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Synthesis and evaluation of *N*-analogs of 1,2-diarylethane as *Helicobacter pylori* urease inhibitors

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ABSTRACT

Therapies based on urease inhibition are now seriously considered as the first line of treatment for infections caused by *Helicobacter pylori*. However, the present inhibitors are ineffective or unstable in highly acidic gastric juice. Here, we report a series of benzylanilines as effective inhibitors of *H. pylori* urease. Out of the obtained twenty-one compounds, *N*-(3,4-dihydroxybenzyl)-4-nitroaniline (**4**) was evaluated in detail and shows promising features for development as anti-*H. pylori* agent. Excellent potency against urease in both cell-free extract and intact cell was observed at low concentrations of **4** (IC_{50} = 0.62 ± 0.04 and 1.92 ± 0.09 μM), which showed over 29- and 54-fold increase in potency with respect to the positive control AHA. The SAR analysis revealed that protection of 3,4-dihydroxy group of **4** as methoxy or changes of 4-NO_2 will result in a moderate to dramatic decrease in potency.

1. Introduction

It is well known that *Helicobacter pylori* infection causes various gastric diseases, including chronic gastritis, gastric lymphoma, peptic ulcer, and stomach cancer.[1] With the help of a constitutive urease production, *H. pylori* create a protective ammonium cloud from urea, which allows the bacteria to survive at the low pH of the stomach during colonization.[2] Urease therefore plays an important role in the pathogenesis of gastric diseases induced by *H. pylori* and is known to be a major virulence factor,[3] and strategies based on urease inhibition are now essential lines of treatment for *H. pylori* infection.[1] Noticeably, ureases have also been isolated from a variety of other pathogenic bacteria such as *Proteus mirabilis*, *Brucella abortus* and *Yersinia enterocolitica*.[4] In infections with these bacteria, urease is considered to be implicated in urolithiasis (stone formation) and be directly involved in the development of acute pyelonephritis and infection-induced reactive arthritis.[5–7] Thus urease inhibition is of very great therapeutic and pharmacological significances.[8]

According to recent statistics, at least half of the world population is estimated to be infected by *H. pylori*, and potent inhibitors of *H. pylori* urease have increasingly attracted the interests of medicinal chemists. In the past decades, many synthetic compounds, natural products and metal complexes were determined as urease inhibitors,[9–13] with phosphoramidates being the most potent compounds. However, direct treatment of *H. pylori* infection with phosphoramidates has been limited by the rapid hydrolysis in the low pH of gastric juice.[14] Therefore, it is of great urgency to discover and develop novel chemical entities with satisfactory stability and effective activity against *H. pylori* urease.

Recently, some novel urease inhibitors[15–17] were determined by our group with 1,2-diarylethane (**1**, Scheme 1) to be noted.[18] This specific inhibitor shows good potency against *H. pylori* urease in both cell-free system and intact cell.[19] It is well known that a relative simple molecular skeleton and low molecular weight indicate a large of optimization space. Based on this consideration, compound **1** was selected as a lead in this work. Molecular docking studies of **1** revealed that the linkage of ethylene does not involve in any interactions with residues in active site of urease. In this docking complex, however, a relative short contact was observed between the ethylene group in **1** and the sulfhydryl group in Cys-321.[19] Structural studies of the enzyme disclosed that Cys-321 is located on a flexible flap composed of residues α313-α346,[20] and is an essential residue for the catalytic activity of

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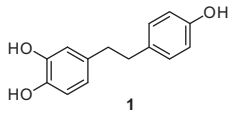


1

Scheme 1. Structure of compound **1**.

H. pylori urease.[21] Motivated by these findings and in continuation of our ongoing efforts on the discovery of new urease inhibitors, a series of benzylanilines, bioisosteres of **1** with substitution of NH for a CH_2, were herein reported, and some showe good potency.

