

# Aminobenzylated 4-Nitrophenols as Antibacterial Agents Obtained from 5-Nitrosalicylaldehyde through a Petasis Borono–Mannich Reaction

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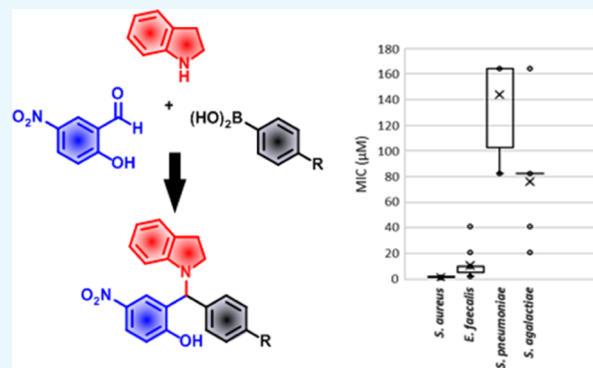
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## Supporting Information

**ABSTRACT:** Multidrug-resistant bacteria are one of the current biggest threats to public health and are responsible for most nosocomial infections. Herein, we report the efficient and facile synthesis of antibacterial agents aminoalkylphenols, derived from 5-nitrosalicylaldehyde and prepared through a Petasis borono–Mannich multicomponent reaction. Minimum inhibitory concentrations (MICs) as low as 1.23  $\mu\text{M}$  for a chlorine derivative were determined for multidrug-resistant Gram-positive bacteria, namely, *Staphylococcus aureus* and *Enterococcus faecalis*, two of the main pathogens responsible for infections in a hospital environment. The most promising antibacterial agents were further tested against eight strains of four Gram-positive species in order to elucidate their antibacterial broadness. In vitro cytotoxicity assays of the most active aminoalkylphenol revealed considerably lower toxicity against mammalian cells, as concentrations one order of magnitude higher than the determined MICs were required to induce human keratinocyte cell death. The phenol moiety was verified to be important in deeming the antibacterial properties of the analyzed compounds, although no correlation between such properties and their antioxidant activity was observed. A density functional theory computational study substantiated the ability of aminoalkylphenols to serve as precursors of *ortho*-quinone methides.



## INTRODUCTION

The discovery and development of antibiotics stands as one of mankind's greatest achievements. However, the number of infections provoked by multidrug-resistant bacteria is increasing at a remarkable pace, a problem that science has not been able to address.<sup>1</sup> The unrestrained use of antibiotics in the last 50 years has been advocated as one of the reasons for the colonization and infection due to drug resistant bacteria.<sup>2,3</sup> It is estimated that in Europe and the United States, 48 000 people die each year because of multidrug-resistant bacterial infections,<sup>4</sup> and that in 2016, there were 600 000 worldwide cases with resistance to rifampicin, of which 490 000 had multidrug-resistant tuberculosis.<sup>5</sup> If no actions are taken to tackle this severe public health issue, it is foreseen that by 2050, the death toll can rise up to 10 million lives when considering drug resistance of only six pathogens.<sup>6</sup> This is even more alarming in the hospital environment or other health care

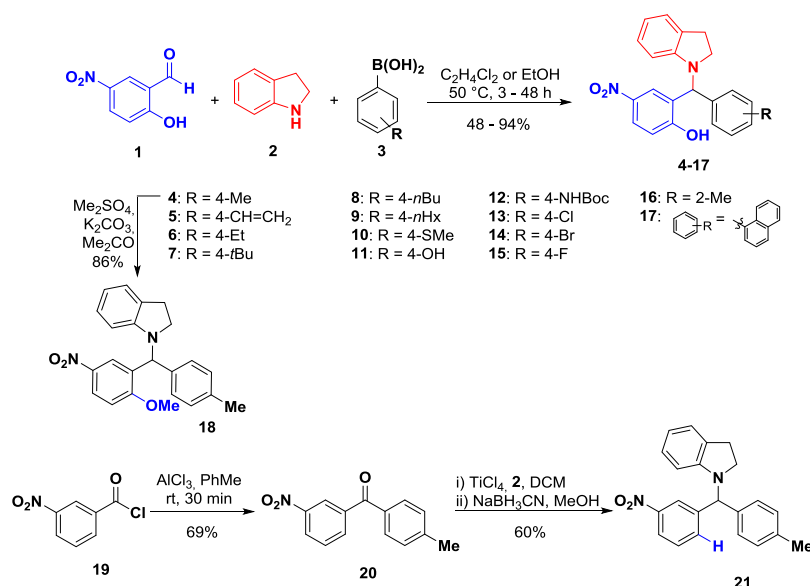
facilities, where acquired infections are associated with significant morbidity and mortality, additional health care expenditure, greater use of broad spectrum antibiotics (which amplifies the emergence and reemergence of drug resistant microorganisms), and increased costs.<sup>7</sup> According to WHO estimates, of every hundred hospitalized patients, 7 in developed and 10 in developing countries will acquire at least one nosocomial infection.<sup>8</sup> Besides intrinsic patient factors (age, duration of hospitalization, or underlying diseases), many extrinsic factors, such as caregivers' practices, surgical operations, the use of invasive devices, administration of broad-spectrum antibiotics, or immunosuppressive agents, are risk predisposers to these infections.<sup>9,10</sup> The majority of

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## Scheme 1. Preparation of Aminoalkylphenols 4–17 and Derivatives 18 and 21

Table 1. Antimicrobial Activity of Aminoalkylphenols 4–17 and Derivatives 18 and 21 against Laboratory-Adapted Strains<sup>a</sup>

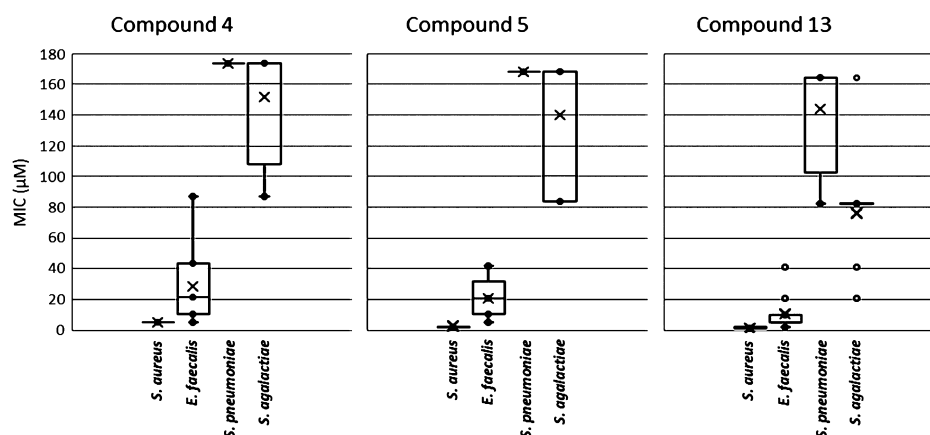
compd.	<i>S. aureus</i> ATCC25923 (MSSA)		<i>S. aureus</i> CIP6538		<i>S. aureus</i> CIP106760 (MRSA)		<i>E. faecalis</i> 29212		<i>E. faecalis</i> ATCC51299 (VRE)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4	21.3	347	10.8	43.4	2.71	5.42	10.8	86.7	21.7	173
5	10.5	168	5.24	41.9	1.31	5.24	5.24	83.9	10.5	168
6	4.93	39.5	2.47	2.47	1.23	<1.23	2.47	19.7	2.47	39.5
7	4.93	39.5	2.47	19.7	1.23	>9.86	2.47	19.7	2.47	>19.7
8	4.93	39.5	2.47	9.86	2.47	<1.23	4.93	>39.5	4.93	39.5
9	9.86	19.7	9.86	39.5	19.7	39.5	9.86	>78.9	4.93	78.90
10	9.86	78.9	2.47	4.93	2.47	4.93	4.93	39.50	4.93	39.5
11	43.1	1379	86.2	689	86.2	1379	172	1379	345	1379
12	67.7	541	33.9	270	67.7	1083	135	1083	135	1083
13	2.47	9.86	1.23	2.47	1.23	<1.23	1.23	>9.86	1.23	19.7
14	2.30	36.8	1.15	4.59	1.15	4.59	1.15	9.19	2.30	9.19
15	10.7	85.8	5.36	21.44	2.68	21.4	2.68	42.90	5.36	171
16	4.93	9.86	1.23	4.93	1.23	<1.23	2.47	>19.7	1.23	39.5
17	4.93	>19.7	1.23	9.86	2.47	<2.47	4.93	>39.5	2.47	>19.7
18	166	1335	83.5	333	333	1335	166	1335	166	1335
21	373	1495	93.5	747	747	1495	186	1495	373	1495
control	5.40 <sup>b</sup>	nd <sup>e</sup>	1.35 <sup>b</sup>	nd <sup>e</sup>	<1.50 <sup>c</sup>	nd <sup>e</sup>	3.07 <sup>c</sup>	nd <sup>e</sup>	<5.83 <sup>d</sup>	nd <sup>e</sup>

<sup>a</sup>MICs and MBCs are shown in  $\mu\text{M}$ . <sup>b</sup>Vancomycin used as a control. <sup>c</sup>Norflaxacin used as a control. <sup>d</sup>Rifampicin used as a control. <sup>e</sup>Not determined.

nosocomial infections are caused by bacteria, with *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus*, and *Streptococcus* spp. leading.<sup>11</sup> Despite the society being in urgent need for new antibacterial agents, the small fraction of yearly revenue per patient generated by such agents when compared with anticancer drugs have pushed the pharmaceutical companies away from their research and development programs on antibiotics.<sup>12,13</sup> Opportunely, the development of antibiotics has been relaunched by small biotech companies, and alternatives to antibiotics, including “non-compound” approaches and small molecule “resistance breakers”, are growing trends in the field.<sup>14</sup>

