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Multicomponent Petasis-borono Mannich Preparation of Alkylaminophenols and Antimicrobial Activity Studies

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In this work we report the antibacterial activity of alkylaminophenols. A series of such compounds was prepared by a multicomponent Petasis-borono Mannich reaction starting from salicylaldehyde and its derivatives. The obtained compounds were tested against a large panel of microorganisms, Gram-positive and Gram-negative bacteria, and a yeast. Among the several tertiary amine derivatives tested, indoline-derived aminophenols containing a nitro group at the *para*-phenol position showed considerable activity against bacteria tested with minimal inhibitory concentrations as low as 1.36 μM against *Staphyloccocus aureus* and *Mycobacterium smegmatis*. Cytotoxicity of the new *para*-nitrophenol derivatives was observed only at concentrations much higher than those required for antibacterial activity.

Introduction

Antimicrobial drugs have been successful therapeutics in treating many life-threatening bacterial infections since the beginning of the 20th century. However, in the last 50 years, the unrestrained use of antibacterial drugs has been cited as the main cause for the emergence of multidrug-resistant bacteria. In some instances, bacteria resistant to more than one antibiotic have been reported. As microorganisms are becoming resistant to antibiotics, the development of new antibacterial agents is of pivotal importance for global health. *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* are some examples of pathogens known to be multidrug-resistant organisms.[1]

Despite the technologies used in the development of new antibacterial drugs, specifically challenging network pharmacology[2] and functional genomics profiling,[3] drugs from natural origin encompass around 75% of all the antibacterial agents discovered between 1981 and 2010,[4] while considerably fewer examples of synthetic antimicrobials have been reported. Despite the many thousands of natural antibiotics discovered, our knowledge about their targets remains very limited. The structural requirements for a compound to penetrate bacterial cells are still obscure, making the process of finding novel penetrating compounds difficult. Despite many efforts in finding new antibacterial agents, there are some hurdles that hamper the process of finding new antibiotics, such as: the narrow selection of chemical compounds and their limited range of mechanisms, the complex mechanisms of action of antibiotics in current use, and the incompatibility of existing antibiotics regarding their physical and chemical properties with conventional medicinal chemistry approaches.[5] Maybe for these reasons most synthetic antibiotics have been discovered outside antibiotics discovery programs.[6]

Many phenolic compounds, either natural products or synthetic molecules, have been reported to have antibacterial activity. Natural phenol derivative arzanol has been identified as a lead structure in the development of new antibacterials,[7] and carvacrol has been reported to act as a biocidal agent by causing disruption of the bacterial membrane and to have antioxidant activities improved by modification into its Schiff bases.[8] Phenolic triterpenoids were reported to have bacteriostatic action against *Staphylococcus epidermidis*,[9] while simple 3-alkylphenols have shown moderate in vitro antibacterial activity.[10] Bromophenols have been shown to be inhibitors of *Candida albicans* isocitrate lyase.[11]

While salicylaldehydes,[12] as well as their Schiff bases,[13] have been reported to have antimicrobial activity, reports on the antibacterial activity of tertiary amines derived from addition to the sp^2 carbon of the iminium are scarce. Jameel et al. reported that the antibacterial activity of *N*-[(5-amino-2-hydroxyphenyl)(phenyl)methyl]-*N*-phenyl amides increased after formation of metal chelates.[14]

As a continuation of our work on the preparation of alkylaminophenols derived from the Petasis-borono Mannich reaction,[15] and considering the aforementioned antibacterial activity of salicylaldehyde derivatives, we envisioned such compounds to also have antibacterial properties. To identify the

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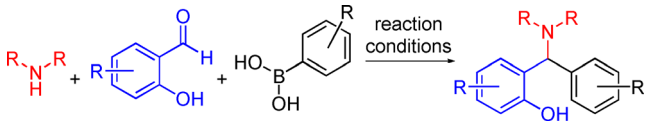
structural elements needed for antibacterial activity, various secondary amines were initially considered, and the substituents of the aromatic rings further tuned. Herein we present the results of our study of the antibacterial activity of alkylaminophenols derived from eight different secondary amines.

Results and Discussion

Synthesis

Compounds **1**–**43** were prepared by a Petasis-borono Mannich (PBM) reaction (Scheme 1),[16] according to previously reported



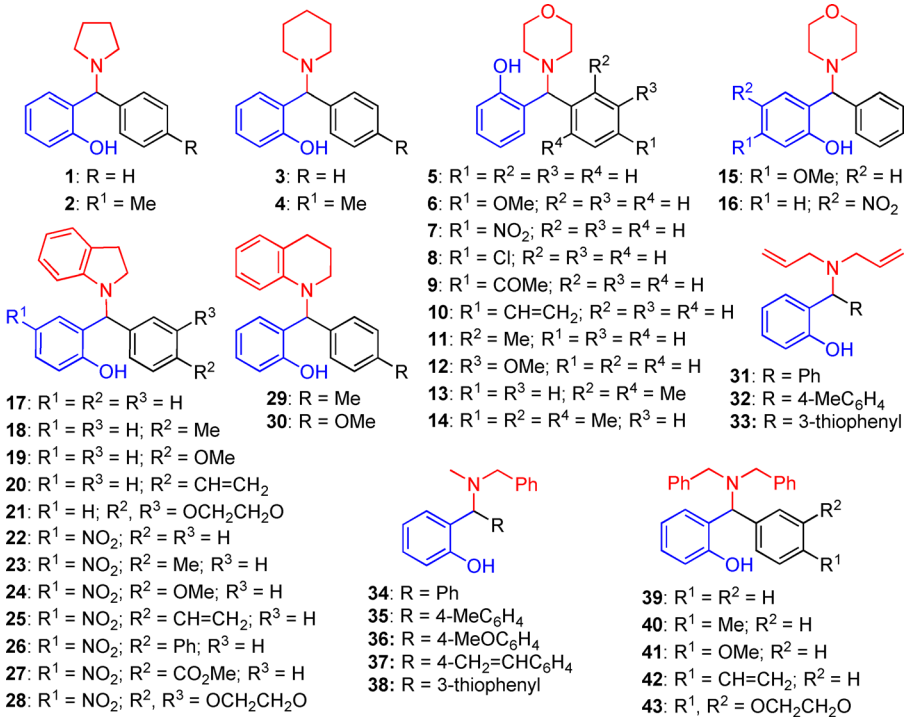
Scheme 1. General method for the preparation of alkylaminophenols.

procedures. The multicomponent character of such methodology allows the rapid preparation of large libraries of compounds by replacement of a single component of the reaction.[17] Furthermore, this method allowed us to rapidly obtain several alkylaminophenols starting from different secondary amines. Cyclic amines such as pyrrolidine, piperidine, morpholine, and indoline, as well as acyclic amines such as diallylamine, methylbenzyl amine, and dibenzyl amine are known to be efficient partners for the PBM reaction. Such amines and tetrahydroquinoline were condensed with different salicylaldehyde derivatives in the presence of various boronic acids, providing a small library of alkylaminophenols for the antimicrobial assays (Scheme 2, Table 1).