2. Results and discussion

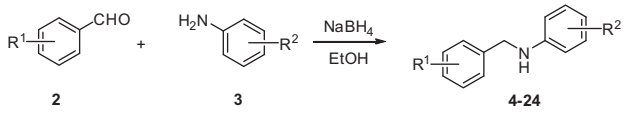
2.1. Chemistry

The general synthetic route for benzylaniline derivatives (**4**–**24**), *N*-analogs of 1,2-arylethanes, is shown in Scheme 2. The synthesis of all benzylanilines begins from aldehydes (**2**), which coupled with anilines (**3**) under Borch reaction conditions to yield target compounds (**4**–**24**).

2.2. Urease inhibitory activity

To understand the SAR for urease inhibition, structurally diverse benzylanilines were profiled in an in vitro *H. pylori* urease assay (Table 1). The results revealed that many compounds were active in assays, and **4** was the most potent compound with an IC_{50} of 0.62 ± 0.04 μM, showing a >2- and 30-fold upward shift in potency with respect to that of the lead compound **1** and the positive control AHA (acetohydroxamic acid), respectively. This is consistent with our earlier observations where the 3,4-dihydroxyphenyl pattern is very important to activity.[19] Movement of the nitro group of **4** from 4- to 3-position resulted in compound **8**, showing moderate (about 4-fold) decrease in potency, while movement to 2-potion rendered the molecule (**15**) significant loss of activity. In comparison with compound **4**, removal of 3-OH led to the compound **5** with a 13-fold decrease in potency. The activity further decreased when 4-OH of **5** was replaced by Cl (**6**) or NO_2 (**7**). These findings indicate that 3,4-dihydroxyl group of benzyl moiety and 4-nitro group of aniline moiety are very essential to urease inhibition, which were confirmed by a partial tolerance of 3,4-dihydroxyl group being protected as methyl ether or 1,3-dioxolane (**8** vs **9** or **10**), and by an intolerance of 4-nitro group being further replaced (**4** vs **16** or **17**).

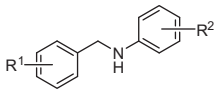
Similar to what was observed for 3,4-disubstituted derivatives, the 4-substituted analog with 3-nitro group in aniline moiety (**12**) showed a moderate decrease in potency when compared to the compound with 4-nitro group (**6**). In *para–meta* series (**11**–**13**), conversion of the chlorine atom in compound **12** to a *N*,*N*-dimethylamino group enhanced potency, while to a nitro group reduced potency. These data together with those from *para–para* series (**5**–**7**) confidently indicated the requirement of an electron-donating group for compounds with 4-monosubstitution pattern in benzyl moiety. Regarding aniline moiety, the nitro group seems to be optimal at both *meta*- and *para*-positions, and any variations of the substitution caused significant degradation of activity or rendered the molecule inactive (**16**–**24**).



2 3 4-24

Scheme 2. Synthesis of benzylanilines.

Table 1
In vitro inhibitory activity data of the synthesized compounds against *H. pylori* urease

Compound			IC_{50} (μM)	
	R^1	R^2	Cell free urease	Urease in intact cell
4	3,4-$(OH)_2$	4-NO_2	0.62 ± 0.04	1.92 ± 0.09
5	4-OH	4-NO_2	8.26 ± 0.48	28.1 ± 1. 8
6	4-Cl	4-NO_2	19.5 ± 1.0	83.8 ± 1.6
7	4-NO_2	4-NO_2	26.3 ± 1.2	126.2 ± 13.1
8	3,4-$(OH)_2$	3-NO_2	2.33 ± 0.11	9.52 ± 0.60
9	3,4-$(OMe)_2$	3-NO_2	50.4 ± 5.0	ND
10	3,4-Methylenedioxy	3-NO_2	13.1 ± 0.9	60.3 ± 5.2
11	4-NMe_2	3-NO_2	16.8 ± 1.1	72.4 ± 6.5
12	4-Cl	3-NO_2	57.5 ± 3.3	ND
13	4-NO_2	3-NO_2	64.9 ± 5.8	ND
14	3-NO_2	3-NO_2	85.0 ± 7.1	ND
15	3,4-$(OH)_2$	2-NO_2	45.1 ± 3.6	ND
16	3,4-$(OMe)_2$	4-Cl	>500	ND
17	3,4-Methylenedioxy	4-Cl	>500	ND
18	4-NMe_2	4-Cl	>500	ND
19	2-NO_2	4-Cl	238.7 ± 14.4	ND
20	4-NO_2	3-Cl	236.5 ± 20.9	ND
21	3-NO_2	3-Cl	410.3 ± 39.8	ND
22	4-NMe_2	4-OMe	>500	ND
23	4-Cl	4-OMe	>500	ND
24	3-NO_2	4-OMe	319.2 ± 18.3	ND
HAH			18.4 ± 1.3	104.8 ± 9.7

