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

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Abstract: 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 salicylaldehyde derivatives. The obtained compounds were tested against a large panel of micro-organisms, 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 the 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 the ones needed 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 has been pointed as the main cause for the appearance of multi-drug resistant bacteria. In some instances, bacteria resistant to more than one antibiotic have been reported. As micro-organisms are becoming resistant to antibiotics, the development of new antibacterial agents is of pivotal importance for community wellbeing. *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 new technologies employed for 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 less examples of synthetic antimicrobials have been reported. Despite the many thousands of natural antibiotics discovered, our knowledge on their targets remains very scarce. The structural requirements to make a compound able to penetrate bacterial cells is still obscure making the process of finding novel penetrating compounds difficult. Despite the 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 complexes mechanisms of action of existing antibiotics and the non-compatibility of existing antibiotics regarding their physical and chemical properties with conventional medicinal chemistry approaches.[5] Maybe for these reasons most of synthetic antibiotics have been discovered outside of antibiotic discovery programs.[6]

Many phenolic compounds, either natural products or completely synthetic molecules, have been reported to have antibacterial activity. Natural phenol derivative Arzanol has been identified as a lead structure in the development on new antibacterials,[7] and carvacrol has been reported to act as 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-alkyl phenols shown moderate *in vitro* antibacterial activity[10] and bromophenols isocitrate lyase inhibitors of *Candida albicans*.[11]

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

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

In order to identify the structural elements needed for antibacterial activity, different secondary amines were firstly

considered and the substituents of the aromatic rings further tuned. Herein we present the results on the study of the antibacterial activity of alkylaminophenols derived from 8 different secondary amines.

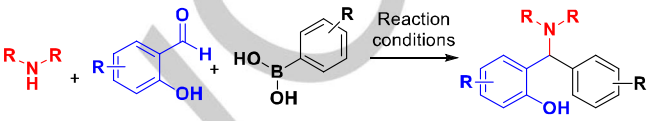
Results and Discussion

Synthesis of alkylaminophenols

Compounds **1-43** were prepared by a Petasis-borono Mannich (PBM) reaction (Scheme 1),[16] according to previously reported procedures. The multicomponent character of such methodology allows the fast 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 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 presence of different 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 and Pan.[18]

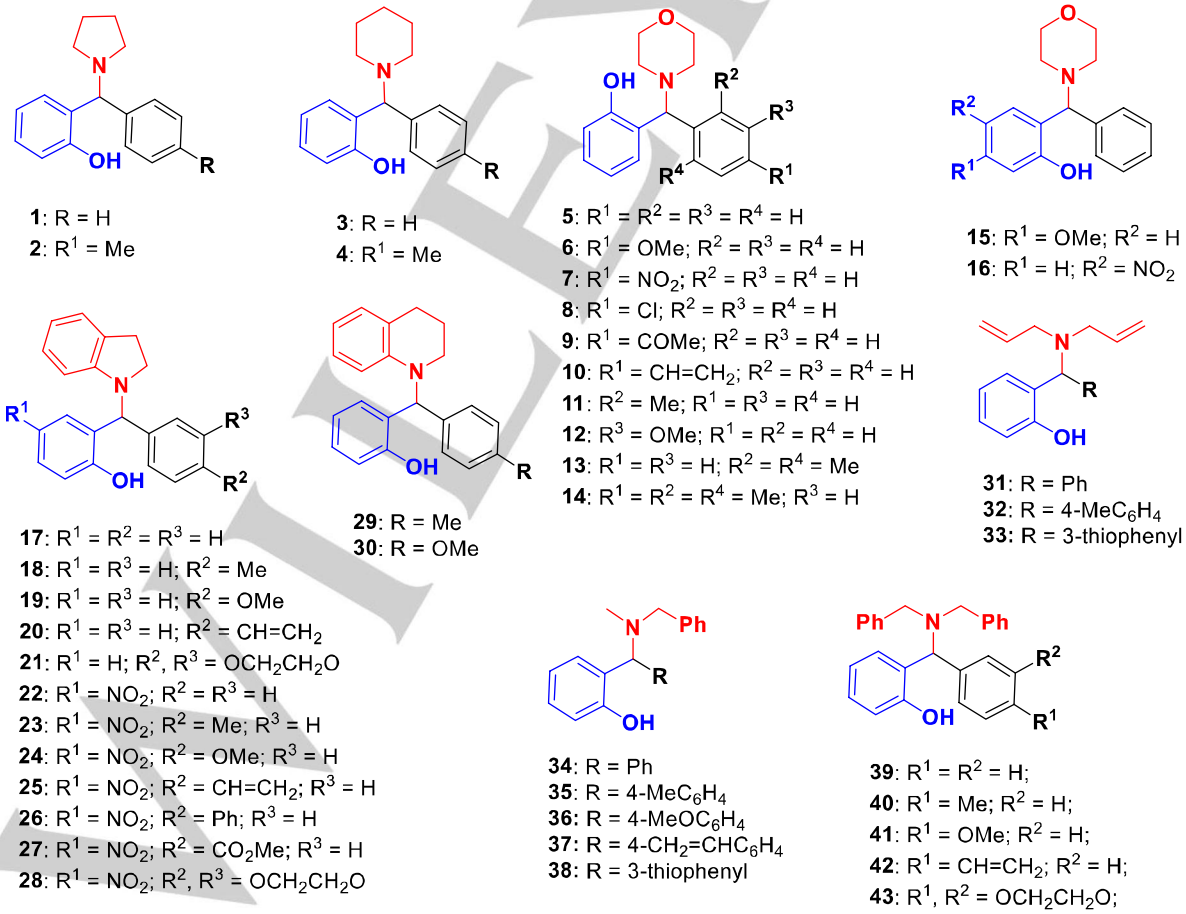
All desired compounds were purified by column chromatography in silica gel. Their chemical structures were confirmed by NMR, and mass spectrometry for all new compounds.