Motivated by the antimicrobial activity of salicylaldehydes<sup>15–17</sup> and their Schiff bases,<sup>18</sup> we have recently reported the preparation and antimicrobial screening of several aminoalkylphenols.<sup>19</sup> From screening of a library of 43

compounds, some structural features pivotal for the antibacterial activity have been identified, namely, indoline as the amine counterpart and a *para*-nitrophenol group. Promising antibacterial activity against several resistant microorganisms such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE), as well as other non-pathogenic Gram-positive strains, was reported for some of such derivatives. Notwithstanding the fact that aminoalkylphenols can be problematic compounds in high-throughput screenings because of their ability to form reactive quinone methides or acting as metal chelators,<sup>20,21</sup> their anticancer and cytotoxic properties continue to be extensively explored.<sup>22,23</sup> Almost simultaneously to our report, Roman and co-workers reported the antibacterial properties of 1-aminoalkyl 2-naphthols, in which similar properties were observed against Gram-positive bacteria.<sup>24</sup> Somewhat different structural



**Figure 1.** MIC values of compounds 4, 5, and 13 for diverse clinical isolates of *S. aureus*, *E. faecalis*, *S. pneumoniae*, and *S. agalactiae*. A boxplot chart was generated using R statistical software v. 3.4.2 and consists of boxes (median and interquartile range) and whiskers that extend to the most extreme data points that were no more than 1.5 times the interquartile range from the box. Boxes represent the variability of MIC values found among all strains from each species. The horizontal black line within each box marks the median, while the lowest and the highest coverage values observed are represented by the extremes of the whisker below and above each box, respectively. Outliers are indicated by open circles.

requirements for antibacterial activity were determined by Roman, as thiophen-2-yl derivatives showed the best activity, regardless of the nature of the amine moiety. The antibacterial properties of other Mannich bases derived from naphthol have been previously explored.<sup>25</sup> Aminoalkylphenols have also been reported to inhibit biofilm formation<sup>26</sup> and classified as anti-infectives rather than traditional antibiotics as they cure *Caenorhabditis elegans* of an *Enterococcus faecalis* infection at significantly lower concentration than the one required for in vitro bacteria growth inhibition.<sup>27</sup> After observing that the concentration required for an antibacterial effect of some promising aminoalkylphenols was generally inferior to the cytotoxic concentrations,<sup>19</sup> we then set to expand our previous library in order to optimize the antibacterial properties.

## RESULTS AND DISCUSSION

As multicomponent reactions are a remarkable tool for the easy preparation of libraries of compounds,<sup>28,29</sup> our previous library of aminoalkylphenols was expanded using the Petasis borono–Mannich<sup>30,31</sup> reaction (Scheme 1). Starting from 5-nitrosalicylaldehyde **1** and indoline **2**, the corresponding iminium was formed in situ and trapped with different aryl boronic acids **3** to provide the desired tertiary amines **4–17**. Ether **18** was prepared by methylation of the phenol functionality upon treatment of **4** with dimethyl sulfate. Tertiary amine **21**, lacking the oxygen functionality in the nitroaryl substituent, was prepared by Friedel–Crafts acylation of toluene with *meta*-nitro benzoyl chloride **19** followed by reductive amination with indoline.

The selection of compounds to be prepared was reasoned based on two aspects, first, by replacing the 4-methyl substituent in **4** with other isosteres because we have previously identified position 4 of the phenyl moiety to be important in conferring the desired antibacterial activity. Therefore, the 4-methyl substituent in **4** was replaced by other alkyl substituents or heteroatoms such as thioether, hydroxyl, carbamate, and halogens. Second, in order to fully disclose the importance of the phenol moiety in the nitroaryl moiety, the methyl ether derivative **18** and tertiary amine **21** were also prepared and their antibacterial properties were evaluated.

The synthesized aminoalkylphenols **4–17** and derivatives **18** and **21** were tested against a large panel of Gram-positive microorganisms (Table 1), namely, three *S. aureus* strains (a reference ATCC, a methicillin-resistant and a non-resistant strain) and two *E. faecalis* strains (a reference ATCC and a vancomycin-resistant strain). An *E. coli* strain, representative of Gram-negative microorganisms, was also tested, although no antibacterial properties were observed for any of the compounds assayed (not shown). The modification of the para-methyl substituent to other alkyl substituents resulted in derivatives **6–8** of a similar antibacterial profile, except when a longer C<sub>6</sub> alkyl chain was introduced in **9**. Replacing the alkyl substituent by a heteroatom functionality such as thioether, alcohol, or a carbamate also had a detrimental effect, as observed for **10–12**, respectively. Gladly, the introduction of a halide such as chloride (**13**) or bromide (**14**) resulted in not only augmenting the bacterial inhibition growth but also in efficacy of killing the bacteria. Notably, a more effective activity was determined for chloro derivative **13**, for which minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) as low as 1.23 µM were determined for most of the bacteria tested. On the other hand, the presence of fluoride (**15**), change of the position of the methyl substituent to the 2-position (**16**), or replacement of the *p*-tolyl moiety by a 1-naphthyl (**17**) did not confer any improvement to the antibacterial characteristics of the aminoalkylphenols. Compounds **18** and **21** were poor antibacterials, as the MIC and MBC values increased by one to two orders of magnitude when compared with parental **4**, hence evidencing the importance of the phenol functionality in conferring the desired properties.

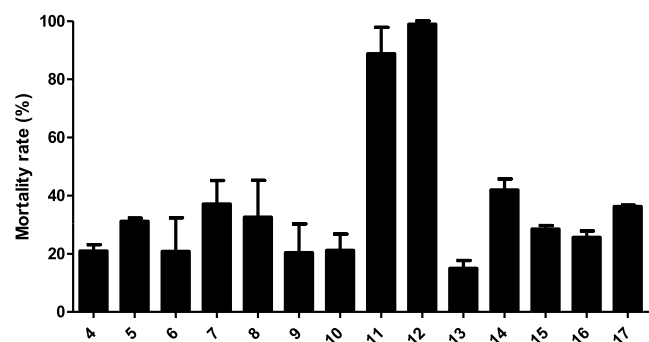
In order to further elucidate the antibacterial broadness of the most promising synthesized aminoalkylphenols, compounds **4**, **5**, and **13** were tested against a larger panel of pathogenic Gram-positive bacterial strains with dissimilar resistance phenotypes. Overall, 32 strains from *S. aureus*, *E. faecalis*, *Streptococcus pneumoniae* (*S. pneumoniae*), and *Streptococcus agalactiae*—group B (GBS) (eight clinical isolates from each species) were tested (Figure 1 and Table S1).

In general, similar MIC values were observed for *S. aureus* and *E. faecalis* clinical isolates when compared with those seen for the ATCC adapted strains. Some exceptions occurred,

where, at some instances, a maximum of fourfold differences were observed, which may be due both to the dissimilar genetic background of the clinical isolates and to the extensive in vitro passaging of the ATCC strains. As clearly shown in Figure 1, all three selected compounds seem to be much more effective against *S. aureus* and *E. faecalis*, displaying a mean of MIC values at least 5.4-fold lower than that observed for *S. pneumoniae* and *S. agalactiae*. For the former species, for which the results were encouraging, compound 13 revealed the higher antimicrobial efficacy, given by lower and more homogeneous MIC values.

*S. aureus* and *E. faecalis* are among the most important bacteria causing infections in the hospital environment.<sup>32–34</sup> While *S. aureus* is the primary cause of lower respiratory tract infections and surgical site infections and the second leading cause of nosocomial bacteremia, pneumonia, and cardiovascular infections, *E. faecalis* is responsible for urinary tract infections (associated with instrumentation and antimicrobial administration) followed by intra-abdominal and pelvic infection, surgical wound infection, bacteremia, endocarditis, neonatal sepsis, and rarely meningitis.<sup>11</sup> Furthermore, because of the large usage of broad-spectrum antibiotics in the hospital environment, both bacteria (mostly *S. aureus*) are able to emerge and reemerge as multidrug-resistant clones, which complicates the treatment of the caused nosocomial infections.<sup>35,36</sup>

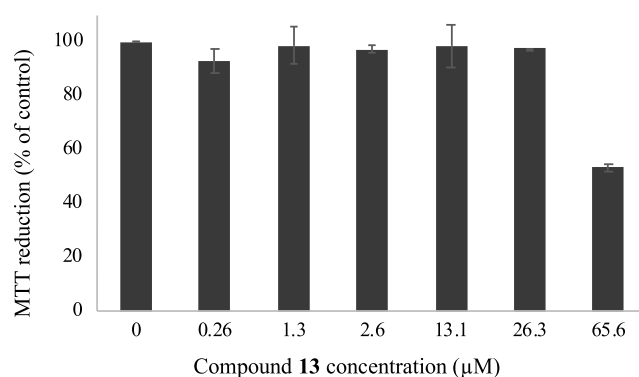
A test of lethality to *Artemia salina* brine shrimp was performed<sup>37</sup> in order to evaluate the toxicity of the different compounds, for which concentrations of 0.1 mg/mL (217–277  $\mu$ M) of each compound in dimethylsulfoxide (DMSO) were tested (Figure 2). Delightfully, a residual 15% mortality



**Figure 2.** General toxicity of 4–17 in the mortality rate of *A. salina* brine shrimp assay was performed. Concentrations of 10  $\mu$ g/mL of each compound were tested. The number of dead larvae was recorded after 24 h and used to calculate the lethal concentration (%).

rate was observed for compound 13, while compounds 11 and 12 were highly toxic with mortality rates of 89 and 98%, respectively. Fortunately, these compounds were not active against any microorganism on the antibacterial assay. Of the remaining compounds, none held a mortality rate higher than 42%, which was observed for the bromide derivative 14, followed by compound 5 with a 31% mortality rate. For compounds 4 and 5, mortality rates of 21 and 31% were observed, respectively.