One should be noted that **4** also shows substantial intact-cell urease assay activity, with over 54-fold improvement in comparison to positive control AHA, indicating that **4** deserves further research as a potential agent to treat diseases caused by *H. pylori* infection.

2.3. Molecular docking

To further understand the results of biological measures, we performed computational docking studies on the top active compound **4**. A detailed representation of the **4**-urease active site is shown in Figures 1 and 2. It is observed that the NH moiety of **4** is involved in an interaction with the key residue Cys-321 on the flex flap, forming a N–H···S hydrogen bond with a H-S distance of 2.863 Å, which confirms our design conception for benzylanilines. In comparison with the previous reported compound **1**, compound **4** docked the urease active site in a similar pose with 3,4-dihydroxyphenyl moiety being oriented toward the entrance cavity and the other phenyl moiety toward the bottom. However, 4-nitrophenyl moiety is more buried in the urea binding pocket with respect to 4-hydroxyphenyl group of **1**. The nitro group drops completely into the bottom and chelates the metal doublet, forming a tetrahedron with distances in a rang of 1.868–2.331 Å. In addition, a hydrophobic interaction between the ring of 4-nitrophenyl moiety and the backbone CH_3 of Ala-365 (3.297 Å)

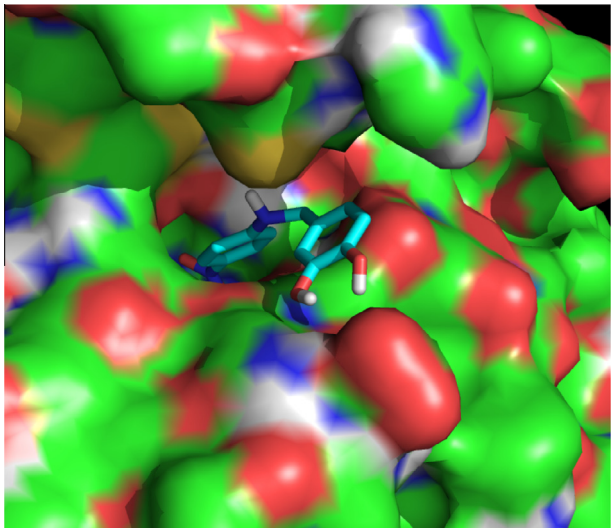


Figure 1. Binding mode of compound **4** with *H. pylori* urease. The enzyme is shown as surface, while **4** docked structure is shown as stick. The figure was made using PyMol.