Scheme 1. General method for preparation of alkylaminophenols.

Antimicrobial activity of alkylaminophenols

All prepared compounds were screened for their antimicrobial activity by the well diffusion assay. This preliminary test aimed to



Scheme 2 Chemical structures of prepared alkylaminophenols **1**-**43**.

Table 1. Preparation and isolated yields of alkylaminophenols

Compound	Method[a]	Yield (%)	Compound	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. of amine and boronic acid in glycerol (1 mL), 50 °C, 24-48 h; Method B: aldehyde (0.5 mmol), 1.0 equiv. of amine and boronic acid in ethanol (5 mL), 50 °C, 48 h. [b] reaction conducted at 80 °C. [c] reaction conducted in refluxing ethanol (8 mL) for 24 h. [d] reaction conducted at 80 °C for 3 h.

identify the antimicrobial compounds comparing with the corresponding positive controls. The antimicrobial activity was evaluated against a large panel of micro-organisms: 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 to select the compounds **22-29** 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 micro-organisms (MRSA and VRE) but also a non-pathogenic strain *Mycobacterium smegmatis,* from the common genus of the *Mycobacterium tuberculosis*, the most important 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**, the corresponding positive control exhibited 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 one (MIC values ranging from <1.36 to 2.72 µM). Gladly, higher antibacterial activity of the indoline derivatives than salicylaldehydes was observed against *S. aureus*.[12b, 12c].

Table 2. Antimicrobial activity of the synthesized alkylaminophenols derivatives.[a]

Compound	*Minimum inhibitory concentrations (µM)*			
	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 represent 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 the ones 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 a higher lipophilicity than the other derivatives (Table 3), an apparent requirement to inhibit the tested bacteria growth.

Considering these indoline compounds **22**-**28**, the *para*-nitro group in the phenol ring is another essential structural feature for the antimicrobial activity. A similar feature was observed for salicylaldehyde derivatives where 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 the derivative with a *para*-methyl group in the phenyl group showed to be the most active compound (**23**). 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**.

In order to assess for a correlation between the antimicrobial activity and the electronic properties of alkylaminophenols, the structures on the series of 5-nitro substituted derivatives **23-29**, and selected unsubstituted alkylaminophenols were geometrically optimized by Density Functional Theory calculations[20] (Table 3). Further analysis of dipole moment and natural bond orbitals suggest a relation between the antibacterial activity of the compounds and its electronic properties, as only more polar compounds with smaller frontier molecular orbital gaps are active.