Although the PBM reaction is known to proceed in a variety of solvents, glycerol was used as solvent in the preparation of most alkylaminophenols.[15a] Reaction of indoline with 5-nitrosalicylaldehyde in glycerol resulted in several instances in formation of side products that hampered the purification of the desired tertiary amine. Replacing glycerol by ethanol and diluting the reaction conditions, it was possible to isolate the desired compounds **25**–**28** in reasonably good yields at 50 °C, namely when employing *para*-substituted aryl boronic acids (Table 1). An attempt to increase the reaction yield by increasing the reaction temperature to reflux ethanol led to considerable formation of the *N*-alkylindoline resultant from the intermolecular hydride transfer as reported by Sun, Pan, and colleagues.[18] All desired compounds were purified by column chromatography in silica gel, and their chemical structures were confirmed by NMR and mass spectrometry.

Antimicrobial activity

All prepared compounds were screened for their antimicrobial activity by the well diffusion assay. This preliminary test aimed to identify the antimicrobial compounds comparing with the corresponding positive controls. The antimicrobial activity was evaluated against a large panel of microorganisms: Gram-positive and Gram-negative bacteria and a yeast (data not shown). The synthesized compounds did not reveal antimicrobial activity against Gram-negative bacteria and the yeast; however, compounds **22**–**29** were active against Gram-positive bacteria. Thus, this primary test allowed us to select compounds **22**–**29**



Scheme 2. Structures of prepared alkylaminophenols **1**–**43**.

Table 1. Preparation and isolated yields of alkylaminophenols.

Compd	Method[a]	Yield [%]	Compd	Method[a]	Yield [%]
1	A[b]	44	**23**	A	57
2	A	70	**24**	A	58
3	A	49	**25**	B	81
4	A	69	**26**	B	80
5	A	77	**27**	B[c]	50
6	A	77	**28**	B	65
7	A[b]	34	**29**	A	13
8	A[b]	72	**30**	A[d]	64
9	A	26	**31**	A	44
10	A	76	**32**	A	62
11	A	75	**33**	A	42
12	A	70	**34**	A[b]	60
13	A	11	**35**	A	70
14	A	17	**36**	A	60
15	A	58	**37**	A	70
16	A	55	**38**	A	66
17	A	94	**39**	A	54
18	A	92	**40**	A	74
19	A	90	**41**	A	75
20	A	97	**42**	A	76
21	A	95	**43**	A	60
22	B	51			

[a] Method A: aldehyde (0.41 mmol), 1.5 equiv amine and boronic acid in glycerol (1 mL), 50 °C, 24–48 h; Method B: aldehyde (0.5 mmol), 1.0 equiv amine and boronic acid in ethanol (5 mL), 50 °C, 48 h. [b] Reaction conducted at 80 °C. [c] Reaction conducted in ethanol (8 mL) at reflux for 24 h. [d] Reaction conducted at 80 °C for 3 h.

for further evaluation of their minimum inhibitory concentration (MIC) values by the microdilution method. The MIC values were tested against a reference MSSA *Staphyloccocus aureus* and selected resistant microorganisms (MRSA and VRE) as well as a non-pathogenic strain *Mycobacterium smegmatis*, from the common genus of the *Mycobacterium tuberculosis*, the most significant mycobacterium that causes human tuberculosis. The obtained results listed in Table 2 were compared with corresponding positive controls (vancomycin for Gram-positive bacteria, and rifampicin for the mycobacteria). Only in the case of compounds **28** and **29** did the corresponding positive control exhibit higher activity, with MIC values ranging from 23.93 to 154.55 μM; compounds **22**–**27** were more active and showed MIC values of <1.36 to 147.93 μM. Considering all the strains tested, compound **23** was the most active (MIC values ranging from <1.36 to 2.72 μM). Higher antibacterial activity of the indoline derivatives than salicylaldehydes was observed against *S. aureus*.[12b,c]

Table 2. Antimicrobial activity of the synthesized alkylaminophenol derivatives.

Compd	MIC [μM][a]			
	S. aureus ATCC25923 (MSSA)	*S. aureus* CIP106760 (MRSA)	*E. faecalis* ATCC51299 (VRE)	*M. smegmatis* ATCC607
22	2.83	2.83	11.29	11.29
23	<1.36	<1.36	2.72	<1.36
24	10.39	2.60	2.60	5.18
25	2.63	2.63	5.24	10.50
26	36.92	2.32	9.25	147.93
27	2.42	9.67	9.67	19.31
28	38.58	77.27	154.55	77.27
29	47.79	23.93	23.93	11.98
vancomycin	5.40	2.70	2.70	–
rifampicin	–	–	–	<0.60

[a] Data are the median values of at least three replicates.

Considering the cyclic amines pyrrolidine, piperidine, morpholine and indoline, as well as the acyclic amines such as diallylamine, methylbenzyl amine, and dibenzyl amine used in the alkylaminophenol derivative synthesis, the more potent derivatives were those with the indoline group, **22**–**28**. This could be considered an essential group for the antimicrobial activity of these new alkylaminophenols. The presence of this aromatic heterocyclic group confers to the antimicrobial compounds higher lipophilicity than the other derivatives (Table 3), an apparent requirement to inhibit the growth of the tested bacteria.

Table 3. Predicted log*P*, dipole moment, and frontier molecular orbital energies of selected compounds.

Compd	log*P*[a]	Dipole [D][b]	E_{HOMO} [eV][b]	E_{LUMO} [eV][b]	$\Delta E_{HOMO-LUMO}$ [eV][b]
1	3.56	3.87	−5.61	−0.23	5.38
3	3.95	2.97	−5.76	−0.16	5.60
5	2.76	2.19	−5.90	−0.27	5.63
16	2.66	6.20	−6.63	−1.82	4.81
17	4.80	3.21	−5.79	−0.28	5.50
22	4.61	7.27	−6.20	−1.78	4.42
23	4.96	7.31	−6.17	−1.75	4.42
24	4.50	7.10	−6.15	−1.74	4.41
25	5.18	7.26	−6.19	−1.78	4.41
26	6.13	7.23	−6.18	−1.77	4.41
27	4.52	7.96	−6.27	−1.84	4.43
28	4.31	7.36	−6.08	−1.71	4.38
29	5.44	7.10	−6.03	−1.78	4.25

[a] Predicted log*P*.[21] [b] Calculated using DFT at the PBE1PBE/6-31G(d,p) level of theory using polarizable continuum model as water solvation model.