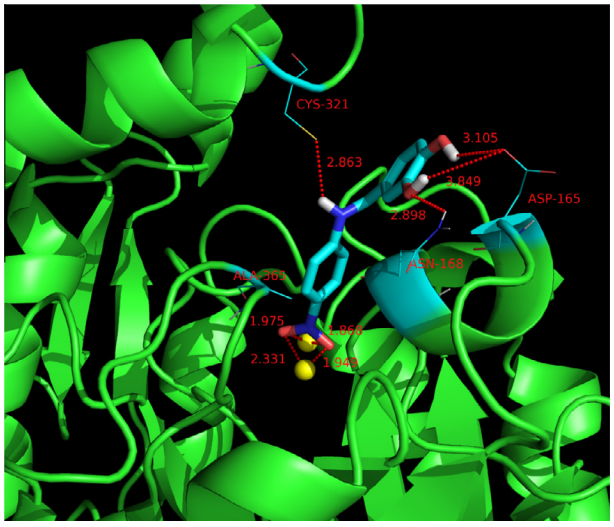


Figure 2. Binding mode of compound **4** with *H. pylori* urease. For clarity, only interacting residues were labeled. Hydrogen bonding interactions are shown in dash. Ni atoms are shown as yellow balls. The figure was made using PyMol.

is observed. It is important to be noted that 3,4-dihydroxyphenyl moiety is involved in three hydrogen bonding interactions with the backbone amino group of Asn-168 and carbonyl group of Asp 165. Additional interactions provided by NH and NO_2 may give a rational explanation for the higher potency of **4** (IC_{50} = 0.62 ± 0.04 μM) than **1** (IC_{50} = 1.5 ± 0.2 μM).

3. Conclusion

Benzylanilines, the bioisostere of 1,2-diarylethane with substitution of NH for CH_2, were determined as *H. pylori* urease inhibitors. Several derivatives showed excellent activity against urease in both cell-free extract and intact cell, with 4-((4-nitrophenylamino)methyl)benzene-1,2-diol (**4**) being the most active, which has the potential to be developed for treatment of gastritis and gastric ulcer caused by *H. pylori* infection. Molecular dockings disclosed that its potency may be attributed to the tight interactions with the enzyme provided by NO_2, NH and 3,4-dihydroxy groups. The SAR analysis also confirmed that any changes of NO_2 or 3,4-dihydroxy group of compound **4**, including substitution and shift, will lead to a moderate to dramatic decrease in potency. Further investigations are in progress to determine the activity in vivo and optimize the pharmacokinetics profile of the compounds.

4. Experimental section

4.1. Materials

Protease inhibitors (Complete mini EDTA-free) were purchased from Roche Diagnostics GmbH (Mannheim, Germany) and Brucella broth was from Becton-Dickinson (Cockeysville, MD). Horse serum was from Hyclone (Utah, American).

4.2. Bacteria

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was grown in Brucella broth supplemented with 10% heat-inactivated horse serum for 24 h at 37 °C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), as our previously described.[22,23]

4.3. Preparation of *H. pylori* urease

For urease inhibition assays, 50 mL broth cultures (2.0×10^8 CFU/mL) were centrifuged (5000 g, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *H. pylori* precipitation was stored at −80 °C. *H. pylori* were returned to room temperature, and after addition of 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation (15,000*g*, 4 °C), the supernatant was desalted through Sephadex G-25 column (PD-10 columns, Amersham Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and stored at 4 °C until use in the experiment.

4.4. Urease activity determination

One unit of urease activity is defined as the amount of enzyme needed to liberate 1.0 μmol of NH_3 from urea per min at pH 7.0 at 25 °C. According to the enzymatic assays of Sigma–Aldrich,[24] the activity of the obtained *H. pylori* urease was determined. Briefly, one mL of 0.5 M urea with 0.05% (w/v) bovine serum albumin solution was pipetted into a test tube and warmed to 25 °C. Then, 0.20 mL of urease solution containing 0.02 M sodium phosphate buffer (pH 7.0 at 25 °C) was added. The obtained mixture was incubated at 25 °C for exactly 5 minutes with magnetic stirring, and followed by addition of 0.20 mL of indicator (4.0 mg/mL of *p*-nitrophenol solution), which was titrated immediately with 0.1 M standardized HCl until the color turns from yellow to colorless. As for blank, only difference is that the urease solution was added after incubation. The urease activity was calculated based on the following formula:

$$\text{U/mL urease} = \frac{0.1\ \text{mmol/mL} \times \Delta V_{\text{HCl}}\ \text{mL} \times 1000\ \mu\text{mol/mmol}}{5\ \text{min} \times 0.2\ \text{mL}}$$