To further characterize the toxicity of the compounds, an in vitro model representative of noncancer human cells was also used. The cytotoxicity profile of compound 13 is shown in Figure 3, while other analogues were previously tested<sup>19</sup> using the same protocol. No cytotoxic effects were observed for

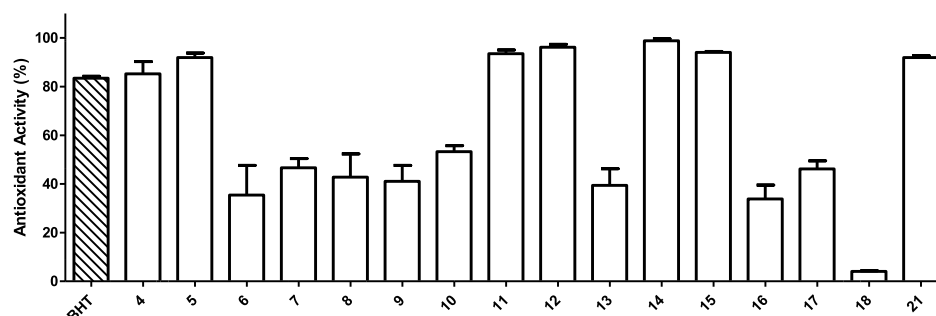


**Figure 3.** Cell viability of HaCat cells exposed to compound 13, as evaluated by the MTT assay. Cells were incubated with increasing concentrations of compound 13 for 24 h. Results are expressed as average values  $\pm$  SD from two independent experiments, each comprising four replicate cultures.

concentrations of compound 13 up to 26.3  $\mu$ M. Only the highest concentration tested (65.6  $\mu$ M) showed relevant cytotoxicity, decreasing cell viability to 53.4  $\pm$  1.5%. Compared with analogue 4 previously tested, for which a concentration of 28  $\mu$ M led to a cell viability of less than 20%,<sup>19</sup> compound 13 shows a better safety profile.

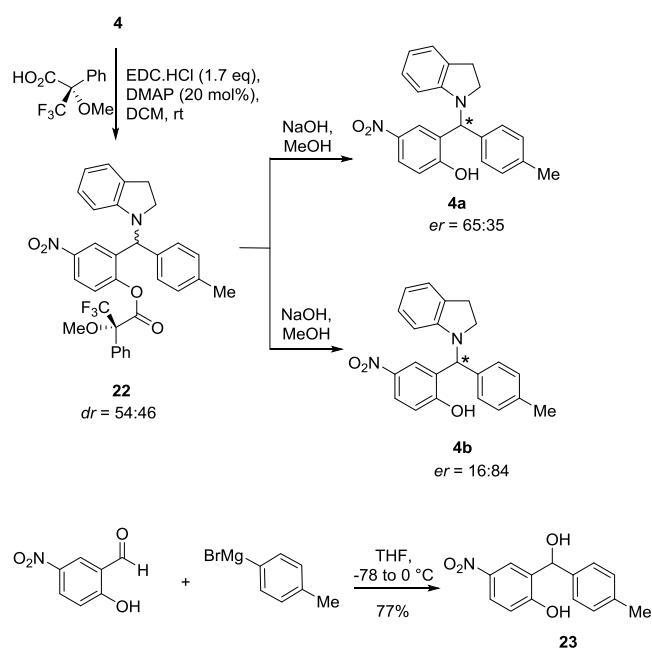
Taking together, the common feature of the presence of the phenol moiety and the lack of significant antibacterial activity of 18 and 21, it was hypothesized that the high activity observed could be related with the antioxidant properties of phenol compounds.<sup>38</sup> Tests of antioxidant activity of compounds 4–17 using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Figure 4) showed that the bromine and fluorine derivatives 14 and 15 had a scavenging activity of 99 and 94%, respectively. Compounds 4 and 5 are also highly antioxidant (85 and 92%, respectively), although not being the case for the chlorine derivative 13. Furthermore, despite the decent antibacterial properties found for derivatives 6–8, their antioxidant activity was rather low (35–47%), opposing the trend observed for compounds 4 and 5. Interestingly, compound 21, lacking the hydroxyl group, showed antioxidant activity in the same range as the most antioxidant species tested. Therefore, a different main mode of action other than an antioxidant mechanism is likely to be the cause of the antibacterial properties observed.

As the identified aminoalkylphenols bear a stereogenic center in the benzylic position, the separation of both enantiomers of 4 was attempted. Hence, 4 was transformed into the corresponding Mosher's ester 22, separated through preparative thin layer chromatography and further hydrolyzed (Scheme 2). Chiral HPLC analysis of the enantiomerically enriched samples of 4 invariably revealed the presence of significant amounts of a minor enantiomer, which equilibrated to a racemic mixture upon standing in solution. Ultimately, the enantiomerically enriched samples 4a and 4b obtained (with enantiomeric ratios of 65:35 and 16:84, respectively) were also tested for their antibacterial activity (Table 2). Triggered by the difficulty in obtaining enantiomerically pure samples of 4, the putative formation of an *ortho*-quinone methide (QM)<sup>39,40</sup> was considered. With this in mind, the benzyl alcohol 23, was also prepared by arylation of the 5-nitrosalicylaldehyde (Scheme 2 bottom) and its action for bacteria inhibition growth also tested (Table 2).



**Figure 4.** Antioxidant activity of 4–18 and 21, evaluated at a concentration of 10  $\mu\text{g/mL}$  (22–29  $\mu\text{M}$ ) by the DPPH method.

### Scheme 2. Preparation of Enantiomeric Rich Samples of 4 and Synthesis of 23



The rather low antimicrobial activity observed for 23 (Table 2) clearly indicates that the antibacterial properties of 4 are not caused by the hydrolyzed QM. On the other hand, both enantiomerically enriched samples 4a and 4b showed similar levels of toxicity against bacteria as the previously tested racemate. Rokita and co-workers have shown that depending on the electronic feature of the QM, they can be stable toward water nucleophilic attack, while being trapped by nucleotides or other biomolecules.<sup>41,42</sup> Specifically, the introduction of an electron-withdrawing group in the *para*-phenol position was shown to decrease the rate of the QM formation, while also decreasing its lifetime due to increased reactivity toward hydrolysis.<sup>42</sup> When monitoring by <sup>1</sup>H NMR a solution of 4 in CDCl<sub>3</sub> in the presence of morpholine, we observed the

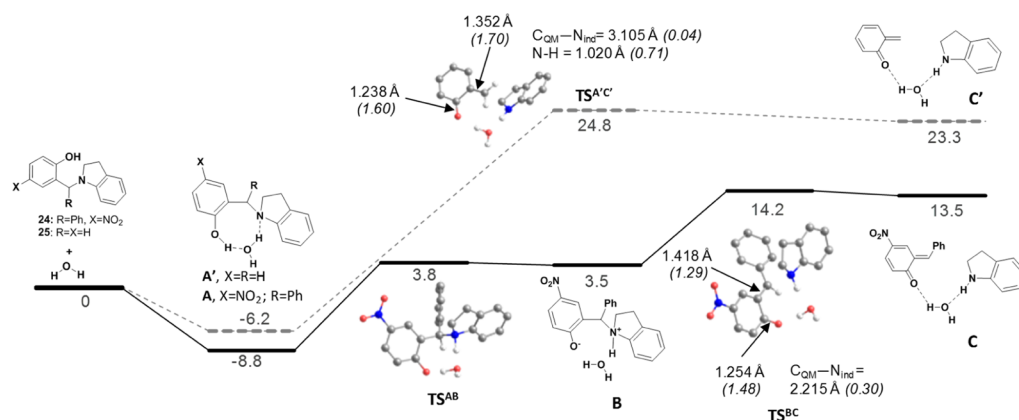
formation of the morpholine derivative in 79% yield after 96 h which was attributed to the chloroform slight acidity (Figure S5 in Supporting Information). Contrastingly, a similar experiment in DMSO-*d*<sub>6</sub> did not show formation of the corresponding morpholine-substituted aminoalkylphenol after 12 h (Figure S4 in Supporting Information). These observations suggest that even though the nitro substituent can decrease the ability of aminoalkylphenols to serve as QM precursors, their formation and fate is highly dependent on the acidity of the medium. An additional stability test showed that 4 remains stable in DMSO-*d*<sub>6</sub> at 35 °C for at least 11 days (Figure S6 in Supporting Information). The ability of water and acids in catalyzing nucleophilic attacks to QMs has been demonstrated both theoretically and experimentally.<sup>43–45</sup> Moreover, the presence of an additional aryl substituent should facilitate the formation of the QM because of additional conjugation.