Table 3. Predicted Log P, dipole moment and frontier molecular orbitals energies of selected compounds.

Compound	Log P[a]	Dipole (Debye)	E_{HOMO} (eV)	E_{LUMO} (eV)	$\Delta E_{HOMO-LUMO}$ (eV)
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.

The results obtained also suggest a relationship of the antimicrobial activity with unsubstituted *meta*-position of the phenyl group as in all active compounds **22-28**. In this series, compound **28** having 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 on 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 *S. aureus* strain, as model of Gram positive bacteria. For compound **23** at 1.36 µM (MIC value <1.36 µM), no inhibitory effect was observed. At 2.72 µM and 4.08 µM concentration values the compound **23** was responsible for a strong delay and decrease of 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.

In order 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[19] so there is an evidence of bacteriostatic properties for compound **23** against *S. aureus*.

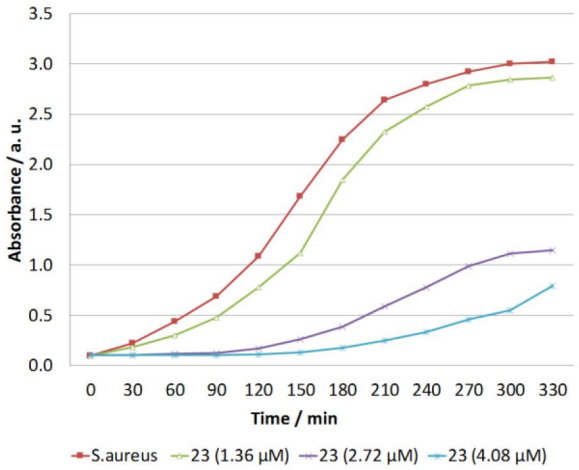


Figure 1. Growth curves of bacterial strains *S. aureus* ATCC 25923 independently challenged with compound **23**. Bacterial growth was assessed in the absence of compound (*S. aureus*) or in the presence of different concentrations of the compound **23**. The optical density was monitored at 620 nm.

Cytotoxicity

The most active compound **23** and two other compounds selected from their antimicrobial properties, **24** and **29**, were evaluated for their cytotoxicity in a human keratinocytes (HaCaT) cell line. The results are depicted in Figure 2. Under 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 mention that the concentrations in 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 a cutaneous application.

Conclusions

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

Antimicrobial activity of the synthesized compounds was evaluated against a large panel of micro-organisms, Gram-positive and Gram-negative bacteria, and a yeast. The

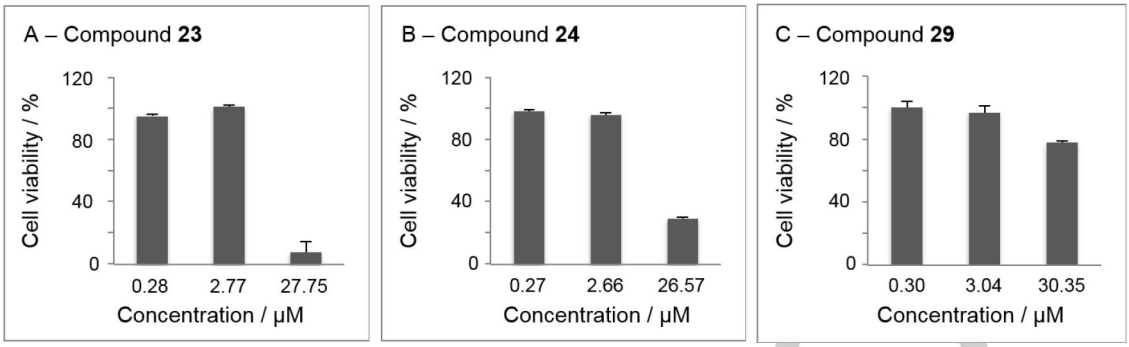


Figure 2. Effect of compounds **23** (A), **24** (B) and **29** (C) on the viability of human keratinocytes, as evaluated by MTT assay. Cells were incubated with increasing concentrations of the compounds for 24h. Results are average values ± SD from two independent experiments, each comprising four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

structure–activity relationship of the synthesized compounds revealed that the compounds **22-28** bearing an indoline group were more 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*-nitro phenol 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 concentrations. 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 supports further studies towards a 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

Syntheses of alkylaminophenols

General Remarks: All reactions using glycerol 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). Bidistilled glycerol (99.5 % w/v) was used as obtained from VWR (0.5 % maximum water content. The reactions were monitored by thin-layer chromatography carried out on pre-coated (Merck TLC silica gel 60 F254) aluminium 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.