4.5. Measurement of urease inhibitory activity

The assay mixture, containing 25 μL (10U) of *H. pylori* urease and 25 μL of the test compound, was pre-incubated for 1.5 h at room temperature in a 96-well assay plate. Urease activity was

determined by measuring ammonia production using the indophenol method as described by Weatherburn.[25]

4.6. Protocol of docking study

The automated docking studies were carried out using AutoDock version 4.2. First, AutoGrid component of the program pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of 56 Å size (x, y, z) with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitor within the macromolecules. The GALS search algorithm (genetic algorithm with local search) was chosen to search for the best conformers. The parameters were set using the software ADT (AutoDockTools package, version 1.5.4) on PC which is associated with AutoDock 4.2. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 2.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and the results of the most favorable free energy of binding were selected as the resultant complex structures.

4.7. Chemistry

All chemicals (reagent grade) used were purchased from Aldrich (U.S.A.) and Sinopharm Chemical Reagent Co., Ltd (China). Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). EI mass spectra were obtained on a Waters GCT mass spectrometer, and ^{1}H NMR spectra were recorded on a Bruker AV-300 or 400 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

4.7.1. General procedure for the preparation of compounds 4-24

A solution of an aniline **3** (10 mmol) and an appropriate substituted benzaldehyde **2** (10 mmol) in methanol (10 mL) was refluxed for 3–5 h. After cooling and addition of $NaBH_4$ (15 mmol), the mixture was further stirred at room temperature for 3–6 h and concentrated under reduced pressure. The residue was dissolved in AcOEt (80 mL), and 40 mL of water was added. The aqueous layer was separated and extracted with AcOEt (2 × 40 mL). The organic layer and extracts were combined, dried over $MgSO_4$, and concentrated under reduced pressure, and the residue was purified by flash column chromatography (silica gel, EtOAc/petroleum ether) to give desired compounds **4**–**24**.

4.7.1.1. 4-((4-Nitrophenylamino)methyl)benzene-1,2-diol (4). Yellow powder, yield 85%, mp 182–183 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 4.21 (d, J = 5.3 Hz, 2H); 6.59 (dd, J = 8.0 Hz, J = 1.8 Hz, 1H); 6.65 (d, J = 9.7 Hz, 2H); 6.68 (d, J = 8.0 Hz, 1H); 6.71 (d, J = 1.8 Hz, 1H); 7.69 (t, J = 5.5 Hz, 1H); 7.97 (d, J = 9.3 Hz, 2H); 8.78 (s, 1H); 8.84 (s, 1H); EIMS m/z 260 (M^+). Anal. Calcd for $C_{13}H_{12}N_2O_4$: C, 60.00; H, 4.65; N, 10.76. Found: C, 59.92; H, 4.65; N, 10.78

4.7.1.2. 4-((4-Nitrophenylamino)methyl)phenol (5). Light yellow powder, yield 53%, mp 141–143 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 4.37 (d, J = 5.2 Hz, 2H); 6.47 (t, J = 5.2 Hz, 1H); 7.06 (s, 4H); 7.42 (d, J = 8.8 Hz, 2H); 8.21 (d, J = 8.9 Hz, 2H); EIMS m/z 244 (M^+). Anal. Calcd for $C_{13}H_{12}N_2O_3$: C, 63.93; H, 4.95; N, 11.47. Found: C, 63.98; H, 4.95; N, 11.45.

4.7.1.3. *N*-(4-Chlorobenzyl)-4-nitroaniline (6). Yellow powder, yield 48%, mp 197–199 °C, ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): 4.32 (s, 2H); 6.52 (d, J = 8.6 Hz, 2H); 7.17 (bs, 1H); 7.24 (s, 4H); 7.91 (d, J = 8.7 Hz, 2H); EIMS m/z 262 (M^+). Anal. Calcd for $C_{13}H_{11}ClN_2O_2$: C, 59.44; H, 4.22; Cl, 13.50; N, 10.66. Found: C, 59.48; H, 4.22; Cl, 13.49; N, 10.65.

