMSO) δ 8.48 (d, J = 8.5 Hz, 1H, aromatic), 8.24 (d, J = 16.1 Hz, 1H, vinyl), 8.05 (dd, J = 7.5, 1.9 Hz, 1H, aromatic), 8.00–7.92 (m, 3H, aromatic), 7.64–7.53 (m, 3H, aromatic), 7.48–7.38 (m, 2H, aromatic); ^{13}C NMR: 101 MHz, DMSO δ ppm 156.08, 143.93, 138.03, 134.28, 133.56, 132.62, 131.66, 130.83, 130.61, 130.53, 130.39, 129.14, 128.19, 128.02, 127.82, 127.08, 122.03; EI-HRMS: found 299.02606 (calc. for $C_{17}H_{11}Cl_2N$: 299.02685).

4.2.1.2.5. 2-[2-(2-Chlorophenyl)vinyl]quinolin-8-ol (5). Method B, an yellow solid, yield 91%, mp 125 °C, m/z = 283.33 $[M+2H]^+$, HPLC purity 97.04%; UV (nm), λ_{max}/log ε: 309.8/3.56; ^{1}H NMR (400 MHz, DMSO) δ 8.45 (d, J = 8.6 Hz, 1H, aromatic), 8.22 (d, J = 16.0 Hz, 1H, vinyl), 8.04 (dd, J = 7.6, 1.8 Hz, 1H, aromatic), 7.88 (dd, J = 7.9, 1.7 Hz, 1H, aromatic), 7.81 (d, J = 8.5 Hz, 1H, aromatic), 7.62–7.53 (m, 4H, aromatic), 7.47–7.37 (m, 2H, aromatic); ^{13}C NMR (101 MHz, DMSO) δ 169.73, 154.89, 147.65, 140.71, 137.52, 134.11, 133.59, 131.16, 130.75, 130.40, 129.79, 128.90, 128.14, 127.74, 126.64, 126.14, 122.41, 122.15; EI-HRMS: found 281.06112 (calc. for $C_{17}H_{12}ClNO$: 281.06074).

4.2.1.2.6. 2-[2-(2-Hydroxyphenyl)vinyl]quinolin-8-ol (6). Method B, an orange solid, yield 30%, mp 316 °C, m/z = 264.55 $[M+2H]^+$, HPLC purity 99.63%; UV (nm), λ_{max}/log ε: 292.6/3.52; ^{1}H NMR (400 MHz, DMSO) δ 11.72 (bs, 1H, OH), 10.61 (s, 1H, OH), 8.82 (d, J = 9.0 Hz, 1H, aromatic), 8.43 (d, J = 7.4 Hz, 1H, aromatic), 8.29 (d, J = 16.4 Hz, 1H, vinyl), 8.09 (d, J = 16.6 Hz, 1H, vinyl), 7.62 (d, J = 5.0 Hz, 3H aromatic), 7.47 (s, 1H, aromatic), 7.30 (t, J = 7.6 Hz, 1H, aromatic), 7.07 (d, J = 8.2 Hz, 1H, aromatic), 6.94 (t, J = 7.5 Hz, 1H, aromatic); ^{13}C NMR (101 MHz, DMSO) δ 158.06, 153.50, 149.07, 144.18, 139.91, 132.63, 129.77, 128.44, 122.25, 120.46, 120.01, 119.15, 118.70, 117.16, 116.60; EI-HRMS: found 263.09370 (calc. for $C_{17}H_{13}NO_2$: 263.09463).