Compounds **1**, **4, 6, 15, 31, 33, 36, 40** have been obtained with same spectral characterization as reported elsewhere.[15c] Characterization of compounds **2, 7, 10, 13, 16-21, 32, 35, 37, 42** have been previously described.[15a] Compounds **3, 34, 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 same spectral characterization as previously described.

General Procedure for syntheses of alkylaminophenols

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 x 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 minutes, 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 (br. s., 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 (br. s., 2H), 2.51 (br. s., 2H), 1.89 – 1.80 (m, 4H) ppm.

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

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 (br. s., 4H), 1.75 - 1.55 (m, 4H), 1.55 - 1.20 (m, 2H) ppm.

2-(piperidin-1-yl(*p*-tolyl)methyl)phenol (**4**): ^{1}H NMR (300 MHz, $CDCl_3$) δ = 12.63 (br. s., 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 (br. s., 4H), 2.33 (s, 3H), 1.67 - 1.56 (m, 4H), 1.57 - 1.38 (m, 2H) ppm.

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

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

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

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

1-(4-((2-hydroxyphenyl)(morpholino)methyl)phenyl)ethan-1-one (**9**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.50 (br. s., 1 H), 7.89 (d, *J*=6.0 Hz, 2 H), 7.55 (d, *J*=8.2 Hz, 2 H), 7.19 - 7.07 (m, 1 H), 6.99 - 6.81 (m, 2 H), 6.76 - 6.71 (m, 1 H), 4.45 (s, 1 H), 3.87 - 3.60 (m, 4 H), 2.79 - 2.52 (m, 5 H), 2.51 - 2.36 (m, 2 H) ppm; ^{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 (br. s, 1 H), 7.41 - 7.33 (m, 4 H), 7.16 - 7.11 (m, 1 H), 6.96 - 6.86 (m, 2 H), 6.76 - 6.62 (m, 2 H), 5.72 (d, *J*=17.6 Hz, 1 H), 5.24 (d, *J*=10.8 Hz, 1 H), 4.41 (s, 1 H), 3.82 - 3.71 (m, 4 H), 2.60 - 2.43 (m, 4 H) ppm.

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

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

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

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 (br. s, 1H), 6.78 - 6.65 (m, 5H), 5.37 (s, 1H), 3.94 – 3.62 (m, 4H), 3.18 (br. s, 1H), 2.55 – 2.45 (m, 5H), 2.32 – 2.16 (m, 6H) ppm; ^{13}C NMR (75MHz, $CDCl_3$) 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 (br. s., 1 H), 7.47 - 7.39 (m, 2 H), 7.33 - 7.25 (m, 3 H), 6.82 (d, *J*=8.5 Hz, 1 H), 6.43 (d, *J*=2.6 Hz, 1 H), 6.30 (dd, *J*=8.3, 2.5 Hz, 1 H), 4.38 (s, 1 H), 3.74 (br. s, 7 H), 2.60 - 2.40 (m, 4 H) ppm

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

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

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

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

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

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 (br. s, 1 H), 7.24 - 7.15 (m, 2 H), 7.07 - 6.83 (m, 8 H), 6.52 (d, *J*=7.9 Hz, 1 H), 5.25 (s, 1 H), 4.23 (s, 4 H), 3.32 – 3.25 (m, 1 H), 3.12 (q, *J*=9.4 Hz, 1 H), 2.97 - 2.91 (m, 2 H) ppm.