4.7.1.4. 4-Nitro-*N*-(4-nitrobenzyl)aniline (7). Light yellow powder, yield 41%, mp 187–188 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 4.19 (d, J = 5.6 Hz, 2H); 6.52 (t, J = 5.5 Hz, 1H); 7.48 (d, J = 8.4 Hz, 2H); 6.57 (d, J = 8.8 Hz, 2H); 7.94 (d, J = 8.9 Hz, 2H); 8.27 (d, J = 8.4 Hz, 2H); EIMS m/z 273 (M^+). Anal. Calcd for $C_{13}H_{11}N_3O_4$: C, 57.14; H, 4.06; N, 15.38. Found: C, 57.19; H, 4.05; N, 15.39.

4.7.1.5. 4-((3-Nitrophenylamino)methyl)benzene-1,2-diol (8). Orange powder, yield 77%, mp 126–128 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 4.15 (s, 2H); 6.61 (d, J = 8.0 Hz, 1H); 6.68 (d, J = 8.0 Hz, 1H); 6.73 (s, 1H); 6.84 (bs, 1H); 6.97 (d, J = 7.0 Hz, 1H); 7.25–7.33 (m, 3H); 8.72 (bs, 1H); 8.80 (bs, 1H); EIMS m/z 260 (M^+). Anal. Calcd for $C_{13}H_{12}N_2O_4$: C, 60.00; H, 4.65; N, 10.76. Found: C, 59.94; H, 4.67; N, 10.77.

4.7.1.6. *N*-(3,4-Dimethoxybenzyl)-3-nitroaniline (9). Yellow powder, yield 37%, mp 146–148 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 3.72 (s, 3H); 3.74 (s, 3H); 4.26 (d, J = 5.9 Hz, 2H); 6.86–6.92 (m, 3H); 6.96–7.02 (m, 2H); 7.29 (t, J = 7.9 Hz, 1H); 7.32 (d, J = 1.8 Hz, 1H); 7.37 (d, J = 2.2 Hz, 1H); EIMS m/z 288 (M^+). Anal. Calcd for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.59; N, 9.72. Found: C, 62.41; H, 5.60; N, 9.73.

4.7.1.7. *N*-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-3-nitroaniline (10). Yellow powder, yield 63%, mp 146–148 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 4.25 (d, J = 5.9 Hz, 2H); 6.12 (s, 2H); 6.84–6.93 (m, 3H); 6.98 (s, 1H); 7.00 (d, J = 8.1 Hz, 1H); 7.29 (t, J = 7.9 Hz, 1H); 7.32 (s, 1H); 7.37 (s, 1H); EIMS m/z 272 (M^+). Anal. Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29. Found: C, 61.69; H, 4.44; N, 10.30.

4.7.1.8. *N,N*-Dimethyl-4-((3-nitrophenylamino)methyl)aniline (11). Light yellow powder, yield 51%, mp 104–106 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 2.85 (s, 6H); 4.19 (d, J = 5.7 Hz, 2H); 6.69 (d, J = 7.5 Hz, 2H); 6.83 (t, J = 5.5 Hz, 1H); 6.98 (d, J = 7.0 Hz, 1H); 7.18 (d, J = 7.7 Hz, 2H); 7.25–7.35 (m, 3H); EIMS m/z 271 (M^+). Anal. Calcd for $C_{15}H_{17}N_3O_2$: C, 66.40; H, 6.32; N, 15.49. Found: C, 66.46; H, 6.33; N, 15.47.