4.2.1.2.7. 2-[2-(3-Hydroxyphenyl)vinyl]quinolin-8-ol (7). Method B, an orange solid, yield 50%, mp 215 °C, m/z = 264.45 $[M+2H]^+$, HPLC purity 99.91%; UV (nm), λ_{max}/log ε: 296.9/3.55; ^{1}H NMR (400 MHz, DMSO) δ 10.93 (bs, 1H, OH), 9.73 (bs, 1H, OH), 8.68 (s, 1H, aromatic), 8.24 (s, 1H, aromatic), 8.14 (d, J = 16.3 Hz, 1H, vinyl), 7.72 (d, J = 16.3 Hz, 1H, vinyl), 7.62–7.50 (m, 2H, aromatic), 7.31 (t, J = 7.8 Hz, 2H, aromatic), 7.17 (d, J = 7.7 Hz, 1H, aromatic), 7.14 (d, J = 18.3 Hz, 1H, aromatic), 6.86 (dd, J = 8.0, 1.8 Hz, 1H, aromatic); ^{13}C NMR (101 MHz, DMSO) δ 158.33, 153.17, 150.63, 141.76, 140.68, 137.34, 130.58, 129.06, 128.46, 123.86, 120.23, 119.37, 118.58, 117.78, 114.58; EI-HRMS: found 263.09391 (calc. for $C_{17}H_{13}NO_2$: 263.09463).

4.2.1.2.8. 2-[2-(2,4-Dihydroxyphenyl)vinyl]quinolin-8-ol (8). Method B, a brick-red solid, yield 58.6%, mp 273 °C, m/z = 301.45 $[M+Na]^+$, HPLC purity 99.65%; UV (nm), λ_{max}/log ε: 361.9/3.58; ^{1}H NMR (400 MHz, DMSO) δ 11.89 (bs, 1H, OH), 10.66 (s, 1H, OH), 10.32 (bs, 1H, OH), 8.78 (d, J = 9.1 Hz, 1H, aromatic), 8.42 (d, J = 9.1 Hz, 1H, aromatic), 8.23 (d, J = 16.2 Hz, 1H, vinyl), 7.95 (d, J = 16.1 Hz, 1H, vinyl), 7.59 (t, J = 6.6 Hz, 2H, aromatic), 7.46 (t, J = 7.4 Hz, 2H, aromatic), 6.55 (d, J = 2.1 Hz, 1H, aromatic), 6.42 (dd, J = 8.6, 2.0 Hz, 1H, aromatic); ^{13}C NMR (101 MHz, DMSO) δ 162.80, 160.31, 154.19, 148.37, 143.88, 141.52, 131.84, 129.27, 128.53, 127.84, 118.75, 116.81, 115.54, 114.33, 109.04, 103.33; EI-HRMS: found 279.08949 (calc. for $C_{17}H_{13}NO_3$: 279.08954).

4.2.1.2.9. 2-[2-(2-Ethoxyphenyl)vinyl]quinolin-8-ol (9). Method B, an yellow solid, yield 57.5%, mp 100 °C, m/z = 313.92 $[M-H+Na]^{l+}$, HPLC purity 90.83%; UV (nm), λ_{max}/log ε: 303.5/3.53; ^{1}H NMR (400 MHz, DMSO) δ 9.53 (s, 1H, OH), 8.28 (d, J = 8.6 Hz, 1H, aromatic), 8.12 (d, J = 16.5 Hz, 1H, vinyl), 7.78 (d, J = 8.6 Hz, 1H, aromatic), 7.72 (t, J = 4.1, 2.1 Hz, 1H, aromatic), 7.52 (d, J = 16.5 Hz, 1H, vinyl), 7.42–7.30 (m, 3H, aromatic), 7.12–7.06 (m, 2H, aromatic), 7.02 (t, J = 7.5 Hz, 1H, aromatic), 4.17 (q, J = 6.9 Hz, 2H, CH_2-ethoxy), 1.46 (t, J = 7.0 Hz, 3H, CH_3-ethoxy); ^{13}C NMR (101 MHz, DMSO) δ 157.12, 154.48, 153.38, 138.69, 136.92, 130.37, 130.13, 129.53, 128.50, 128.14, 127.41, 125.44, 121.13, 120.94, 118.09, 113.02, 111.79, 64.15, 15.13; EI-HRMS: found 291.12606 (calc. for $C_{19}H_{17}NO_2$: 291.12593).