Considering these indoline compounds **22**–**28**, the *para*-nitro group in the phenol ring is another essential structural feature for antimicrobial activity. A similar feature was observed for salicylaldehyde derivatives in which the 5-nitro group conferred some antibacterial activity.[12b] However, the nitro group on other derivatives (e.g., **7** and **16**) did not confer antimicrobial activity. It is also notable that **23**, the derivative with a *para*-methylphenyl group was the most active compound. This structural relationship is verified on compound **29**, which, although not having an indoline moiety, still presents antimicrobial activity probably due to the structural similarities with compound **23**.

To test for a correlation between the antimicrobial activity and the electronic properties of alkylaminophenols, the structures of the series of 5-nitro-substituted derivatives **23**–**29** and selected unsubstituted alkylaminophenols were geometrically optimized by density functional theory (DFT) calculations[19] (Table 3). Further analysis of dipole moments and natural bond

orbitals suggest a relation between the antibacterial activity of the compounds and their electronic properties, as only more polar compounds with smaller frontier molecular orbital gaps are active.

The results obtained also suggest a relationship between antimicrobial activity and the unsubstituted *meta* position of the phenyl group as in all active compounds **22**–**28**. In this series, compound **28**, with an ethylenedioxy moiety, proved to be the less active amongst the *para*-nitro derivatives. For all this, it is possible to identify important structural relationships in the new alkylaminophenols and the antimicrobial activity tested, namely the indoline group, the *para*-nitrophenol, and the *para*-methyl group in the phenyl ring. These structural features conferred to these derivatives potent antimicrobial activity that should be further explored to study and identify their mode of action.

The analysis of the effect of compound **23** (Figure 1) on bacterial growth over time was performed for an *S. aureus* strain,

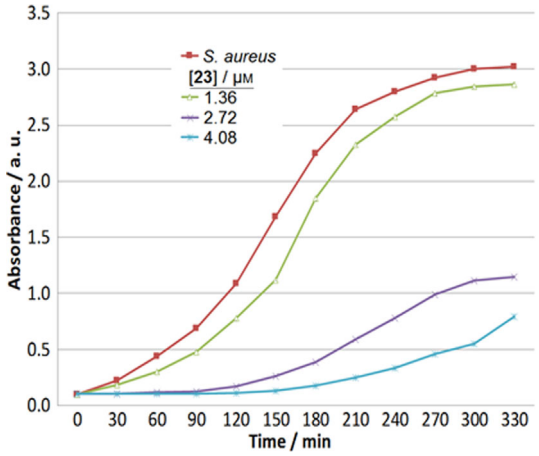


Figure 1. Growth curves of bacterial strains *S. aureus* ATCC 25923 independently challenged with compound **23**. Bacterial growth was assessed in the absence of compound (*S. aureus*) or in the presence of **23** at various concentrations as indicated. The optical density was monitored at 620 nm.

as a model of Gram-positive bacteria. For compound **23** at 1.36 μM (MIC < 1.36 μM), no inhibitory effect was observed. At 2.72 and 4.08 μM, compound **23** was responsible for a strong delay and decrease in the growth rate of the Gram-positive strain. At these concentrations, the growth profiles of *S. aureus* differed from the control (cells grown in the absence of compound). This behavior could be explained by the adaptation of the bacterial cells to the presence of the compound, decreasing its antimicrobial activity.

To address the bacteriostatic and bactericidal properties of compound **23** against *S. aureus*, the MBC value was also evaluated. The MBC value (130.56 μM) was much higher than the MIC value (< 1.36 μM) of the compound tested. A compound is usually regarded as bactericidal if the MBC is no more than four times the MIC value,[20] so there is evidence of bacteriostatic properties for compound **23** against *S. aureus*.

Cytotoxicity

The most active compound **23** and two other compounds, **24** and **29**, selected based on their antimicrobial properties, were evaluated for their cytotoxicity in a human keratinocyte (HaCaT) cell line. The results are depicted in Figure 2. Under

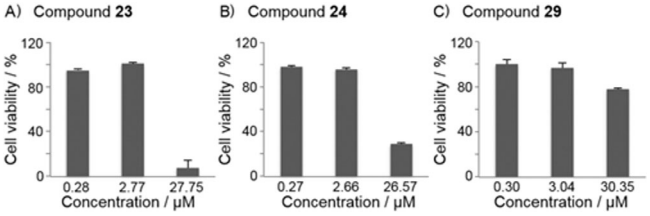


Figure 2. Effect of compounds **23** (A), **24** (B), and **29** (C) on the viability of human keratinocytes, as evaluated by MTT assay. Cells were incubated with increasing concentrations of the compounds for 24 h. Results are average values ± SD from two independent experiments, each comprising four replicate cultures.

the conditions tested, the three compounds did not show relevant cytotoxicity at 0.3 and 2.7–3.0 μM. However, the viability of HaCaT cells exposed to the highest concentration tested (27.8–30.4 μM) decreased considerably, especially for compounds **23** and **24**. It is important to note that the concentrations at which the compounds exhibited antibacterial properties were generally lower than those presenting cytotoxicity. The obtained results, although preliminary, suggest that these compounds should be safe for cutaneous application.

Conclusions

The objective of this study was to synthesize novel alkylaminophenol derivatives, to screen their antimicrobial and cytotoxic activities, and to obtain new antimicrobial structural entities. A series of new alkylaminophenol analogues was synthesized through convenient and efficient synthetic procedures.

The antimicrobial activity of the synthesized compounds was evaluated against a large panel of microorganisms, Gram-positive and Gram-negative bacteria, and a yeast. The structure–activity relationship of the synthesized compounds revealed that the compounds **22**–**28** bearing an indoline group were the most potent derivatives. The influence of the indoline moiety in antimicrobial activity of these compounds may be explained by the hydrophobicity. In addition to the indoline group, it was also possible to identify other important structural relationships on the new alkylaminophenols and the antimicrobial activity tested. The *para*-nitrophenol group, the *para*-methyl substituent, and *meta*-unsubstitution of the phenyl group were identified as beneficial for higher antibacterial activity.

The compounds that showed highest activity against Gram-positive bacteria were not cytotoxic to human keratinocytes at MIC values. Considering the obtained results from the growth inhibition and MBC values, there is a suggestion of bacteriostatic properties for compound **23** against *S. aureus*.

The data obtained herein support further studies toward the potential use of these compounds in the topical treatment of skin infections. The preparation of derivatives of **23**, their antibacterial activity, and mode of action as well as the identification of specific targets of bacterial cell will be reported in due course.