With the previous observations in mind, the formation of two QMs from 24 and 25, the latter lacking the additional phenyl ring and the nitro moiety, was considered by density functional theory (DFT)<sup>46a</sup> studies in the gas phase (Figure 5), by taking into account the previously described role of bulk water in the alkylation of nitrogen nucleophiles.<sup>43,47,48</sup> As expected, the formation of the QMs is a thermodynamically nonfavored process, as the overall  $\Delta G_{\text{form}}$  of QM from 24 is +16.4 and  $\Delta G_{\text{form}}$  of QM from 25 is 25.8 kcal/mol (Figures S1 and S2 in Supporting Information). Comparison of energy profiles in Figures S1–S3 reveals the stabilization effect of the phenyl substituent on the transition states when considering water as a proton shuttle. Additionally, this substituent and the nitro moiety induce the stabilization of the QMs, as the 25-derived QM is 10.2 kcal/mol less stable than the one derived from 24. It is worth noting that the presence of the nitro substituent induces a two-step mechanism, as zwitterion species B is a local minimum of the energy profile (see Figure S3 of Supporting Information for a similar study without the nitro substituent). On the other hand, the formation of the simplest QM from 25 seems to occur by a synchronous process where water acts as a proton shuttle resulting in indoline release. The interaction of the starting tertiary amines

**Table 2.** Minimum Inhibitory and Bactericidal Concentrations ( $\mu\text{M}$ ) of 4a, 4b, and 23<sup>a</sup>

compd.	<i>S. aureus</i> ATCC25923 (MSSA)		<i>S. aureus</i> CIP106760 (MRSA)		<i>E. faecalis</i> 29212		<i>E. faecalis</i> ATCC51299 (VRE)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4a (er = 65:35)	5.42	43.4	10.8	43.4	1.35	10.8	10.8	86.7
4b (er = 16:84)	10.8	86.7	5.42	43.4	5.42	43.4	5.42	43.4
23	168	1342	335	1342	83.9	671	167	1342

<sup>a</sup>see Table 1 for controls.



**Figure 5.** Gas phase energy profile (PBE1PBE/6-31G\*\*) for formation of quinone methides from 2-(indolin-1-yl(phenyl)methyl)-4-nitrophenol (**24**, solid line) and 2-(indolin-1-ylmethyl)phenol (**25**, dashed line). Transition states are presented with bond distances and Wiberg indexes (in italics) for the more relevant bonds. Energy values are presented in kcal/mol, referring to the initial pair of tertiary amines and water.

with water results in an electronic stabilization due to formation of hydrogen bonds, even though this results in Gibbs energy increase due to the entropy contribution. The transition state for the proton exchange step in **24**,  $TS^{AB}$ , is described by a shortening of the C–O bond of the phenol moiety (1.29 Å in  $TS^{AB}$  vs 1.34 Å in **A**) accompanied by a Wiberg index<sup>b</sup> increase (WI = 1.28 in  $TS^{AB}$  vs 1.09 in **A**). Simultaneously, the O–H bond elongates considerably (1.31 Å in  $TS^{AB}$  vs 1.00 Å in **A**; WI = 0.27 in  $TS^{AB}$  vs 0.59 in **A**), while a new N–H bond is established ( $d = 1.16$  Å, WI = 0.43 in  $TS^{AB}$  vs  $d = 1.81$  Å, WI = 0.06 in **A**). The cleavage of the C–N bond represents the higher energetic barrier, characterized by the  $TS^{BC}$ , where the C–O bond becomes stronger ( $d = 1.25$  Å, WI = 1.48 in  $TS^{BC}$  vs  $d = 1.28$  Å, WI = 1.33 in **B**), and the C–N bond weakens ( $d = 2.22$  Å, WI = 0.30 in  $TS^{BC}$  and  $d = 1.55$  Å, WI = 0.83 in **B**). When similarly taking into account the water-assisted decomposition of **25** to the corresponding QM, a single late transition state  $TS^{A'C'}$  was encountered. The abovementioned events are compacted in a single transition state, namely, shortening of the C–O bond ( $d = 1.24$  Å, WI = 1.60 in  $TS^{A'C'}$  vs  $d = 1.34$  Å, WI = 1.07 in **A**), disruption of the O–H bond ( $d = 1.77$  Å, WI = 0.07 in  $TS^{A'C'}$  vs  $d = 0.99$  Å, WI = 0.61 in **A**), formation of the N–H bond ( $d = 1.02$  Å, WI = 0.71 in  $TS^{A'C'}$  vs  $d = 1.72$  Å, WI = 0.10 in **A**), and cleavage of the C–N bond ( $d = 3.11$  Å, WI = 0.04 in  $TS^{A'C'}$  vs  $d = 1.50$  Å, WI = 0.92 in **A**).

Considering the low activation energy barrier for formation of the **25**-derived QM together with their increased stability as compared to the simplest QM, widely used as a nucleotide alkylating agent,<sup>49,50</sup> is reasonable to suggest this path to be responsible for the antibacterial properties of the Mannich bases reported herein. Moreover, several antibiotics, namely, natural products, are known to have their antibacterial properties ground on their capability to form quinone methides and subsequently alkylate macromolecules.<sup>51,52</sup> Contrarily to the recently reported 1-aminoalkyl 2-naphthols by Roman,<sup>24</sup> the antibacterial properties of the compounds described herein are highly dependent on the amine moiety.<sup>19</sup> As indoline is released upon QM formation, other unforeseen modes of action might be the cause of the antibacterial properties. The rather lower cytotoxic levels of the studied compounds are surely dependent on the different metabolisms of the organisms tested and could be explained by a faster

kinetic profile toward QM formation in the bacterial media.<sup>53,54</sup>

## CONCLUSIONS

In summary, through use of Petasis borono–Mannich multicomponent reaction, starting from 5-nitrosalicylaldehyde, we were able to expand our library of aminoalkylphenols and find more potent antibacterials than the previously reported. The most active compound synthesized **13** was demonstrated to be selectively active against important bacteria responsible for nosocomial infections (*S. aureus* and *E. faecalis*), while only moderately active against *S. pneumoniae* and *S. agalactiae*. Gladly, the most active compounds against bacteria showed cytotoxicity against mammalian cells only at concentrations considerably higher than the determined MICs, as verified through an in vitro model. The phenol moiety was verified to be essential to achieve the desired antibacterial properties; however, such properties do not relate with the antioxidant properties of the compounds tested. As Mannich bases have been demonstrated to be precursors of quinone methides, a computational study was performed, and the results found are in line with such a hypothesis. As quinone methides are highly reactive, they can play a role as antibacterial agents, although not encompassing the hydrolyzed adduct. This aspect is also in agreement with the determined MICs of mixtures of different enantiomeric ratios, as both enantiomers can form a similar reactive intermediate. Nevertheless, the antibacterial mode of action of these compounds remains obscure, and further studies will be reported in due course.

## EXPERIMENTAL SECTION

**General Remarks.** All reagents were obtained from Sigma-Aldrich or TCI and were used without further purification. The reactions were performed under argon atmosphere and monitored by thin-layer chromatography carried out on precoated (Merck TLC silica gel 60 F254) aluminium plates by using UV light as a visualizing agent and cerium molybdate solution or ninhydrin as developing agents. Flash column chromatography was performed on silica gel 60 (Merck, 0.040–0.063 mm). NMR spectra were recorded with Varian Mercury 300 MHz or JEOL ECZR 500 instruments using  $CDCl_3$  or  $DMSO-d_6$  as solvents and calibrated using tetramethylsilane as internal standard. Chemical shifts ( $\delta$ ) are reported in ppm referenced to the  $CDCl_3$  residual peak ( $\delta$

7.26) or TMS peak ( $\delta$  0.00) for  $^1\text{H}$  NMR, to  $\text{CDCl}_3$  ( $\delta$  77.16) for  $^{13}\text{C}$  NMR. The following abbreviations were used to describe peak splitting patterns: s = singlet, d = doublet, t = triplet, m = multiplet. Coupling constants,  $J$ , were reported in hertz. High-resolution mass spectra were recorded on a Waters ESI-TOF MS spectrometer. Elemental analysis (C, N, and H) was performed on Elementar vario EL III. All compounds tested for antibacterial activity, with exception of **12**, were established to be >95% pure upon elemental analysis.

**General Method for the Synthesis of Aminoalkylphenols 4–17.** Indoline (56  $\mu\text{L}$ , 0.50 mmol) was added to a solution of 2-hydroxy-5-nitrobenzaldehyde (83.6 mg, 0.50 mmol) and arylboronic acid (0.50 mmol) in 1,2-dichloroethane (DCE) or ethanol (EtOH; 5.0 mL) at 50  $^\circ\text{C}$ . The reaction was stirred at the same temperature until it was complete as judged by TLC. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography to give the pure aminoalkylphenol.

**2-((Indolin-1-yl(*p*-tolyl)methyl)-4-nitrophenol (4).** After 5 h reaction in DCE, purification by column chromatography [hexane/dichloromethane (DCM) 2:3] gave **4** (155 mg, 86% yield) as an off-white solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.87 (br s, 1H), 8.10 (dd,  $J$  = 9.1, 2.6 Hz, 1H), 7.97 (d,  $J$  = 2.9 Hz, 1H), 7.33–7.29 (m, 2H), 7.19–7.16 (m, 3H), 7.05–7.00 (m, 1H), 6.97–6.90 (m, 2H), 6.52 (d,  $J$  = 7.62 Hz, 1H), 5.32 (s, 1H), 3.27–3.20 (m, 1H), 3.09–2.90 (m, 3H), 2.34 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.9, 150.4, 140.9, 138.9, 135.2, 132.6, 130.0, 128.8, 127.7, 127.1, 125.2, 125.1, 124.9, 122.6, 117.8, 112.5, 70.6, 53.7, 28.6, 21.3. Elemental analysis: calcd for  $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_3 \cdot 0.19\text{H}_2\text{O}$ : C, 72.64; H, 5.65; N, 7.70; found: C, 72.64; H, 5.49; N, 7.77. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 361.1547; found, 361.1566.