4.2.1.2.10. 2-[2-(4-Ethoxyphenyl)vinyl]quinolin-8-ol (10). Method B, an yellow solid, yield 20.5%, mp 130 °C, m/z = 313.77 $[M-H+Na]^{l+}$, HPLC purity 97.18%; UV (nm), λ_{max}/log ε: 306.8/3.51; ^{1}H NMR (400 MHz, DMSO) δ 8.37 (d, J = 8.7 Hz, 1H, aromatic), 7.87–7.82 (m, 2H, aromatic), 7.77 (d, J = 16.3 Hz, 1H, vinyl), 7.68 (d, J = 8.7 Hz, 2H, aromatic), 7.57–7.47 (m, 2H, aromatic), 7.30 (d, J = 16.3 Hz, 1H, vinyl), 6.99 (d, J = 8.7 Hz, 2H, aromatic), 4.13–4.04 (q, 2H, CH_2-ethoxy), 1.35 (t, J = 7.0 Hz, 3H, CH_3-ethoxy); ^{13}C NMR (101 MHz, DMSO) δ 191.71, 159.52, 154.28, 153.28, 138.60, 136.83, 134.61, 132.28, 129.44, 129.11, 127.96, 127.31, 126.06, 121.25, 118.02, 115.29, 111.56, 64.20, 15.10; EI-HRMS: found 291.12481 (calc. for $C_{19}H_{17}NO_2$: 291.12593).

4.2.1.2.11. 5,7-Dichloro-2-[2-(2-chlorophenyl)vinyl]quinolin-8-ol (11). Method A, a beige crystals, yield 47.0%, mp 205–210 °C, m/z = 348.27 $[M]^-$; HPLC purity 98.74%; UV (nm), λ_{max}/log ε: 319.9/3.61; ^{1}H NMR (400 MHz, DMSO) δ 8.48 (d, J = 8.7 Hz, 1H, aromatic), 8.36 (d, J = 16.2 Hz, 1H, vinyl), 7.98 (d, J = 8.8 Hz, 1H, aromatic), 7.94 (dd, J = 7.6; 2.0 Hz, 1H, aromatic); 7.77 (s, 1H, aromatic); 7.58–7.50 (m, 2H, aromatic); 7.47–7.36 (m, 2H, aromatic); ^{13}C NMR: 101 MHz, DMSO δ ppm 155.17, 149.33, 139.39, 134.81, 133.99, 133.57, 132.06, 131.14, 130.83, 130.44, 128.27, 128.17, 127.85, 124.37, 123.01, 119.63, 116.28; EI-HRMS: found 348.98262 (calc. for $C_{17}H_{10}Cl_3NO$: 348.98280).

4.2.1.2.12. 5,7-Dichloro-2-[2-(2-hydroxyphenyl)vinyl]quinolin-8-ol (12). Yield 87% of a beige crystalline compound; mp. 141 °C; Anal. Calcd: C, 61.47; H, 3.34; N, 4.22. Found: C, 61.87; H, 3.05; N, 4.12%; HPLC purity 98.10%; UV (nm), λ_{max}/log ε: 309.9/3.61.[22]

4.2.1.2.13. 5,7-Dichloro-2-[2-(4-hydroxyphenyl)vinyl]quinolin-8-ol (13). Yield 86% of a beige crystalline compound; mp. 198 °C; Anal. Calcd: C, 61.47; H, 3.34; N, 4.22. Found: C, 61.54; H, 3.32; N, 4.28; HPLC purity 98.52%; UV (nm), λ_{max}/log ε: 309.9/3.58.[22]

4.2.1.2.14. 5,7-Dichloro-2-[2-(2,4-dihydroxyphenyl)vinyl]quinolin-8-ol (14). Yield 83% of a beige crystalline compound; mp. 102–1030 °C; HPLC purity 98.96%; UV (nm), λ_{max}/log ε: 321.9/3.65.[22]

4.2.1.2.15. 5,7-Dichloro-2-[2-(3,4-dihydroxyphenyl)vinyl]quinolin-8-ol (15). Yield 44% of an ocher crystals, mp 250–252 °C.[52]

4.2.1.2.16. 5,7-Dichloro-2-[2-(3,5-dihydroxyphenyl)vinyl]quinolin-8-ol (16). Yield 93% of a beige crystalline compound, mp. 206 °C; HPLC purity 98.59%; UV (nm), λ_{max}/log ε: 314.5/3.68.[22]