Experimental Section

Synthesis

General: All reactions using glycerol as solvent were performed in air atmosphere in long, capped test tubes. The reagents and solvents were used as obtained from the suppliers (Sigma–Aldrich, Fluka and TCI). Bi-distilled glycerol (99.5% *w/v*) was used as obtained from VWR (0.5% maximum water content). The reactions were monitored by thin-layer chromatography (TLC) carried out on pre-coated (Merck TLC silica gel 60 F_{254}) aluminum plates by using UV light as visualizing agent and cerium molybdate solution as developing agent. Flash column chromatography was performed on silica gel 60 (Merck, 0.040–0.063 mm). NMR spectra were recorded with Varian Mercury 300 MHz instrument using $CDCl_3$ as solvent and calibrated using tetramethylsilane as internal standard. Chemical shifts are reported in ppm relative to TMS, and coupling constants are reported in Hz. High-resolution mass analysis (ES, positive) was determined on a WatersSynapt G1 instrument.

Compounds **1**, **4**, **6**, **15**, **31**, **33**, **36**, and **40** were obtained with the same spectral characterization as reported elsewhere.[15c] The characterization of compounds **2**, **7**, **10**, **13**, **16**–**21**, **32**, **35**, **37**, and **42** has been previously described.[15a] Compounds **3**, **34**, and **39** have been described elsewhere.[22] Other compounds such as **5**,[23] **8**,[24] **11**,[25] **12**,[26] **23**,[27] and **41**[28] have been obtained with the same spectral characterization as previously described.

Method A: A long, capped test tube containing a magnetic stirrer was charged with boronic acid (1.5 equiv) and pure glycerol (1.0 mL). The boronic acid was left to dissolve for 5 min at 50 or 80 °C, after which the aldehyde (0.41 mmol) was added and left stirring for 2 min at the same temperature, followed by addition of amine (1.5 equiv). The reactions were left stirring at that temperature, at the longest, for 48 h or until complete consumption of the aldehyde, as monitored by TLC. After cooling at room temperature, the reaction was quenched with addition of 1.0 mL of water and 1.0 mL of saturated $NaHCO_3$ solution and then extracted diethyl ether (3 to 5×5 mL) until no product was visible on TLC. Solvent was removed under reduced pressure, and the product further purified by flash chromatography on silica gel using a mixture of ethyl acetate/hexane as solvent.

Method B: Aryl boronic acid (0.5 mmol) was dissolved in ethanol (5 mL), followed by addition of the aldehyde (1.0 equiv). After stirring at 50 °C for 5 min, the amine (1.0 equiv) was added and the mixture left stirring at that temperature for 48 h. The solvent was evaporated under reduced pressure and the desired compound isolated by flash chromatography in toluene.

2-(phenyl(pyrrolidin-1-yl)methyl)phenol (**1**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.27 (brs, 1H), 7.47 (d, J = 7.3 Hz, 2H), 7.37–7.17 (m, 3H), 7.17–7.05 (m, 1H), 6.96 (d, J = 7.6 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 6.71 (t, J = 7.3 Hz, 1H), 4.38 (s, 1H), 2.65 (brs, 2H), 2.51 (brs, 2H), 1.89–1.80 ppm (m, 4H).

2-(pyrrolidin-1-yl(*p*-tolyl)methyl)phenol (**2**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.27 (brs, 1H), 7.39 (d, J = 7.9 Hz, 2H), 7.15–7.09 (m, 3H), 6.98 (dd, J = 7.47, 1.61 Hz, 1H), 6.88 (dd, J = 8.2, 0.9 Hz, 1H), 6.72 (td, J = 7.3, 1.2 Hz, 1H), 4.39 (s, 1H), 2.66–2.51 (m, 4H), 2.31 (s, 3H), 1.90–1.81 ppm (m, 4H).

2-(phenyl(piperidin-1-yl)methyl)phenol (**3**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 13.02–11.99 (m, 1H), 7.40 (d, J = 6.4 Hz, 2H), 7.36–7.19 (m, 3H), 7.18–7.04 (m, 1H), 6.94–6.82 (m, 2H), 6.69 (t, J = 7.4 Hz, 1H), 4.48 (s, 1H), 2.42 (brs, 4H), 1.75–1.55 (m, 4H), 1.55–1.20 ppm (m, 2H).

2-(piperidin-1-yl(*p*-tolyl)methyl)phenol (**4**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.63 (brs, 1H), 7.39–7.20 (m, 2H), 7.20–7.05 (m, 3H), 6.88 (t, J = 8.2 Hz, 2H), 6.69 (t, J = 7.6 Hz, 1H), 4.47 (s, 1H), 2.43 (brs, 4H), 2.33 (s, 3H), 1.67–1.56 (m, 4H), 1.57–1.38 ppm (m, 2H).

2-(morpholino(phenyl)methyl)phenol (**5**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.74 (brs, 1H), 7.45–7.42 (m, 2H), 7.37–7.21 (m, 3H), 7.16–7.10 (m, 1H), 6.97–6.86 (m, 2H), 6.73 (td, J = 7.5, 1.2 Hz, 1H), 4.41 (s, 1H), 3.78–3.71 (m, 4H), 2.61–2.43 ppm (m, 4H).

2-((4-methoxyphenyl)(morpholino)methyl)phenol (**6**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.81 (brs, 1H), 7.34 (d, J = 8.5 Hz, 2H), 7.15–7.09 (m, 1H), 6.93 (dd, J = 7.6, 1.6 Hz, 1H), 6.87–6.82 (m, 3H), 6.73 (td, J = 7.6, 1.2 Hz, 1H), 4.38 (s, 1H), 3.76–3.74 (brs, 7H), 2–59–2.41 ppm (m, 4H).

2-(morpholino(4-nitrophenyl)methyl)phenol (**7**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.29 (brs, 1H), 8.16 (d, J = 9.1 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.19–7.13 (m, 1H), 6.96–6.87 (m, 2H), 6.76 (t, J = 7.5 Hz, 1H), 4.50 (s, 1H), 3.77–3.83 (m, 4H), 2.63 (brs, 2H), 2.49–2.42 ppm (m, 2H).

2-((4-chlorophenyl)(morpholino)methyl)phenol (**8**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.58 (brs, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.5 Hz, 2H), 7.18–7.12 (m, 1H), 6.94–6.86 (m, 2H), 6.75 (t, J = 7.6 Hz, 1H), 4.39 (s, 1H), 3.83–3.72 (m, 4H), 2.60–2.41 ppm (m, 4H).

1-(4-((2-hydroxyphenyl)(morpholino)methyl)phenyl)ethan-1-one (**9**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.50 (brs, 1H), 7.89 (d, J = 6.0 Hz, 2H), 7.55 (d, J = 8.2 Hz, 2H), 7.19–7.07 (m, 1H), 6.99–6.81 (m, 2H), 6.76–6.71 (m, 1H), 4.45 (s, 1H), 3.87–3.60 (m, 4H), 2.79–2.52 (m, 5H), 2.51–2.36 ppm (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 197.7, 156.1, 144.9, 137.0, 129.4, 129.3, 128.8, 124.2, 120.1, 117.4, 76.7, 67.0, 52.5, 26.9 ppm; HRMS (ESI^+): calcd for $C_{19}H_{22}NO_3$ [M+H^+]: 312.1600, found: 312.1628.