**2-((Indolin-1-yl(4-vinylphenyl)methyl)-4-nitrophenol (5).** After 48 h reaction in EtOH, purification by column chromatography (toluene) gave **5** (150 mg, 81% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.80 (br s, 1H), 8.12 (dd,  $J$  = 8.9, 2.8 Hz, 1H), 8.02 (d,  $J$  = 2.6 Hz, 1H), 7.42 (s, 4H), 7.20 (d,  $J$  = 7.0 Hz, 2H), 7.07–6.92 (m, 3H), 6.72 (dd,  $J$  = 17.7, 11.0 Hz, 1H), 6.55 (d,  $J$  = 7.9 Hz, 1H), 5.79 (d,  $J$  = 17.6 Hz, 1H), 5.38 (s, 1H), 5.31 (d,  $J$  = 10.8 Hz, 1H), 3.31–3.24 (m, 1H), 3.13–3.02 (m, 1H), 2.99–2.91 (m, 2H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.8, 150.2, 140.8, 138.1, 137.5, 135.9, 132.5, 129.1, 127.6, 127.0, 126.7, 125.2, 125.0, 124.8, 122.5, 117.7, 115.1, 112.4, 70.3, 53.6, 28.5. Elemental analysis: calcd for  $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}_3 \cdot 0.46\text{H}_2\text{O}$ : C, 72.55; H, 5.54; N, 7.36; found: C, 72.55; H, 5.58; N, 7.15. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{23}\text{H}_{21}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 373.1547; found, 373.1541.

**2-((4-Ethylphenyl)(indolin-1-yl)methyl)-4-nitrophenol (6).** After 48 h reaction in EtOH, purification by column chromatography (hexane/toluene 1:3) gave **6** (132 mg, 71% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.87 (br s, 1H, ArOH), 8.10 (dd,  $J$  = 8.9, 2.8 Hz, 1H), 8.00 (d,  $J$  = 2.6 Hz, 1H), 7.35 (d,  $J$  = 8.2 Hz, 2H), 7.22–7.17 (m, 3H), 7.05–6.90 (m, 3H), 6.53 (d,  $J$  = 7.6 Hz, 1H), 5.34 (s, 1H), 3.28–3.22 (m, 1H), 3.10–2.91 (m, 3H), 2.66 (q,  $J$  = 7.6 Hz, 2H), 1.24 (t,  $J$  = 7.6 Hz, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.9, 150.4, 145.0, 140.9, 135.4, 132.5, 128.8, 128.7, 127.6, 127.1, 125.1, 125.0, 124.9, 122.5, 117.7, 112.4, 70.6, 53.7, 28.6, 15.4. Elemental analysis: calcd for  $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 0.67\text{H}_2\text{O}$ : C, 71.49; H, 6.09; N, 7.25; found: C, 71.49; H, 6.10; N, 7.08. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 375.1703; found, 375.1712.

**2-((4-(*tert*-Butyl)phenyl)(indolin-1-yl)methyl)-4-nitrophenol (7).** After 48 h reaction in EtOH, purification by column chromatography (hexane/toluene 3:7) gave **7** (156 mg, 78% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.89 (br s, 1H), 8.11 (dd,  $J$  = 8.9, 2.8 Hz, 1H), 8.02 (d,  $J$  = 2.6 Hz, 1H), 7.38 (s, 4H), 7.19 (d,  $J$  = 7.3 Hz, 1H), 7.05–6.90 (m, 3H), 6.54 (d,  $J$  = 7.9 Hz, 1H), 5.34 (s, 1H), 3.28–3.20 (m, 1H), 3.09–2.90 (m, 3H), 1.33 (s, 9H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.9, 151.9, 150.5, 140.9, 135.2, 132.5, 128.5, 127.6, 127.1, 126.1, 125.1, 125.0, 124.9, 122.4, 117.7, 112.4, 70.6, 53.8, 34.7, 31.3, 28.5. Elemental analysis: calcd for  $\text{C}_{25}\text{H}_{26}\text{N}_2\text{O}_3 \cdot 0.16\text{H}_2\text{O}$ : C, 74.08; H, 6.54; N, 6.91; found: C, 74.08; H, 6.56; N, 6.76. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{25}\text{H}_{27}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 403.2016; found, 403.2020.

**2-((4-Butylphenyl)(indolin-1-yl)methyl)-4-nitrophenol (8).** After 48 h reaction in EtOH, purification by column chromatography (hexane/toluene 1:4) gave **8** (41 mg, 19% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.88 (br s, 1H), 8.10 (dd,  $J$  = 9.1, 2.9 Hz, 1H), 7.99 (d,  $J$  = 2.6 Hz, 1H), 7.34–7.31 (m, 2H), 7.19–7.16 (m, 3H), 7.05–6.90 (m, 3H), 6.51 (d,  $J$  = 7.6 Hz, 1H), 5.31 (s, 1H), 3.26–3.18 (m, 1H), 3.09–2.90 (m, 3H), 2.60 (t,  $J$  = 7.6 Hz, 2H), 1.64–1.54 (m, 2H), 1.42–1.29 (m, 2H), 0.93 (t,  $J$  = 7.3 Hz, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.9, 150.5, 143.8, 140.9, 135.4, 132.6, 129.3, 128.8, 127.6, 127.1, 125.2, 125.1, 124.9, 122.5, 117.7, 112.5, 70.8, 53.7, 35.4, 33.5, 28.6, 22.5, 14.1. Elemental analysis: calcd for  $\text{C}_{25}\text{H}_{26}\text{N}_2\text{O}_3 \cdot 0.19\text{H}_2\text{O}$ : C, 73.97; H, 6.55; N, 6.90; found: C, 73.97; H, 6.55; N, 6.73. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{25}\text{H}_{27}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 403.2016; found, 403.2020.

**2-((4-Hexylphenyl)(indolin-1-yl)methyl)-4-nitrophenol (9).** After 48 h reaction in EtOH, purification by column chromatography (hexane/toluene 1:4) gave **9** (140 mg, 65% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.87 (br s, 1H), 8.10 (dd,  $J$  = 8.9, 2.8 Hz, 1H), 8.00 (d,  $J$  = 2.6 Hz, 1H), 7.35–7.32 (m, 2H), 7.19–7.16 (m, 3H), 7.05–6.90 (m, 3H), 6.52 (d,  $J$  = 7.9 Hz, 1H), 5.33 (s, 1H), 3.26–3.18 (m, 1H), 3.09–2.90 (m, 3H), 2.60 (t,  $J$  = 7.3 Hz, 2H), 1.66–1.56 (m, 2H), 1.37–1.31 (m, 6H), 0.91–0.87 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.8, 150.4, 143.8, 140.8, 135.4, 132.5, 129.2, 128.7, 127.5, 127.1, 125.1, 125.0, 124.8, 122.4, 117.6, 112.4, 70.5, 53.6, 35.7, 31.7, 31.3, 29.1, 28.5, 22.6, 14.1. Elemental analysis: calcd for  $\text{C}_{27}\text{H}_{30}\text{N}_2\text{O}_3 \cdot 0.30\text{H}_2\text{O}$ : C, 74.40; H, 7.07; N, 6.43; found: C, 74.40; H, 6.94; N, 6.37. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{27}\text{H}_{31}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 431.2329; found, 431.2318.

**2-((Indolin-1-yl(4-(methylthio)phenyl)methyl)-4-nitrophenol (10).** After 48 h reaction in EtOH, purification by column chromatography (hexane/EtOAc 3:1) gave **10** (167 mg, 85% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.76 (br s, 1H), 8.11 (dd,  $J$  = 9.1, 2.9 Hz, 1H), 7.97 (d,  $J$  = 2.6 Hz, 1H), 7.35–7.32 (m, 2H), 7.26–7.17 (m, 3H), 7.05–6.90 (m, 3H), 6.51 (d,  $J$  = 7.6 Hz, 1H), 5.32 (s, 1H), 3.27–3.21 (m, 1H), 3.10–2.91 (m, 3H), 2.47 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.8, 150.2, 140.9, 139.8, 134.5, 132.5, 129.3, 127.7, 126.74, 126.71, 125.3, 125.1, 124.8, 122.6, 117.8, 112.4, 70.2, 53.6, 28.6, 15.4. Elemental analysis: calcd for  $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_3\text{S} \cdot 0.63\text{H}_2\text{O}$ : C, 65.44; H, 5.31; N, 6.94; found: C, 65.44; H, 5.31; N, 6.65. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_3\text{S}^+ [\text{M} + \text{H}]^+$ , 393.1267; found, 393.1273.

**2-((4-Hydroxyphenyl)(indolin-1-yl)methyl)-4-nitrophenol (11).** After 24 h reaction in DCE, purification by column chromatography (hexane/EtOAc 4:1) gave **11** (144 mg, 80%

yield) as a yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.11 (dd,  $J = 9.1, 2.6$  Hz, 1H), 7.96 (d,  $J = 2.9$  Hz, 1H), 7.28 (d,  $J = 7.6$  Hz, 2H), 7.18 (d,  $J = 7.0$  Hz, 1H), 7.05–6.90 (m, 3H), 6.81 (d,  $J = 8.8$  Hz, 2H), 6.50 (d,  $J = 8.2$  Hz, 1H), 5.30 (s, 1H), 3.25–3.18 (m, 1H), 3.10–2.90 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ) 162.9, 156.1, 150.2, 140.9, 132.6, 130.4, 127.7, 127.1, 125.3, 125.1, 124.9, 122.6, 117.8, 116.1, 112.6, 70.2, 53.5, 28.6. Elemental analysis: calcd for  $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_4 \cdot 0.35\text{H}_2\text{O}$ : C, 68.42; H, 5.11; N, 7.60; found: C, 68.42; H, 5.10; N, 7.32. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_4^+ [\text{M} + \text{H}]^+$ , 363.1339; found, 363.1339.