2-(indolin-1-yl(phenyl)methyl)-4-nitrophenol (**22**): ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 11.83 (br. s., 1 H), 8.11 (dd, *J*=9.1, 2.9 Hz, 1 H), 7.98 (d, *J*=2.6 Hz, 1 H), 7.51 - 7.31 (m, 5 H), 7.19 (d, *J*=7.3 Hz, 1 H), 6.83 - 7.09 (m, 3 H), 6.51 (d, *J*=7.6 Hz, 1 H), 5.34 (s, 1 H), 3.29 - 3.14 (m, 1 H), 3.11 - 2.80 (m, 3 H) ppm; ^{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 (br. s, 1 H), 8.10 (dd, *J*=9.1, 2.6 Hz, 1 H), 7.99 (d, *J*=2.6 Hz, 1 H), 7.33 (d, *J*=7.9 Hz, 2 H), 7.17 - 7.09 (m, 3 H), 7.09 - 6.85 (m, 3 H), 6.53 (d, *J*=7.9 Hz, 1 H), 5.34 (s, 1 H), 3.33 - 3.18 (m, 1 H), 3.00 - 3.13 (m, 1 H), 3.00 - 2.82 (m, 2 H), 2.35 (s, 3 H) ppm.

2-(indolin-1-yl(4-methoxyphenyl)methyl)-4-nitrophenol (**24**): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 11.89 (br. s., 1 H), 8.10 (dd, J=8.9, 2.8 Hz, 1 H), 7.96 (d, J=3.5 Hz, 1 H), 7.38 - 7.28 (m, 2 H), 7.17 (d, J=6.4 Hz, 1 H), 7.08 - 6.82 (m, 5 H), 6.50 (d, J=7.9 Hz, 1 H), 5.31 (s, 1 H), 3.80 (s, 3 H), 3.28 - 3.12 (m, 1 H), 3.12 - 2.98 (m, 1 H), 2.98 - 2.81 (m, 2 H) ppm; ^{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 (br. s., 1 H), 8.12 (dd, *J*=8.9, 2.8 Hz, 1 H), 8.02 (d, *J*=2.6 Hz, 1 H), 7.42 (s, 4 H), 7.20 (d, *J*=7.3 Hz, 1 H), 7.07 - 6.92 (m, 3 H), 6.72 (dd, *J*=17.7, 11.0 Hz, 1 H), 6.55 (d, *J*=7.9 Hz, 1 H), 5.79 (d, *J*=17.6 Hz, 1 H), 5.38 (s, 1 H), 5.31 (d, *J*=10.8 Hz, 1 H), 3.31 - 3.24 (m, 1 H), 3.13 - 3.01 (m, 1 H), 2.99 - 2.92 (m, 2 H) ppm; ^{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 (br. s., 1 H), 8.15 (dd, *J*=8.8, 2.6 Hz, 1 H),

8.09 (d, *J*=2.3 Hz, 1 H), 7.65 - 7.61 (m, 4 H), 7.56 - 7.46 (m, 4 H), 7.41 (d, *J*=7.0 Hz, 1 H), 7.23 (d, *J*=5.9 Hz, 1 H), 7.11 - 6.95 (m, 3 H), 6.60 (d, *J*=7.6 Hz, 1 H), 5.44 (s, 1 H), 3.37 - 3.30 (m, 1 H), 3.18 - 3.08 (m, 1 H), 3.02 - 2.96 (m, 2 H) ppm; ^{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 (br. s, 1 H), 8.12 (dd, *J*=9.1, 2.6 Hz, 1 H), 8.04 (d, *J*=8.2 Hz, 2 H), 7.94 (d, *J*=2.6 Hz, 1 H), 7.52 (d, *J*=8.2 Hz, 2 H), 7.18 (d, *J*=7.0 Hz, 1 H), 7.04 - 6.91 (m, 3 H), 6.48 (d, *J*=7.9 Hz, 1 H), 5.41 (s, 1 H), 3.91 (s, 3 H), 3.27 - 3.18 (m, 1 H), 3.06 - 3.92 (m, 3 H) ppm; ^{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 (br. s, 1 H), 8.10 (dd, *J*=8.9, 2.8 Hz, 1 H), 7.99 (d, *J*=2.6 Hz, 1 H), 7.30 - 7.15 (m, 1 H), 7.05 - 7.00 (m, 1 H), 7.05 - 6.83 (m, 5 H), 6.51 (d, *J*=7.6 Hz, 1 H), 5.25 (s, 1 H), 4.25 (s, 4 H), 3.32 - 3.25 (m, 1 H), 3.12 - 2.91 (m, 3 H) ppm; ^{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, 4 H), 7.16 - 7.03 (m, 2 H), 6.92 (t, *J*=4.4 Hz, 1 H), 6.87 - 6.82 (m, 3 H), 6.59 (d, *J*=5.0 Hz, 2 H), 5.46 (s, 1 H), 3.20 - 3.17 (m, 2 H), 2.80 (t, *J*=6.3 Hz, 2 H), 2.35 (s, 3 H), 1.90 - 1.82 (m, 2 H) ppm; ^{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, 1 H), 7.09 – 7.04 (m, 2 H), 6.86 – 6.81 (m, 5 H), 6.72 – 6.69 (m, 2 H), 6.41 (d, *J*=7.9 Hz, 1 H), 5.43 (s, 1 H), 3.79 (s, 3 H), 3.30 – 3.26 (m, 2 H), 2.68 (t, *J*=6.4 Hz, 2 H), 1.95 – 1.87 (m, 2 H) ppm; ^{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 (ES^+): 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 (m, 2H) ppm.