2-(morpholino(4-vinylphenyl)methyl)phenol (**10**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.71 (brs, 1H), 7.41–7.33 (m, 4H), 7.16–7.11 (m, 1H), 6.96–6.86 (m, 2H), 6.76–6.62 (m, 2H), 5.72 (d, J = 17.6 Hz, 1H), 5.24 (d, J = 10.8 Hz, 1H), 4.41 (s, 1H), 3.82–3.71 (m, 4H), 2.60–2.43 ppm (m, 4H).

2-(morpholino(*o*-tolyl)methyl)phenol (**11**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.92 (brs, 1H), 7.63–7.60 (m, 1H), 7.10–7.19 (m, 4H), 6.95–6.87 (m, 2H), 6.72 (t, J = 7.5 Hz, 1H), 4.90 (s, 1H), 3.78–3.75 (m, 4H), 2.59–2.48 ppm (m, 7H).

2-((3-methoxyphenyl)(morpholino)methyl)phenol (**12**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.68 (brs, 1H), 7.22 (t, J = 7.5 Hz, 1H), 7.15–7.10 (m, 1H), 7.05–6.94 (m, 3H), 6.87 (d, J = 8.2, 1H), 6.81–6.70 (m, 2H), 4.36 (s, 1H), 3.76 (brs, 7H), 2.60–2.45 ppm (m, 4H).

2-((2,6-dimethylphenyl)(morpholino)methyl)phenol (**13**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.30 (brs, 1H), 7.10–7.05 (m, 3H), 7.02–6.89 (m, 1H), 6.76 (d, J = 9.0 Hz, 1H), 6.72–6.64 (m, 2H), 5.41 (s, 1H), 3.90–3.63 (m, 4H), 3.19 (brs, 1H), 2.57–2.20 ppm (m, 9H).

2-(mesityl(morpholino)methyl)phenol (**14**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.35 (s, 1H), 7.10–7.05 (m, 1H), 6.92 (brs, 1H), 6.78–

6.65 (m, 5H), 5.37 (s, 1H), 3.94–3.62 (m, 4H), 3.18 (brs, 1H), 2.55–2.45 (m, 5H), 2.32–2.16 ppm (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.9, 137.7, 137.7, 134.2, 131.3, 129.6, 128.0, 122.6, 119.4, 117.0, 105.0, 69.8, 21.0 ppm; HRMS (ESI$^+$): calcd for $C_{20}H_{26}NO_2$ [$M+H^+$]: 312.1964, found: 312.1934

5-methoxy-2-(morpholino(phenyl)methyl)phenol (**15**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.85 (brs, 1H), 7.47–7.39 (m, 2H), 7.33–7.25 (m, 3H), 6.82 (d, J = 8.5 Hz, 1H), 6.43 (d, J = 2.6 Hz, 1H), 6.30 (dd, J = 8.3, 2.5 Hz, 1H), 4.38 (s, 1H), 3.74 (brs, 7H), 2.60–2.40 ppm (m, 4H)

2-(morpholino(phenyl)methyl)-4-nitrophenol (**16**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 13.24 (brs, 1H), 8.04 (dd, J = 8.9, 2.8 Hz, 1H), 7.90 (d, J = 2.6 Hz, 1H), 7.39–7.31 (m, 5H), 6.91 (d, J = 8.8 Hz, 1H), 4.55 (s, 1H), 3.77 (brs, 4H), 2.61–2.46 ppm (m, 4H).

2-(indolin-1-yl(phenyl)methyl)phenol (**17**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 10.12 (brs, 1H), 7.48–7.44 (m, 2H), 7.37–7.25 (m, 3H), 7.23–7.14 (m, 2H), 7.02–6.79 (m, 5H), 6.50 (d, J = 7.9 Hz, 1H), 5.33 (s, 1H), 3.25–3.17 (m, 1H), 3.09 (q, J = 9.2 Hz, 1H), 2.96–2.89 ppm (m, 2H).

2-(indolin-1-yl(*p*-tolyl)methyl)phenol (**18**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 10.24 (brs, 1H), 7.43 (d, J = 8.0 Hz, 2H), 7.28–7.20 (m, 4H), 7.10–6.87 (m, 5H), 6.59 (d, J = 7.9 Hz, 1H), 5.38 (s, 1H), 3.32–3.27 (m, 1H), 3.15 (q, J = 9.2 Hz, 1H), 3.00–2.94 (m, 2H), 2.40 ppm (s, 3H).

2-(indolin-1-yl(4-methoxyphenyl)methyl)phenol (**19**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 10.18 (brs, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.25–7.17 (m, 2H), 7.06–6.84 (m, 7H), 6.54 (d, J = 7.9 Hz, 1H), 5.34 (s, 1H), 3.81 (s, 3H), 3.28–3.21 (m, 1H), 3.12 (q, J = 9.2 Hz, 1H), 2.97–2.91 ppm (m, 2H).

2-(indolin-1-yl(4-vinylphenyl)methyl)phenol (**20**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 10.13 (brs, 1H), 7.50–7.41 (m, 4H), 7.28–7.19 (m, 2H), 7.08–6.85 (m, 5H), 6.75 (dd, J = 17.7, 11.0 Hz, 1H), 6.57 (d, J = 7.6 Hz, 1H), 5.80 (d, J = 17.6 Hz, 1H), 5.39 (s, 1H), 5.31 (d, J = 10.8 Hz, 1H), 3.32–3.25 (m, 1H), 3.14 (q, J = 9.5 Hz, 1H), 2.99–2.93 ppm (m, 2H).

2-((2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)(indolin-1-yl)methyl)phenol (**21**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 10.08 (brs, 1H), 7.24–7.15 (m, 2H), 7.07–6.83 (m, 8H), 6.52 (d, J = 7.9 Hz, 1H), 5.25 (s, 1H), 4.23 (s, 4H), 3.32–3.25 (m, 1H), 3.12 (q, J = 9.4 Hz, 1H), 2.97–2.91 ppm (m, 2H).

2-(indolin-1-yl(phenyl)methyl)-4-nitrophenol (**22**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.83 (brs, 1H), 8.11 (dd, J = 9.1, 2.9 Hz, 1H), 7.98 (d, J = 2.6 Hz, 1H), 7.51–7.31 (m, 5H), 7.19 (d, J = 7.3 Hz, 1H), 6.83–7.09 (m, 3H), 6.51 (d, J = 7.6 Hz, 1H), 5.34 (s, 1H), 3.29–3.14 (m, 1H), 3.11–2.80 ppm (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 163.0, 150.4, 141.0, 138.4, 132.7, 129.4, 129.1, 129.0, 127.8, 127.0, 125.4, 125.2, 125.0, 122.7, 117.9, 112.6, 71.1, 53.9, 28.7 ppm; HRMS (ESI$^+$): calcd for $C_{21}H_{19}N_2O_3$ [$M+H^+$]: 347.1342, found: 347.1385.