*tert*-Butyl (4-((2-Hydroxy-5-nitrophenyl)(indolin-1-yl)methyl)phenyl)carbamate (**12**). After 24 h reaction in DCE, purification by column chromatography (hexane/EtOAc 85:15) gave **12** (215 mg, 82% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.80 (br s, 1H), 8.10 (dd,  $J = 8.8, 2.9$  Hz, 1H), 7.95 (d,  $J = 2.9$  Hz, 1H), 7.38–7.31 (m, 4H), 7.17 (d,  $J = 7.0$  Hz, 1H), 7.04–6.89 (m, 3H), 6.51–6.49 (m, 2H, ArH), 5.31 (s, 1H, CH), 3.25–3.19 (m, 1H), 3.10–3.01 (m, 1H), 2.95–2.89 (m, 2H), 1.51 (s, 9H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.9, 152.7, 150.2, 141.0, 139.0, 132.6, 132.5, 129.7, 127.7, 126.9, 125.3, 125.1, 124.9, 122.6, 119.0, 117.8, 112.5, 81.1, 70.2, 53.6, 28.6, 28.4. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{26}\text{H}_{26}\text{N}_3\text{O}_5^- [\text{M} - \text{H}]^-$ , 460.1878; found, 460.1863.

2-((4-Chlorophenyl)(indolin-1-yl)methyl)-4-nitrophenol (**13**). After 150 min reaction in DCE, purification by column chromatography (hexane/DCM, gradient from 4:6 to 3:7) gave **13** (127 mg, 67% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.65 (br s, 1H), 8.13 (dd,  $J = 9.4, 2.9$  Hz, 1H), 7.95 (d,  $J = 2.3$  Hz, 1H), 7.40–7.33 (m, 4H), 7.19 (d,  $J = 7.0$  Hz, 1H), 7.05–6.91 (m, 3H), 6.49 (d,  $J = 8.2$  Hz, 1H), 5.34 (s, 1H), 3.26–3.18 (m, 1H), 3.10–2.92 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.6, 149.9, 140.9, 136.5, 134.9, 132.3, 130.1, 129.4, 127.6, 126.2, 125.4, 125.1, 124.6, 122.7, 117.8, 112.3, 69.9, 53.6, 28.5. Elemental analysis: calcd for  $\text{C}_{21}\text{H}_{17}\text{N}_2\text{O}_3\text{Cl} \cdot 0.14\text{H}_2\text{O}$ : C, 65.80; H, 4.54; N, 7.31; found: C, 65.80; H, 4.61; N, 7.12. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_3\text{Cl}^+ [\text{M} + \text{H}]^+$ , 381.1000; found, 381.1006.

2-((4-Bromophenyl)(indolin-1-yl)methyl)-4-nitrophenol (**14**). After 24 h reaction in DCE, purification by column chromatography (hexane/EtOAc 3:1) gave **14** (154 mg, 72% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.59 (br s, 1H), 8.12 (dd,  $J = 8.7, 2.8$  Hz, 1H), 7.94 (d,  $J = 2.3$  Hz, 1H), 7.53–7.48 (m, 2H), 7.33–7.28 (m, 2H), 7.19 (d,  $J = 7.6$  Hz, 1H), 7.04–6.91 (m, 3H), 6.48 (d,  $J = 8.2$  Hz, 1H), 5.33 (s, 1H), 3.26–3.18 (m, 1H), 3.10–2.92 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.7, 150.0, 141.0, 137.1, 132.5, 132.4, 130.5, 127.7, 126.3, 125.5, 125.2, 124.7, 123.1, 122.7, 117.9, 112.4, 70.0, 53.7, 28.6. Elemental analysis: calcd for  $\text{C}_{21}\text{H}_{17}\text{N}_2\text{O}_3\text{Br} \cdot 0.68\text{H}_2\text{O}$ : C, 57.66; H, 4.23; N, 6.40; found: C, 57.66; H, 4.24; N, 6.23. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_3\text{Br}^+ [\text{M} + \text{H}]^+$ , 425.0495; found, 425.0498.

2-((4-Fluorophenyl)(indolin-1-yl)methyl)-4-nitrophenol (**15**). After 24 h reaction in DCE, purification by column chromatography (hexane/EtOAc 3:1) gave **15** (172 mg, 94% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.68 (br s, 1H), 8.12 (dd,  $J = 8.8, 2.7$  Hz, 1H), 7.94 (d,  $J = 2.3$  Hz, 1H), 7.43–7.38 (m, 2H), 7.19 (d,  $J = 7.6$  Hz, 1H), 7.09–6.91 (m, 5H), 6.49 (d,  $J = 8.2$  Hz, 1H), 5.35 (s, 1H), 3.24–3.18 (m, 1H), 3.10–2.91 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  164.6, 162.8, 161.3, 150.1, 141.0, 134.1, 134.0,

132.5, 130.7, 130.6, 127.7, 126.7, 125.4, 125.2, 124.8, 122.8, 117.9, 116.5, 116.2, 112.5, 70.0, 53.7, 28.6. Elemental analysis: calcd for  $\text{C}_{21}\text{H}_{17}\text{N}_2\text{O}_3\text{F} \cdot 0.87\text{H}_2\text{O}$ : C, 66.38; H, 4.97; N, 7.37; found: C, 66.38; H, 4.80; N, 7.14. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_3\text{F}^+ [\text{M} + \text{H}]^+$ , 365.1296; found, 365.1314.

2-(Indolin-1-yl(*o*-tolyl)methyl)-4-nitrophenol (**16**). After 48 h reaction in EtOH, purification by column chromatography (toluene) gave **16** (87 mg, 48% yield) as a yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.31 (br s, 1H), 8.11 (dd,  $J = 8.9, 2.8$  Hz, 1H), 7.93 (d,  $J = 2.6$  Hz, 1H), 7.43 (d,  $J = 7.3$  Hz, 1H), 7.26–7.19 (m, 4H), 7.05–6.89 (m, 3H), 6.44 (d,  $J = 7.6$  Hz, 1H), 5.76 (s, 1H), 3.31–3.25 (m, 1H), 3.08–2.95 (m, 3H), 2.42 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.8, 150.6, 141.0, 136.8, 136.0, 131.9, 131.2, 128.59, 128.57, 127.7, 127.21, 127.19, 125.1, 125.0, 124.6, 122.1, 117.6, 111.6, 64.6, 53.2, 28.5, 20.6. Elemental analysis: calcd for  $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_3 \cdot 0.27\text{H}_2\text{O}$ : C, 72.35; H, 5.67; N, 7.67; found: C, 72.35; H, 5.63; N, 7.38. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 361.1547; found, 361.1556.

2-(Indolin-1-yl(naphthalen-1-yl)methyl)-4-nitrophenol (**17**). After 48 h reaction in EtOH, purification by column chromatography (toluene) gave **17** (142 mg, 72% yield) as a yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.10 (br s, 1H), 8.12 (dd,  $J = 8.9, 2.8$  Hz, 1H), 8.03–8.00 (m, 1H), 7.94–7.86 (m, 3H), 7.59–7.42 (m, 4H), 7.20 (d,  $J = 7.0$  Hz, 1H), 7.08–7.00 (m, 2H), 6.95–6.90 (m, 1H), 6.54 (d,  $J = 7.6$  Hz, 1H), 6.41 (s, 1H), 3.29–3.22 (m, 1H), 3.13–2.84 (m, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.9, 150.6, 141.2, 134.1, 133.8, 131.8, 129.6, 129.5, 127.9, 127.4, 127.3, 127.2, 126.2, 125.8, 125.3, 125.1, 125.0, 123.0, 121.9, 117.7, 111.2, 63.3, 52.6, 28.6. Elemental analysis: calcd for  $\text{C}_{25}\text{H}_{20}\text{N}_2\text{O}_3 \cdot 0.33\text{H}_2\text{O}$ : C, 74.61; H, 5.18; N, 6.96; found: C, 74.61; H, 5.05; N, 6.76. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{25}\text{H}_{21}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 397.1547; found, 397.1563.

1-((2-Methoxy-5-nitrophenyl)(*p*-tolyl)methyl)indoline (**18**). Dimethyl sulfate (27.9  $\mu\text{L}$ , 0.30 mmol, 1.25 equiv) was added to a solution of **4** (85.0 mg, 0.236 mmol) and  $\text{K}_2\text{CO}_3$  (81.5 mg, 0.59 mmol, 2.5 equiv) in acetone (5.0 mL) at room temperature under argon, and the reaction mixture was stirred for 19 h. The reaction was quenched by adding 10 mL of 5% aq NaOH, and the resulting aqueous solution was extracted with DCM ( $4 \times 5$  mL). The organic extracts were combined, dried over  $\text{MgSO}_4$ , filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (hexane/DCM 2:3) to give **18** (85 mg, 0.227 mmol, 96% yield) as a light yellow solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.39 (d,  $J = 2.3$  Hz, 1H), 8.18 (dd,  $J = 9.2, 2.9$  Hz, 1H), 7.18 (d,  $J = 8.0$  Hz, 2H), 7.12–7.07 (m, 3H), 6.95–6.89 (m, 2H), 6.65 (t,  $J = 7.4$  Hz, 1H), 6.07 (d,  $J = 8.0$  Hz, 1H), 5.78 (s, 1H), 3.87 (s, 3H), 3.24–3.19 (m, 1H), 3.10 (q,  $J = 8.8$  Hz, 1H), 3.02–2.89 (m, 2H), 2.33 (s, 3H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta$  161.7, 151.8, 141.8, 137.4, 136.4, 131.9, 130.7, 129.3, 128.8, 127.2, 124.9, 124.5, 124.5, 118.3, 110.5, 108.2, 60.3, 56.4, 52.2, 28.5, 21.3. Elemental analysis: calcd for  $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 0.35\text{H}_2\text{O}$ : C, 72.56; H, 6.01; N, 7.36; found: C, 72.56; H, 5.63; N, 7.58. HRMS (ESI/TOF):  $m/z$  calcd for  $\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_3^+ [\text{M} + \text{H}]^+$ , 375.1703; found, 375.1703.