4.2.1.2.17. 5,7-Dichloro-2-[2-(2-ethoxyphenyl)vinyl]quinolin-8-ol (17). Method A, a beige crystalline compound, yield 26%, mp 100–102 °C, HPLC purity 98.24%; UV (nm), λ_{max}/log ε: 317.5/3.62; ^{1}H NMR (400 MHz, DMSO) δ 8.44 (d, J = 8.83 Hz, 1H, aromatic), 8.14 (d, J = 16.49 Hz, 1H, vinyl), 7.79 (d, J = 8.84 Hz, 1H, aromatic), 7.71 (dd, J = 7.71, 1.53 Hz, 1H, aromatic), 7.52 (s, 1H, aromatic), 7.46 (d, J = 16.38 Hz, 1H, vinyl), 7.34 (t, J = 7.82, 7.82 Hz, 1H, vinyl), 7.03 (t, J = 7.50, 7.50 Hz, 1H. aromatic), 6.96 (d, J = 8.27 Hz, 1H, aromatic), 4.18 (q, J = 6.96, 6.96, 6.95 Hz, 2H, CH_2-ethoxy), 1.56 (t, J = 6.97, 6.97 Hz, 3H, CH_3-ethoxy); ^{13}C NMR (101 MHz, DMSO) δ 157.40, 156.29, 149.13, 139.36, 133.75, 132.51, 130.87, 129.11, 128.55, 127.39, 125.12, 124.06, 122.51, 121.17, 119.64, 116.09, 113.08, 64.21, 15.15; EI-HRMS: found 359.04682 (calc. for $C_{19}H_{15}NO_2$: 359.04798).

4.2.1.2.18. 5,7-Dichloro-2-[2-(4-ethoxyphenyl)vinyl]quinolin-8-ol (18). Method A, a beige crystalline compound, yield 9%, mp 156–158 °C, HPLC purity 96.79%; UV (nm), λ_{max}/log ε: 315.8/3.57; ^{1}H NMR (400 MHz, DMSO) δ 8.43 (d, J = 8.76 Hz, 1H, aromatic), 8.24 (d, J = 16.11 Hz, 1H, vinyl), 7.89 (d, J = 8.81 Hz, 1H, aromatic), 7.72 (s, 1H, aromatic), 7.67 (d, J = 8.70 Hz, 2H, aromatic), 7.36 (d, J = 16.13 Hz, 1H, vinyl), 7.02 (d, J = 8.73 Hz, 2H, aromatic), 4.09 (q, J = 6.97, 6.95, 6.95 Hz, 2H, CH_2-ethoxy), 1.36 (t, J = 6.96, 6.96 Hz, 3H, CH_3-ethoxy); ^{13}C NMR (101 MHz, DMSO) δ 159.88, 156.11, 149.02, 139.26, 136.72, 133.68, 129.44, 129.15, 127.15, 124.92, 123.89, 122.79, 119.64, 115.80, 115.38, 100.00, 63.68, 15.11; EI-HRMS: found 359.04723 (calc. for $C_{19}H_{15}NO_2$: 359.04798).

4.2.1.2.19. 5,7-Dichloro-2-(2-{4-[2-(5,7-dichloro-8-hydroxyquinolin-2-yl)vinyl]phenyl}vinyl)quinolin-8-ol (19). Yield 69% of a dark brown crystalline compound; mp 300 °C; HPLC purity 98.68%; UV (nm), λ_{max}/log ε: 309.9/3.67.[22]

Complexes [$ReOCl_2$(L)(PPh_3)] (**20**) and [$ReOBr_2$(L)(PPh_3)] (**21**), L = 5,7-Dichloro-2-[2-(2-chlorophenyl)vinyl]quinolin-8-ol (**11**), were obtained according to a previously described procedure.[30]