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

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

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 (s, 3H) ppm.

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

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

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

2-((benzyl(methyl)amino)(thiophen-3-yl)methyl)phenol (**38**): ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 12.27 (br. s., 1 H), 7.41 - 7.26 (m, 8 H), 7.22 - 7.17 (m, 1 H), 6.95 (m, 2 H), 6.79 - 6.74 (m, 1 H), 4.97 (s, 1 H), 3.59 (br. s., 2 H), 2.20 (s, 3 H) ppm; ^{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 (d, *J*=13.5 Hz, 2H) ppm.

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

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

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

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, 1 H), 7.40 – 7.29 (m, 10 H), 7.20 (t, *J*=7.6 Hz, 1 H), 6.98 – 6.89 (m, 5 H), 6.78 – 6.73 (m, 1 H), 5.06 (s, 1 H), 4.29 (s, 4 H), 3.96 (d, *J*=13.5 Hz, 2 H), 3.47 (d, *J*=13.2 Hz, 2 H) ppm; ^{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 generalised gradient approximation (GGA), including 25 % mixture of Hartree-Fock[30] exchange with DFT[20] exchange-correlation, given by Perdew, Burke and Ernzerhof functional (PBE).[31] The optimised 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 coworkers[33] as implemented on Gaussian 09, with radii and non-electrostatic terms for Truhlar and coworkers' SMD solvation model.[34] A Natural Population Analysis (NPA)[35] was used to study the electronic structure of the optimised species as implemented on Gaussian 09. Atomic coordinates for all the optimised species can be found in supporting information.

Biological Assays

Dimethylsulphoxide (DMSO) was purchased from Merck, (Darmstadt, Germany). Trypsin, Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin solution, foetal bovine serum and thiazolyl blue tetrazolium bromide (MTT) were obtained from Sigma-Aldrich, (Saint Louis, MO, U.S.A.)

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 5mm in diameter were made in the agar plates with a sterile glass Pasteur pipette and 50 µL of each compound (1mg/mL), previously reconstituted by dissolving in DMSO, was added into wells. DMSO was used as a negative control, while vancomycin (1mg/mL) and norfloxacin (1mg/mL) were used as positive controls for Gram-positive and Gram-negative bacteria, respectively and nystatin for the yeast. The plates were then incubated at 37ºC for 24 hours. 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 two fold serial broth microdilution assay.[36] The compounds, dissolved in DMSO, were diluted at concentrations ranging from 500 to 0.488 µg/mL, 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 micro-organisms, after 24h of incubation at 37 ºC, and are presented in µM. 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 micro-organism.