1-((3-Nitrophenyl)(*p*-tolyl)methyl)indoline (**21**). Anhydrous  $\text{AlCl}_3$  (0.934 g, 7.01 mmol, 1.3 equiv) was added to a solution of 3-nitrobenzoyl chloride (1.00 g, 5.39 mmol) and dry toluene (4.0 mL). The reaction was stirred at room temperature for 30 min and then quenched by pouring the



reaction mixture into water. The aqueous layer was extracted with DCM, and the organic extract was washed with water and brine, dried over  $\text{MgSO}_4$ , filtered, and evaporated under reduced pressure to afford 4-methyl-3'-nitrobenzophenone (**20**) (0.90 g, 69% yield) as a white solid. The product was obtained with the same spectral characterization as previously described.<sup>55</sup>  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.60 (t,  $J = 1.8$  Hz, 1H), 8.43 (dd,  $J = 7.6, 2.3$  Hz, 1H), 8.12 (dt,  $J = 7.9, 1.3$  Hz, 1H), 7.73–7.67 (m, 3H), 7.33 (d,  $J = 7.6$  Hz, 2H), 2.47 (s, 3H).

To a solution of **20** (0.20 g, 0.83 mmol) in dry DCM (4.5 mL) was added  $\text{TiCl}_4$  (0.10 mL, 0.90 mmol, 1.1 equiv) in dry DCM (0.90 mL). The mixture was cooled to 0 °C, and indoline (0.20 mL, 1.79 mmol, 2.16 equiv) was added. The reaction was stirred at room temperature under argon for 3 h, and sodium cyanoborohydride (0.98 mmol, 0.06 g, 1.18 equiv) in 1.5 mL of methanol was added. After an hour, the mixture was made basic (pH 10) by adding 5 M aq NaOH and filtered. The filtrate was partitioned between DCM (15 mL) and water (15 mL). The organic layer was separated, washed with water (2 × 15 mL) and brine (15 mL), dried over  $\text{MgSO}_4$ , filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc 92:8) to give **21** (168 mg, 60% yield) as a light yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.26–8.25 (m, 1H), 8.14–8.10 (m, 1H), 7.75 (d,  $J = 7.6$  Hz, 1H), 7.50 (t,  $J = 8.2$  Hz, 1H), 7.19–7.9 (m, 5H), 6.94 (t,  $J = 7.0$  Hz, 1H), 6.68 (t,  $J = 7.0$  Hz, 1H), 6.15 (d,  $J = 7.6$  Hz, 1H), 5.56 (s, 1H), 3.28–2.88 (m, 4H), 2.34 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  151.6, 148.7, 144.6, 137.8, 136.7, 134.2, 130.7, 129.6, 128.9, 127.2, 124.7, 123.0, 122.4, 118.4, 108.4, 66.4, 51.8, 28.5, 21.2. Elemental analysis: calcd for  $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_2 \cdot 0.33\text{H}_2\text{O}$ : C, 75.41; H, 5.94; N, 7.99; found: C, 75.41; H, 5.70; N, 7.88. HRMS (ESI/Q-TOF):  $m/z$  calcd for  $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_2^+ [\text{M} + \text{H}]^+$ , 345.1598; found, 345.1593.

**2-(Hydroxy(*p*-tolyl)methyl)-4-nitrophenol (23)**. A suspension of magnesium chips (194 mg, 8.00 mmol, 4 equiv) and a crystal of iodine in dry tetrahydrofuran (THF; 6.0 mL) was stirred and heated to reflux under argon. 1-Bromo-4-methylbenzene (1.37 g, 8.00 mmol, 4 equiv) in dry THF (2.0 mL) was added dropwise. The reaction mixture stirred under reflux for 50 min and then allowed to cool to room temperature. The resulting solution of *p*-tolylmagnesium bromide in THF was added dropwise to a solution of 2-hydroxy-5-nitrobenzaldehyde (2.00 mmol, 334 mg) in dry THF (4.0 mL) at –78 °C under argon. The reaction mixture was stirred for 15 min and then moved to 0 °C for another 15 min. The reaction was quenched by adding 5 mL of saturated aq  $\text{NH}_4\text{Cl}$  and then 5 mL of  $\text{H}_2\text{O}$ . Aqueous and organic layers were separated and the aqueous layer was extracted with  $\text{Et}_2\text{O}$  (2 × 10 mL). Organic extracts were combined, dried over  $\text{MgSO}_4$ , filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (DCM/EtOAc 95:5) to give **23** (400 mg, 1.54 mmol, 77% yield) as an off-white solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 9.21 (s, 1H), 8.07 (dd,  $J = 9.0, 2.7$  Hz, 1H), 7.76 (d,  $J = 2.5$  Hz, 1H), 7.29–7.20 (m, 4H), 6.96 (d,  $J = 8.9$  Hz, 1H), 6.06 (d,  $J = 1.8$  Hz, 1H), 3.03 (d,  $J = 2.5$  Hz, 1H), 2.36 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  161.8, 140.7, 139.2, 137.8, 130.0, 127.0, 126.9, 125.4, 124.6, 118.0, 77.0, 21.3. Elemental analysis: calcd for  $\text{C}_{14}\text{H}_{13}\text{NO}_4 \cdot 0.32\text{H}_2\text{O}$ : C, 63.46; H, 5.19; N, 5.29; found: C, 63.46; H, 4.99; N, 5.09. HRMS (ESI/Q-TOF):  $m/z$  calcd for  $\text{C}_{14}\text{H}_{12}\text{NO}_4^+ [\text{M} - \text{H}]^+$ , 258.0772; found, 258.0776.

**Chiral Resolution of 2-(Indolin-1-yl(*p*-tolyl)methyl)-4-nitrophenol (4)**. **4** (108 mg, 0.30 mmol, 1 equiv) in dry DCM (1 mL) was added to a solution of (*R*)-(+)-Mosher's acid (84.3 mg, 0.36 mmol, 1.2 equiv), *N*-(3-dimethylamino-propyl)-*N'*-ethylcarbodiimide hydrochloride (98 mg, 0.51 mmol, 1.7 equiv), and DMAP (7.9 mg, 0.06 mmol, 0.2 equiv) in dry DCM (2 mL) at room temperature under argon. The reaction mixture was stirred for 72 h and then diluted with 10 mL of DCM. The solution was washed with 10 mL of saturated aq  $\text{NaHCO}_3$  and the layers were separated. Additional 2 × 5 mL of DCM was used to extract the aqueous layer. Organic extracts were combined, dried over  $\text{MgSO}_4$ , filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc, gradient from 85:15 to 80:20) to give **22** (78.0 mg, 0.14 mmol, 45% yield) as a 46:54 mixture of diastereomers, determined by  $^{19}\text{F}$  NMR.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 8.55 (d,  $J = 2.3$  Hz, 1H), 8.49 (d,  $J = 2.9$  Hz, 1H), 8.23 (dd,  $J = 2.9, 1.8$  Hz, 1H), 8.20 (dd,  $J = 2.6, 1.5$  Hz, 1H), 7.53 (t,  $J = 8.2$  Hz, 4H), 7.45–7.27 (m, 9 H), 7.11–7.03 (m, 7 H), 6.94–6.89 (m, 4H), 6.80 (d,  $J = 8.2$  Hz, 3H), 6.73–6.66 (m, 2H), 5.98 (d,  $J = 8.2$  Hz, 1H), 5.93 (d,  $J = 8.2$  Hz, 1H), 5.50 (s, 1H), 5.19 (s, 1H), 3.53 (d,  $J = 1.2$  Hz, 4H), 3.44 (d,  $J = 1.2$  Hz, 3H), 3.21–3.04 (m, 4H), 2.99–2.85 (m, 6 H), 2.32 (s, 3H), 2.31 (s, 3H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ ):  $\delta$  164.8, 164.7, 152.4, 152.0, 151.3, 151.2, 146.5, 146.4, 137.8, 137.0, 136.5, 135.3, 135.1, 131.4, 131.0, 130.7, 130.4, 130.3, 130.2, 129.5, 129.4, 129.0, 128.9, 128.6, 128.6, 127.5, 127.3, 127.3, 125.2, 124.9, 124.5, 124.5, 124.4, 124.3, 123.9, 123.7, 123.2, 123.2, 122.1, 122.0, 118.7, 118.5, 108.5, 108.1, 85.5, 85.3, 85.0, 84.8, 84.6, 60.7, 60.1, 56.0, 55.7, 52.5, 51.9, 28.4, 21.2.  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  –70.91 (s,  $\text{CF}_3$ ), –71.30 (s,  $\text{CF}_3$ ). The diastereomers were separated by preparative thin layer chromatography, by multiple elutions with hexane/toluene 4:6 and the resulting separated diastereomers further purified from the hydrolyzed product by column chromatography (hexane/EtOAc 85:15).