2-(indolin-1-yl(*p*-tolyl)methyl)-4-nitrophenol (**23**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.81 (brs, 1H), 8.10 (dd, J = 9.1, 2.6 Hz, 1H), 7.99 (d, J = 2.6 Hz, 1H), 7.33 (d, J = 7.9 Hz, 2H), 7.17–7.09 (m, 3H), 7.09–6.85 (m, 3H), 6.53 (d, J = 7.9 Hz, 1H), 5.34 (s, 1H), 3.33–3.18 (m, 1H), 3.00–3.13 (m, 1H), 3.00–2.82 (m, 2H), 2.35 ppm (s, 3H).

2-(indolin-1-yl(4-methoxyphenyl)methyl)-4-nitrophenol (**24**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.89 (brs, 1H), 8.10 (dd, J = 8.9, 2.8 Hz, 1H), 7.96 (d, J = 3.5 Hz, 1H), 7.38–7.28 (m, 2H), 7.17 (d, J = 6.4 Hz, 1H), 7.08–6.82 (m, 5H), 6.50 (d, J = 7.9 Hz, 1H), 5.31 (s, 1H), 3.80 (s, 3H), 3.28–3.12 (m, 1H), 3.12–2.98 (m, 1H), 2.98–2.81 ppm (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 162.7, 159.7, 150.1, 140.7, 132.4, 130.0, 130.0, 127.4, 127.0, 125.0, 124.9, 124.7, 122.3, 117.5, 114.3, 112.2, 69.8, 55.2, 53.3, 28.4 ppm; HRMS (ESI$^+$): calcd for $C_{22}H_{21}N_2O_4$ [$M+H^+$]: 377.1501, found: 377.1498.

2-(indolin-1-yl(4-vinylphenyl)methyl)-4-nitrophenol (**25**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.80 (brs, 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 = 7.3 Hz, 1H), 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), 3.13–3.01 (m, 1H), 2.99–2.92 ppm (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 163.0, 150.5, 141.1, 138.4, 137.7, 136.2, 132.7, 129.2, 127.8, 127.2, 127.0, 125.4, 125.3, 125.0, 122.7, 117.9, 115.3, 112.6, 70.6, 53.9, 28.7 ppm; HRMS (ESI$^+$): calcd for $C_{23}H_{21}N_2O_3$ [$M+H^+$]: 373.1552, found: 373.1559.

2-([1,1′-biphenyl]-4-yl(indolin-1-yl)methyl)-4-nitrophenol (**26**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.87 (brs, 1H), 8.15 (dd, J = 8.8, 2.6 Hz, 1H), 8.09 (d, J = 2.3 Hz, 1H), 7.65–7.61 (m, 4H), 7.56–7.46 (m, 4H), 7.41 (d, J = 7.0 Hz, 1H), 7.23 (d, J = 5.9 Hz, 1H), 7.11–6.95 (m, 3H), 6.60 (d, J = 7.6 Hz, 1H), 5.44 (s, 1H), 3.37–3.30 (m, 1H), 3.18–3.08 (m, 1H), 3.02–2.96 ppm (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 163.0, 150.6, 142.0, 141.1, 140.3, 137.3, 132.7, 129.4, 129.2, 128.1, 128.0, 127.9, 127.4, 127.1, 125.5, 125.3, 125.1, 122.8, 118.0, 112.6, 70.7, 54.0, 28.8 ppm; HRMS (ESI$^+$): calcd for $C_{27}H_{23}N_2O_3$ [$M+H^+$]: 423.1709, found: 423.1692.

methyl 4-((2-hydroxy-5-nitrophenyl)(indolin-1-yl)methyl)benzoate (**27**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.52 (brs, 1H), 8.12 (dd, J = 9.1, 2.6 Hz, 1H), 8.04 (d, J = 8.2 Hz, 2H), 7.94 (d, J = 2.6 Hz, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.18 (d, J = 7.0 Hz, 1H), 7.04–6.91 (m, 3H), 6.48 (d, J = 7.9 Hz, 1H), 5.41 (s, 1H), 3.91 (s, 3H), 3.27–3.18 (m, 1H), 3.06–3.92 ppm (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 166.6, 162.8, 150.2, 143.0, 141.1, 132.5, 130.8, 130.7, 129.0, 127.9, 126.2, 125.7, 125.3, 124.9, 122.9, 118.1, 112.5, 70.4, 53.9, 52.6, 28.7 ppm; HRMS (ESI$^+$): calcd for $C_{23}H_{21}N_2O_5$ [$M+H^+$]: 405.1450, found: 405.1442.

2-((2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)(indolin-1-yl)methyl)-4-nitrophenol (**28**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.80 (brs, 1H), 8.10 (dd, J = 8.9, 2.8 Hz, 1H), 7.99 (d, J = 2.6 Hz, 1H), 7.30–7.15 (m, 1H), 7.05–7.00 (m, 1H), 7.05–6.83 (m, 5H), 6.51 (d, J = 7.6 Hz, 1H), 5.25 (s, 1H), 4.25 (s, 4H), 3.32–3.25 (m, 1H), 3.12–2.91 ppm (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 163.0, 150.4, 144.2, 144.0, 141.0, 132.7, 131.5, 127.8, 127.2, 125.3, 125.2, 125.0, 122.6, 122.1, 118.1, 117.9, 117.9, 112.5, 70.3, 64.5, 53.7, 28.7 ppm; HRMS (ESI$^+$): calcd for $C_{23}H_{21}N_2O_5$ [$M+H^+$]: 405.1450, found: 405.1440.

2-((3,4-dihydroquinolin-1(2*H*)-yl)(*p*-tolyl)methyl)phenol (**29**): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 7.16–7.11 (m, 4H), 7.16–7.03 (m, 2H), 6.92 (t, J = 4.4 Hz, 1H), 6.87–6.82 (m, 3H), 6.59 (d, J = 5.0 Hz, 2H), 5.46 (s, 1H), 3.20–3.17 (m, 2H), 2.80 (t, J = 6.3 Hz, 2H), 2.35 (s, 3H), 1.90–1.82 ppm (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 154.1, 142.0, 138.1, 136.5, 130.1, 129.4, 129.2, 129.1, 128.4, 128.1, 127.3, 126.6, 122.8, 120.8, 117.2, 116.4, 46.0, 42.4, 27.5, 21.9, 21.1 ppm; HRMS (ESI$^+$): calcd for $C_{23}H_{24}NO$ [$M+H^+$]: 330.1858, found: 330.1848.