4.3. In vitro antifungal susceptibility testing

The broth microdilution test[53–55] was used for the assessment of in vitro antifungal activity of the synthesized compounds against *Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156 (CT), *Candida krusei* ATCC 6258 (CK), *Candida glabrata* 20/I (CG), *Trichosporonbeigelii* 1188 (TB), *Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC), and *Trichophyton mentagrophytes* 445 (TM). Fluconazole (FLU) was used as the standard since it is a clinically used antimycotic drug. The procedure was performed with a twofold dilution of the compounds in RPMI 1640 (Sevapharma a.s., Prague, Czech Republic) buffered to pH 7.0 with 0.165 mol of 3-morpholino-propane-1-sulphonic acid (MOPS, Sigma, Germany). The final concentrations of the compounds ranged from 500 to 0.975 μmol/L. Drug-free controls were included. The minimum inhibitory concentration (MIC) determination was performed according to the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) reference protocol M27-A2 for yeasts (IC_{80} value) and M38-A for moulds (IC_{50} value). IC_{80} and IC_{50} were defined as an 80% resp. 50% or greater reduction of growth in comparison with the control. The values of MICs were determined after 24 and 48 h of static incubation at 35 °C. For *T. mentagrophytes*, the final MICs were determined after 72 and 120 h of incubation. The results are summarized in Tables 1 and 2.

4.4. In vitro antibacterial susceptibility testing

The synthesized compounds were evaluated for in vitro antibacterial activity against *Staphylococcus aureus* CCM 4516/08 (SA), methicillin-resistant *Staphylococcus aureus* H 5996/08 (MRSA), *Staphylococcus epidermidis* H 6966/08 (SE) and *Enterococcus sp.* J 14365/08 (EF). Bacitracin (BAC), penicillin V (PEN) and ciprofloxacin (CPX) were used as the standards since it is a clinically used antibacterial drug. All strains were sub-cultured on nutrient agar (HiMedia) and maintained on the same medium at 4 °C. Prior to testing, each strain was passaged onto nutrient agar, and bacterial inocula were prepared by suspending a small portion of bacterial colony in sterile 0.85% saline. The cell density was adjusted to 0.5 McFarland units using a densitometer (Densi-La-Meter, PLIVA Lachema Diagnostika, Czech Republic). The final inoculum was made by 1:20 dilution of the suspension with the test medium (Mueller–Hinton broth). The compounds were dissolved in DMSO, and the anti-bacterial activity was determined using Mueller–Hinton broth (MH broth, HiMedia, pH 7.0 ± 0.2). Controls consisted of MH broth and DMSO alone. The final concentration of DMSO in the MH broth did not exceed 1% (v/v) of the total solution composition. The activity of the studied compounds was determined as the minimal inhibition concentration (MIC) according to NCCLS guidelines[56] using broth microdilution test. The MICs were defined as 90% inhibition of bacterial growth compared to the control and were determined after 24 and 48 h of static incubation at 37 °C. After incubation MICs were read visually as an absorbance at 540 nm. The results are shown in Tables 1 and 2.

4.5. Lipophilicity determination using HPLC (capacity factor *k*/calculated log*k*)

A Waters Alliance 2695 XE HPLC separation module and a Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, USA) were used. A Symmetry® C_{18} 5 μm, 4.6 × 250 mm, Part No. WAT054275 (Waters Corp., Milford, MA, USA) chromatographic column was used. The HPLC separation process was monitored by Empower™ 2 Chromatography Data Software, Waters 2009 (Waters Corp., Milford, MA, USA). A mixture of MeOH p.a. (55%) and H_2O-HPLC – Mili-Q Grade (45%) was used as a mobile phase. The total flow of the column was 0.9 mL/min, injection volume, 30 μL, column temperature, 30 °C, and sample temperature, 10 °C. The detection wavelength of 210 nm was chosen. The KI methanolic solution was used for the dead time (t_D) determination. Retention times (t_R) were measured in minutes. The capacity factors k were calculated using the Empower™ 2 Chromatography Data Software according to formula $k = (t_R - t_D)/t_D$, where t_R is the retention time of the solute, whereas t_D denotes the dead time obtained using an unretained analyte. Log k, calculated from the capacity factor k, is used as the lipophilicity index converted to log$P$ scale. The log$k$ values of the individual compounds are shown in Table 3.

4.6. Lipophilicity calculations

Log P, *i.e.* the logarithm of the partition coefficient for *n*-octanol/water, was calculated using the programs CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) and ACD/LogP ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). Clog P values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were generated by means of CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) software. The results are shown in Table 3.

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