**22<sub>Major</sub>**  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.54 (d,  $J = 2.3$  Hz, 1H, ArH), 8.20 (dd,  $J = 8.8, 2.9$  Hz, 1H, ArH), 7.53 (d,  $J = 7.6$  Hz, 2H, ArH), 7.40–7.35 (m, 2H, ArH), 7.32–7.27 (m, 2H, ArH), 7.08 (d,  $J = 7.0$  Hz, 1H, ArH), 7.03 (d,  $J = 7.6$  Hz, 2H, ArH), 6.90 (t,  $J = 7.3$  Hz, 1H, ArH), 6.79 (d,  $J = 8.2$  Hz, 2H, ArH), 6.69 (t,  $J = 7.0$  Hz, 1H, ArH), 5.91 (d,  $J = 7.6$  Hz, 1H, ArH), 5.17 (s, 1H, CH), 3.51 (d,  $J = 1.2$  Hz, 3H,  $\text{OCH}_3$ ), 3.12–3.04 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.96–2.84 (m, 3H,  $\text{NCH}_2\text{CH}_2$ ), 2.30 (s, 3H,  $\text{ArCH}_3$ ).  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  –71.31 (s). **22<sub>Minor</sub>**  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.48 (d,  $J = 2.9$  Hz, 1H, ArH), 8.20 (dd,  $J = 9.1, 2.6$  Hz, 1H, ArH), 7.50 (d,  $J = 7.6$  Hz, 2H, ArH), 7.44–7.30 (m, 4H, ArH), 7.08 (d,  $J = 7.0$  Hz, 1H, ArH), 7.04 (d,  $J = 8.2$  Hz, 2H, ArH), 6.92–6.87 (m, 3H, ArH), 6.68 (t,  $J = 7.6$  Hz, 1H, ArH), 5.97 (d,  $J = 8.2$  Hz, 1H, ArH), 5.48 (s, 1H, CH), 3.42 (d,  $J = 1.2$  Hz, 3H,  $\text{OCH}_3$ ), 3.20–3.11 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 3.08–3.02 (m, 1H,  $\text{NCH}_2\text{CH}_2$ ), 2.98–2.90 (m, 2H,  $\text{NCH}_2\text{CH}_2$ ), 2.31 (s, 3H,  $\text{ArCH}_3$ ).  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  –70.93 (s).

**22<sub>Minor</sub>** (11.6 mg, 20  $\mu\text{mol}$ , 1 equiv) was hydrolyzed by adding 0.01 M NaOH in methanol (2.2 mL, 1.1 equiv). The reaction mixture was stirred for 15 min and then neutralized by adding 0.1 M aq HCl (0.22 mL, 1.1 equiv). The resulting mixture was diluted with brine (3 mL) and extracted with  $\text{Et}_2\text{O}$  (3 × 3 mL). Organic extracts were combined, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc, gradient from 85:15 to 80:20) to yield **4b** (7.3 mg, 20

$\mu\text{mol}$ ). A 16:84 enantiomeric ratio was determined by HPLC analysis of the acetate derivative of **4**, using a Chiralpak IA column (hexane/iProH 99:1, 1.0 mL/min,  $\lambda = 254 \text{ nm}$ ,  $t_{\text{r,minor}} = 12.54 \text{ min}$ ,  $t_{\text{r,major}} = 14.16 \text{ min}$ ). A similar procedure was applied to **22**<sub>Major</sub> (8.8 mg, 15  $\mu\text{mol}$ ), affording **4a** (4.9 mg, 13.5  $\mu\text{mol}$ ) in a 65:35 enantiomeric ratio determined upon subsequent derivatization to the acetate derivative and chiral HPLC analysis. The procedure for derivatization is as follows: enantiomerically enriched samples of **4** (1 mg, 2.8  $\mu\text{mol}$ , 1 equiv) in 0.25 mL of dry DCM were treated with acetyl chloride (1.8  $\mu\text{L}$ , 16.6  $\mu\text{mol}$ , 6 equiv) and  $\text{Et}_3\text{N}$  (0.25  $\mu\text{L}$ , 2.8  $\mu\text{mol}$ , 1.1 equiv) in dry DCM (50  $\mu\text{L}$ ) at room temperature under argon. The reaction was stirred for 25 min and quenched by adding saturated aq  $\text{NaHCO}_3$  (0.25 mL). To the resulting solution was added DCM and  $\text{H}_2\text{O}$  (0.75 mL each). The layers were separated and the aqueous layer further extracted with DCM ( $2 \times 1 \text{ mL}$ ). Organic extracts were combined and filtered through silica, and the solvent was removed under reduced pressure.

**Antibacterial Assays.** For selective purposes, the antimicrobial activity of the diverse compounds was first tested against five bacterial Gram-positive strains obtained from American Type Culture Collection (ATCC): *S. aureus* ATCC25923 (MSSA), *S. aureus* CIP6538, *S. aureus* CIP106760 (MRSA), *E. faecalis* 29212, and *E. faecalis* ATCC51299 (VRE). For the most promising compounds (i.e., compounds **4**, **5**, and **13**), the antimicrobial activity was further evaluated against eight distinct clinical isolates of *S. aureus*, *E. faecalis*, *S. pneumoniae*, and *S. agalactiae* (group B) that belong to the wide collection of pathogenic Gram-positive strains of the Portuguese National Institute of Health (NIH).

For each compound, the minimum inhibitory concentration (MIC) was determined by the broth microdilution method,<sup>56</sup> according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.<sup>57</sup> Before each experiment, frozen stocks of all strains were subcultured three times in appropriate culture medium (CAMHB for *S. aureus* and *E. faecalis* and CAMHB-LHB 3.5% for streptococci) to check strain viability and to avoid any negative growth effect from congelation, and inocula of  $1.5 \times 10^8 \text{ cfu/mL}$  (the equivalent to 0.5 McFarland) were prepared accordingly. For each microorganism, a 1:20 dilution of the prepared inoculum was used. Twofold serial dilutions of concentrated stock compound solutions (1 mM) were prepared in the required medium into 96-well plates. A control without compounds was also prepared. All cultures were incubated for 16–24 h at 35–37 °C with 5%  $\text{CO}_2$ . Purity check and colony or viable cell counts of the inoculum suspensions were also evaluated in order to ensure that the final inoculum density closely approximates the intended number. This was obtained by subculturing a diluted aliquot from the growth control well (without compound) immediately after inoculation onto a suitable nonselective agar plate for simultaneous incubation. The MIC was determined as the lowest compound concentration at which no visible growth was observed. The bacterial growth was measured with an absorbance microplate reader (Thermo Scientific Multiskan FC, Loughborough, UK) set to 620 nm.

The MBC was also evaluated. Briefly, after MIC assessment, the bacterial suspension on the wells was homogenized, serial-diluted, triplicate spread on appropriate medium and incubated. The MBC attributed to the compound concentration resulting in a 99.9% reduction in bacterial numbers. All

assays were carried out in triplicate for each tested microorganism.

**DPPH Method for Antioxidant Activity.** The antioxidant activity of the compounds was tested as previously described.<sup>58</sup> To evaluate the compounds' antioxidant potential through the free radical scavenging test, the change in optical density of DPPH radicals was monitored. The absorbance was measured at 517 nm against a corresponding blank and the antioxidant activity was calculated using the following equation

$$\text{Scavenging activity (\%)} = \frac{\text{absorbance control} - \text{absorbance sample}}{\text{absorbance control}} \times 100$$

The reference standard used for this procedure was butylated hydroxytoluene (BHT).

**A. salina Mortality Bioassay.** A test of mortality was performed on *A. salina* brine shrimp, of the compounds **4**–**17**, as previously described.<sup>59</sup> For this assay, an aquarium air pump (HI-FLOTM Single Type 4000), a thermostat Cabinet Aqua Lytic, and a stereomicroscope (CETI Belgium) were used. The number of dead *nauplii* was recorded after 24 h and used to calculate the percentage (%) of mortality rate, according to the equation

$$\text{Mortality rate (\%)} = \frac{\text{total}_{\text{nauplii}} - \text{alive}_{\text{nauplii}}}{\text{total}_{\text{nauplii}}}$$

**Cell Culture and Cytotoxicity Assessment.** The cytotoxicity profile of the compound **13** was characterized in the normal-like human keratinocytes (HaCaT cell line). HaCat cells were routinely cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Cells were kept at 37 °C, under an atmosphere containing 5%  $\text{CO}_2$  in air. Cell viability was evaluated by the MTT assay, using a 24 h incubation protocol, according to a previously published procedure.<sup>60</sup> Two independent experiments were carried out, each comprising four replicate cultures.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02381.

Computational details, NMR spectra, and HPLC traces (PDF)

SMILES strings of tested compounds (CSV)

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### Author Contributions

T.R. and J.R.V. synthesized the compounds. J.A., E.N., A.S.F., and A.N. performed the biological assays. J.R. isolated and propagated the bacteria clinical isolates. J.P.G., A.N., P.R., A.S.F., and N.R.C. conceived and managed all studies. The manuscript was written through contributions of all authors.

## Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS

DPPH, 2,2-diphenyl-1-picrylhydrazyl; MBC, minimum bactericidal concentration; MSSA, methicillin-sensitive *Staphylococcus aureus*; QM, ortho-quinone methide

## ADDITIONAL NOTES

<sup>a</sup>DFT calculations performed at the PBE1PBE/6-31G(d,p) level with the use of the Gaussian 09 package. A complete account of the computational details and the correspondence list of references are provided in the [Supporting Information](#).

<sup>b</sup>Wiberg indices are electronic parameters related to the electron density between atoms. They can be obtained from a natural population analysis and provide an indication of the bond strength.

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