2-((3,4-dihydroquinolin-1(2*H*)-yl)(4-methoxyphenyl)methyl)phenol (**30**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.16–7.11 (m, 1H), 7.09–7.04 (m, 2H), 6.86–6.81 (m, 5H), 6.72–6.69 (m, 2H), 6.41 (d, J = 7.9 Hz, 1H), 5.43 (s, 1H), 3.79 (s, 3H), 3.30–3.26 (m, 2H), 2.68 (t, J = 6.4 Hz, 2H), 1.95–1.87 ppm (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 158.3, 154.0, 143.7, 135.4, 131.5, 130.9, 130.5, 130.5, 127.9, 127.8, 122.1, 120.7, 116.5, 114.8, 114.1, 55.4, 50.0, 42.3, 27.2, 22.4 ppm; HRMS (ESI$^+$): calcd for $C_{23}H_{24}NO_2$ [$M+H^+$]: 346.1807, found: 346.1795.

2-((diallylamino)(phenyl)methyl)phenol (**31**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.13 (s, 1H), 7.53–7.21 (m, 5H), 7.20–7.04 (m, 1H),

6.97–6.75 (m, 2H), 6.75–6.57 (m, 1H), 6.09–5.72 (m, 2H), 5.32–5.09 (m, 4H), 5.06 (s, 1H), 3.42–3.34 (m, 2H), 3.07–3.00 ppm (m, 2H).

2-((diallylamino)(*p*-tolyl)methyl)phenol (**32**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.23 (s, 1H), 7.35–7.32 (m, 2H), 7.26–7.13 (m, 3H), 6.92–6.84 (m, 2H), 6.73–6.68 (m, 1H), 6.01–5.87 (m, 2H), 5.28–5.98 (m, 5H), 3.42 (dd, J = 14.1, 5.9 Hz, 2H), 3.06 (dd, J = 13.8, 7.6 Hz, 2H), 2.38 ppm (s, 3H).

2-((diallylamino)(thiophen-3-yl)methyl)phenol (**33**): 1H NMR ($CDCl_3$, 300 MHz): δ = 12.00 (br s, 1H), 7.35 (dd, J = 5.1, 3.1 Hz, 1H), 7.25 (dd, J = 3.1, 1.3 Hz, 1H), 7.18–7.12 (m, 2H), 6.87 (dd, J = 8.2, 1.2 Hz, 1H), 6.82–6.79 (m, 1H), 6.73–6.67 (m, 1H), 5.91 (m, 2H), 5.31 (s, 1H), 5.26–5.14 (m, 4H), 3.44–3.37 (m, 2H), 2.95 ppm (dd, J = 13.9, 8.1 Hz, 2H).

2-((benzyl(methyl)amino)(phenyl)methyl)phenol (**34**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.38 (br s, 1H), 7.50 (d, J = 7.3 Hz, 2H), 7.42–7.28 (m, 8H), 7.16 (t, J = 7.8 Hz, 1H), 7.00–6.89 (m, 2H), 6.74 (t, J = 7.3 Hz, 1H), 4.73 (s, 1H), 3.58 (br s, 2H), 2.19 ppm (s, 3H).

2-((benzyl(methyl)amino)(*p*-tolyl)methyl)phenol (**35**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.46 (br s, 1H) 7.43–7.31 (m, 7H), 7.16–7.22 (m, 3H), 6.99–6.95 (m, 2H), 6.77 (t, J = 7.0 Hz, 1H), 4.75 (s, 1H), 3.60 (br s, 2H), 2.38 (s, 3H), 2.21 ppm (s, 3H).

2-((benzyl(methyl)amino)(4-methoxyphenyl)methyl)phenol (**36**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.48 (br s, 1H), 7.43–7.29 (m, 7H), 7.20–7.14 (m, 1H), 6.96–6.90 (m, 4H), 6.78–6.72 (m, 1H), 4.73 (s, 1H), 3.81 (s, 3H), 3.58 (br s, 2H), 2.18 ppm (s, 3H).

2-((benzyl(methyl)amino)(4-vinylphenyl)methyl)phenol (**37**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.39 (br s, 1H), 7.31–7.51 (m, 9H), 7.22–7.17 (m, 1H), 6.96–6.99 (m, 2H), 6.80–6.69 (m, 2H), 5.79 (d, J = 17.6 Hz, 1H), 5.29 (d, J = 11.7 Hz, 1H), 4.75 (s, 1H), 3.61 (s, 2H), 2.22 ppm (s, 3H).

2-((benzyl(methyl)amino)(thiophen-3-yl)methyl)phenol (**38**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.27 (br s, 1H), 7.41–7.26 (m, 8H), 7.22–7.17 (m, 1H), 6.95 (m, 2H), 6.79–6.74 (m, 1H), 4.97 (s, 1H), 3.59 (br s, 2H), 2.20 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 157.4, 138.9, 137.4, 129.6, 129.2, 129.1, 128.9, 128.1, 127.9, 126.7, 125.3, 124.6, 119.4, 117.1, 69.4, 59.5, 38.9 ppm; HRMS (ESI$^+$): calcd for $C_{19}H_{20}NOS$ [$M+H^+$]: 310.1266, found: 310.1302.

2-((dibenzylamino)(phenyl)methyl)phenol (**39**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.12 (s, 1H), 7.49–7.28 (m, 15H), 7.19 (t, J = 7.3 Hz, 1H), 6.96 (d, J = 7.9 Hz, 1H), 6.83 (d, J = 7.0 Hz, 1H), 6.72 (t, J = 7.6 Hz, 1H), 5.16 (s, 1H), 3.97 (d, J = 13.2 Hz, 2H), 3.42 ppm (d, J = 13.5 Hz, 2H).

2-((dibenzylamino)(*p*-tolyl)methyl)phenol (**40**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.19 (s, 1H), 7.39–7.24 (m, 14H), 7.21–7.15 (m, 1H), 6.95 (d, J = 8.1 Hz, 1H), 6.83 (d, J = 7.6 Hz, 1H), 6.71 (t, J = 7.6 Hz, 1H), 5.13 (s, 1H), 3.96 (d, J = 13.2 Hz, 2H), 3.40 (d, J = 13.2 Hz, 2H), 2.42 ppm (s, 3H).

2-((dibenzylamino)(4-methoxyphenyl)methyl)phenol (**41**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.27 (s, 1H), 7.41–7.18 (m, 13H), 7.02–6.98 (m, 3H), 6.86 (d, J = 7.3 Hz, 1H), 6.75 (t, J = 7.5 Hz, 1H), 5.16 (s, 1H), 3.98 (d, J = 13.2 Hz, 2H), 3.88 (s, 3H), 3.43 ppm (d, J = 13.2, 2H).