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 ºC 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 OD620nm. Aliquots were taken at 30 min intervals and incubated at 37ºC for 24h. 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 24h 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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References:

[1] S. N. Khan, A. U. Khan, *Front. Microbiol.* **2016**, *7*, Article 174.
[2] A. L. Hopkins, *Nat. Chem. Biol.* **2008**, *4*, 682-690.
[3] A. Brunschweiger, J. Hall, *ChemMedChem* **2012**, *7*, 194-203.
[4] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2012**, *75*, 311-335.
[5] a) K. Lewis, *Nat Rev Drug Discov* **2013**, *12*, 371-387; b) E. D. Brown, G. D. Wright, *Nature* **2016**, *529*, 336-343.
[6] R. J. Fair, Y. Tor, *Perspect. Medicin. Chem.* **2014**, *6*, 25-64.
[7] O. Taglialatela-Scafati, F. Pollastro, G. Chianese, A. Minassi, S. Gibbons, W. Arunotayanun, B. Mabebie, M. Ballero, G. Appendino, *J. Nat. Prod.* **2013**, *76*, 346-353.
[8] Beena, D. Kumar, D. S. Rawat, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 641-645.
[9] M. R. López, L. de León, L. Moujir, *Planta Med.* **2011**, *77*, 726-729.
[10] Z. Ali, I. A. Khan, *Phytochemistry* **2011**, *72*, 2075-2080.
[11] K.-B. Oh, H. B. Jeon, Y.-R. Han, Y.-J. Lee, J. Park, S.-H. Lee, D. Yang, M. Kwon, J. Shin, H.-S. Lee, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6644-6648.
[12] a) H. Elo, M. Kuure, E. Pelttari, *Eur. J. Med. Chem.* **2015**, *92*, 750-753; b) E. Pelttari, M. Lehtinen, H. Elo, in *Zeitschrift für Naturforschung C, Vol. 66*, **2011**, p. 571; c) E. Pelttari, E. Karhumäki, J. Langshaw, H. Peräkylä, H. Elo, in *Zeitschrift für Naturforschung C, Vol. 62*, **2007**, p. 487.
[13] L. Shi, H.-M. Ge, S.-H. Tan, H.-Q. Li, Y.-C. Song, H.-L. Zhu, R.-X. Tan, *Eur. J. Med. Chem.* **2007**, *42*, 558-564.
[14] A. A. Jameel, M. S. A. Padusha, K. S. S. Ibrahim, *Asian J. Chem.* **2011**, *23*, 1269 - 1272.
[15] a) T. Rosholm, P. M. Gois, R. Franzen, N. R. Candeias, *ChemistryOpen* **2015**, *4*, 39-46; b) N. R. Candeias, P. M. S. D. Cal, V. Andre, M. T. Duarte, L. F. Veiros, P. M. P. Gois, *Tetrahedron* **2010**, *66*, 2736-2745; c) N. R. Candeias, L. F. Veiros, C. A. M. Afonso, P. M. P. Gois, *Eur. J. Org. Chem.* **2009**, 1859-1863.
[16] a) N. A. Petasis, A. Goodman, I. A. Zavialov, *Tetrahedron* **1997**, *53*, 16463-16470; b) N. A. Petasis, I. A. Zavialov, *J. Am. Chem. Soc.* **1997**, *119*, 445-446; c) N. A. Petasis, I. A. Zavialov, *J. Am. Chem. Soc.* **1998**, *120*, 11798-11799.
[17] a) N. R. Candeias, F. Montalbano, P. M. S. D. Cal, P. M. P. Gois, *Chem. Rev.* **2010**, *110*, 6169-6193; b) J. Zhu, H. Bienaymé, *Multicomponent Reactions* Wiley-VCH, Weinheim, **2005**.
[18] H. Mao, R. Xu, J. Wan, Z. Jiang, C. Sun, Y. Pan, *Chem. Eur. J.* **2010**, *16*, 13352-13355.
[19] G. L. French, *J. Antimicrob. Chemother.* **2006**, *58*, 1107-1117.
[20] R. G. Parr, W. Yang, *Density Functional Theory of Atoms and Molecules*, Oxford University Press, New York, **1989**.
[21] a) I. V. Tetko, J. Gasteiger, R. Todeschini, A. Mauri, D. Livingstone, P. Ertl, V. A. Palyulin, E. V. Radchenko, N. S. Zefirov, A. S. Makarenko, V. Y. Tanchuk, V. V. Prokopenko, *J. Comput.-Aided Mol. Des.* **2005**, *19*, 453-463; b) *VCCLAB, Virtual Computational Chemistry Laboratory*, http://www.vcclab.org

[22] Y. Liu, L. Wang, Y. Sui, J. Yu, *Chin. J. Chem.* **2010**, *28*, 2039-2044.
[23] Q. Wang, M. G. Finn, *Org. Lett.* **2000**, *2*, 4063-4065.
[24] W.-Y. Han, J. Zuo, X.-M. Zhang, W.-C. Yuan, *Tetrahedron* **2013**, *69*, 537-541.
[25] P. Nun, J. Martinez, F. Lamaty, *Synthesis* **2010**, *2010*, 2063-2068.
[26] N. J. McLean, H. Tye, M. Whittaker, *Tetrahedron Lett.* **2004**, *45*, 993-995.
[27] P. Doan, A. Karjalainen, J. G. Chandraseelan, O. Sandberg, O. Yli-Harja, T. Rosholm, R. Franzen, N. R. Candeias, M. Kandhavelu, *Eur. J. Med. Chem.* **2016**, *120*, 296-303.
[28] W.-Y. Han, Z.-J. Wu, X.-M. Zhang, W.-C. Yuan, *Org. Lett.* **2012**, *14*, 976-979.
[29] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalman, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian 09, Revision D.01, Gaussian, Inc., Wallingford CT, **2009**.
[30] W. J. Hehre, L. Radom, P. v. R. Schleyer, J. Pople, *Ab initio molecular orbital theory*, John Wiley & Sons, New York, **1986**.
[31] a) J. P. Perdew, *Phys. Rev. B: Condens. Matter* **1986**, *33*, 8822-8824; b) J. P. Perdew, K. Burke, M. Ernzerhof, *Phys. Rev. Lett.* **1997**, *78*, 1396-1396.
[32] a) R. Ditchfield, W. J. Hehre, J. A. Pople, *The Journal of Chemical Physics* **1971**, *54*, 724-728; b) W. J. Hehre, R. Ditchfield, J. A. Pople, *The Journal of Chemical Physics* **1972**, *56*, 2257-2261; c) P. C. Hariharan, J. A. Pople, *Mol. Phys.* **1974**, *27*, 209-214; d) M. S. Gordon, *Chem. Phys. Lett.* **1980**, *76*, 163-168; e) P. C. Hariharan, J. A. Pople, *Theor. Chim. Acta* **1973**, *28*, 213-222.
[33] a) E. Cancès, B. Mennucci, J. Tomasi, *J. Chem. Phys.* **1997**, *107*, 3032-3041; b) B. Mennucci, J. Tomasi, *The Journal of Chemical Physics* **1997**, *106*, 5151-5158; c) M. Cossi, V. Barone, B. Mennucci, J. Tomasi, *Chem. Phys. Lett.* **1998**, *286*, 253-260.
[34] A. V. Marenich, C. J. Cramer, D. G. Truhlar, *The Journal of Physical Chemistry B* **2009**, *113*, 6378-6396.
[35] a) J. E. Carpenter, F. Weinhold, *Journal of Molecular Structure: THEOCHEM* **1988**, *169*, 41-62; b) J. E. Carpenter, University of Wisconsin (Madison WI), **1987**; c) J. P. Foster, F. Weinhold, *J. Am. Chem. Soc.* **1980**, *102*, 7211-7218; d) A. E. Reed, F. Weinhold, *The Journal of Chemical Physics* **1983**, *78*, 4066-4073; e) A. E. Reed, F. Weinhold, *The Journal of Chemical Physics* **1985**, *83*, 1736-1740; f) A. E. Reed, R. B. Weinstock, F. Weinhold, *The Journal of Chemical Physics* **1985**, *83*, 735-746; g) A. E. Reed, L. A. Curtiss, F. Weinhold, *Chem. Rev.* **1988**, *88*, 899-926; h) F. Weinhold, J. E. Carpenter, *The Structure of Small Molecules and Ions* (Eds.: R. Naaman, Z. Vager), Plenum, New York, **1988**, pp. 227-236.
[36] P. A. Wayne, *Performance standards for antimicrobial susceptibility testing: Twenty Fifth International Supplement M100-S25.*, Clinical and Laboratory Standards Institute, **2015**.
[37] T. A. L. Wagemaker, P. Rijo, L. M. Rodrigues, P. M. B. G. Maia Campos, A. S. Fernandes, C. Rosado, *Int. J. Cosmetic Sci.* **2015**, *37*, 506-510.

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