2-((dibenzylamino)(4-vinylphenyl)methyl)phenol (**42**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 12.18 (br s, 1H), 7.54 (d, J = 8.8 Hz, 2H), 7.45–7.31 (m, 12H), 7.24 (t, J = 7.9 Hz, 1H), 7.02 (d, J = 7.9 Hz, 1H), 6.90–6.75 (m, 3H), 5.87 (d, J = 17.6 Hz, 1H), 5.37 (d, J = 11.1 Hz, 1H), 5.20 (s, 1H), 4.02 (d, J = 13.2 Hz, 2H), 3.46 ppm (d, J = 13.2 Hz, 2H).

2-((dibenzylamino)(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)methyl)-phenol (**43**): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 12.16 (s, 1H), 7.40–7.29 (m, 10H), 7.20 (t, J = 7.6 Hz, 1H), 6.98–6.89 (m, 5H), 6.78–6.73 (m, 1H), 5.06 (s, 1H), 4.29 (s, 4H), 3.96 (d, J = 13.5 Hz, 2H), 3.47 ppm (d, J = 13.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 157.7, 143.8, 143.7, 137.3, 130.0, 129.9, 129.1, 128.9, 127.8, 124.9, 124.0, 119.6, 119.3, 117.4, 116.9, 68.0, 64.6, 64.6, 54.0 ppm; HRMS (ESI$^+$): calcd for $C_{29}H_{28}NO_3$ [$M+H^+$]: 438.2069, found: 438.2045.

Computational details

All calculations were performed using the Gaussian 09 software package,[29] without symmetry constraints. The PBE1PBE functional was employed in the geometry optimizations. That functional uses a hybrid generalized gradient approximation (GGA), including 25% mixture of Hartree–Fock[30] exchange with DFT[19] exchange-correlation, given by Perdew, Burke and Ernzerhof functional (PBE).[31] The optimized geometries were obtained with a standard 6-31G(d,p)[32] basis set and solvent effects (water) were considered using the Polarizable Continuum Model (PCM) initially devised by Tomasi and co-workers[33] as implemented on Gaussian 09, with radii and non-electrostatic terms for Truhlar and co-workers' SMD solvation model.[34] A Natural Population Analysis (NPA)[35] was used to study the electronic structure of the optimized species as implemented on Gaussian 09. Atomic coordinates for all the optimized species can be found in the Supporting Information.

Biological assays

Dimethyl sulfoxide (DMSO) was purchased from Merck, (Darmstadt, Germany). Trypsin, Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin solution, fetal bovine serum and thiazolyl blue tetrazolium bromide (MTT) were obtained from Sigma–Aldrich, (Saint Louis, MO, USA).

Microbial strains: The in vitro antimicrobial study was carried out using Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *S. aureus* CIP106760 (MRSA), *Enterococcus faecalis* ATCC 51299, *E. faecalis* ATCC 29212 and *Mycobacterium smegmatis* ATCC 607), Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and a yeast (*Candida albicans* ATCC 10231).

Well diffusion assay: The well diffusion assay was used to determine and screen the antimicrobial activity of all compounds. Petri dishes containing 20 mL Mueller-Hinton culture medium were inoculated with 0.1 mL of a bacterial cell suspension matching a 0.5 McFarland standard solution. The suspension was uniformly spread using a sterile swab over the surface of the medium. Wells of 5 mm in diameter were made in the agar plates with a sterile glass Pasteur pipette and 50 μL of each compound (1 mg mL^{-1}), previously reconstituted by dissolving in DMSO, was added into wells. DMSO was used as a negative control, while vancomycin (1 mg mL^{-1}) and norfloxacin (1 mg mL^{-1}) were used as positive controls for Gram-positive and Gram-negative bacteria, respectively and nystatin for the yeast. The plates were then incubated at 37 °C for 24 h. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the wells in mm. Each assay was performed at least in duplicate.

Microdilution method: The minimum inhibitory concentrations (MICs) of antimicrobial compounds evaluated previously by the well diffusion assay, was determined by means of the twofold serial broth microdilution assay.[36] The compounds, dissolved in

DMSO, were diluted at concentrations ranging from 500 to 0.488 μg mL^{-1}, with Mueller-Hinton broth medium. The antimicrobial activity of the solvent DMSO was evaluated. Vancomycin, norfloxacin, rifampicin, and nystatin were used as controls. The MIC values were taken as the lowest concentration of the compound that inhibited the growth of the microorganisms, after 24 h of incubation at 37 °C, and are presented in micromolar. The microbial growth was measured with an Absorvance Microplate Reader set to 620 nm (Termo scientific Multiskan FC). Assays were carried out in triplicate for each tested microorganism.

Minimum bactericidal concentration (MBC): To determine the minimum bactericidal concentration (MBC) for each set of wells in the MIC determination, a loopful of broth was collected from those wells which did not show any growth and inoculated on sterile Mueller–Hilton medium broth (for bacteria) by streaking. Plates inoculated with bacteria were incubated at 37 °C for 24 h. After incubation, the lowest concentration was noted as MBC (for bacteria) at which no visible growth was observed.

Inhibition of growth: The growth curves of *S. aureus* ATCC 25923 in the absence and in the presence of compound **23**, at the respective MIC, 2×MIC and 3×MIC concentrations, were monitored along time at an OD620 nm. Aliquots were taken at 30 min intervals and incubated at 37 °C for 24 h. Assays were carried out in duplicate.

Cytotoxicity: The cytotoxicity profile of the selected compounds was characterized in the human keratinocyte cell line HaCaT, using a 24 h incubation protocol. The compounds were initially solubilized in DMSO and then further diluted in PBS. The final concentration of DMSO in culture medium was 0.5 %. Cell viability was evaluated by the MTT assay, according to a procedure described in Wagemaker et al.[37] Two independent experiments were carried out, each comprising four replicate cultures.

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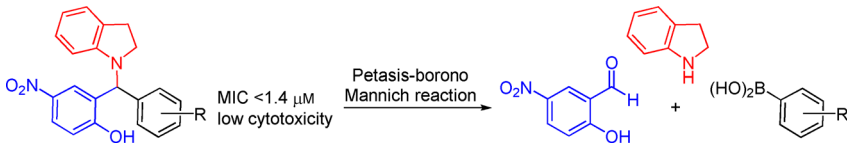
Multicomponent Petasis-borono Mannich Preparation of Alkylaminophenols and Antimicrobial Activity Studies



Antibiotics by MCR: We disclose a new family of antibacterial agents derived from *para*-nitrophenols and indoline, prepared by a multicomponent reaction (MCR). The new alkylaminophenols show minimum inhibitory concentrations lower than 1.4 μM against selected resistant microorganisms. Cytotoxicity assays demonstrated that such compounds are viable candidates as antibacterials, as no cytotoxicity was observed at these concentrations.