4.7.1.9. *N*-(4-Chlorobenzyl)-3-nitroaniline (12). Light yellow powder, yield 83%, mp 108–110 °C, ^{1}H NMR (400 MHz, $CDCl_3$): 4.30 (d, J = 5.3 Hz, 2H); 4.36 (bs, 1H); 6.79 (dd, J = 7.9 Hz, J = 1.9 Hz, 1H); 7.18–7.27 (m, 5H); 7.34 (t, J = 1.9 Hz, 1H); 7.47 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H); EIMS m/z 262 (M^+). Anal. Calcd for $C_{13}H_{11}ClN_2O_2$: C, 59.44; H, 4.22; Cl, 13.50; N, 10.66. Found: C, 59.49; H, 4.22; Cl, 13.48; N, 10.65.

4.7.1.10. 3-Nitro-*N*-(4-nitrobenzyl)aniline (13). Orange powder, yield 69%, mp 129–131 °C, ^{1}H NMR (400 MHz, $CDCl_3$): 4.55 (d, J = 4.6 Hz, 2H); 4.6 (bs, 1H); 6.86 (d, J = 7.9 Hz, 1H); 7.29 (t, J = 8.0 Hz, 1H); 7.40 (s, 1H); 7.54 (d, J = 8.3 Hz, 2H); 7.58 (d, J = 8.0 Hz, 1H); 8.23 (d, J = 8.4 Hz, 2H); EIMS m/z 273 (M^+). Anal. Calcd for $C_{13}H_{11}N_3O_4$: C, 57.14; H, 4.06; N, 15.38. Found: C, 57.08; H, 4.07; N, 15.40.

4.7.1.11. 3-Nitro-*N*-(3-nitrobenzyl)aniline (14). Light yellow powder, yield 79%, mp 130–131 °C, ^{1}H NMR (400 MHz, $CDCl_3$): 4.54 (d, J = 4.2 Hz, 2H); 6.88 (d, J = 7.8 Hz, 1H); 7.30 (t, J = 8.0 Hz, 1H); 7.41 (s, 1H); 7.54 (d, J = 7.8 Hz, 1H); 7.58 (d, J = 6.9 Hz, 1H); 7.71 (d, J = 7.2 Hz, 1H); 8.17 (d, J = 7.9 Hz, 1H); 8.24 (s, 1H); EIMS m/z 273 (M^+). Anal. Calcd for $C_{13}H_{11}N_3O_4$: C, 57.14; H, 4.06; N, 15.38. Found: C, 57.19; H, 4.06; N, 15.37.

4.7.1.12. 4-((2-Nitrophenylamino)methyl)benzene-1,2-diol (15). Yellow powder, yield 58%, mp 109–111 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 4.41 (d, J = 5.9 Hz, 2H); 6.59–6.69 (m, 3H); 6.72 (d, J = 2.0 Hz, 1H); 6.92 (d, J = 8.8 Hz, 1H); 7.44 (t, J = 7.5 Hz, 1H); 8.05 (dd, J = 8.6 Hz, J = 1.5 Hz, 1H); 8.48 (t, J = 5.7 Hz, 1H); 8.80 (bs, 2H); EIMS m/z 260 (M^+). Anal. Calcd for $C_{13}H_{12}N_2O_4$: C, 60.00; H, 4.65; N, 10.76. Found: C, 60.08; H, 4.64; N, 10.74.

4.7.1.13. 4-Chloro-*N*-(3,4-dimethoxybenzyl)aniline (16). White powder, yield 75%, mp 119–121 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 3.71 (s, 2H); 3.76 (s, 3H); 3.72 (s, 3H); 4.15 (d, J = 5.9 Hz, 2H); 6.32 (t, J = 6.0 Hz, 1H); 6.57 (d, J = 9.0 Hz, 2H); 6.84 (dd, J = 8.4 Hz, J = 1.5 Hz, 1H); 6.88 (d, J = 8.0 Hz, 1H); 6.95 (d, J = 1.3 Hz, 1H); 7.04 (d, J = 8.8 Hz, 2H); EIMS m/z 277 (M^+). Anal. Calcd for $C_{15}H_{16}ClNO_2$: C, 64.87; H, 5.81; Cl, 12.76; N, 5.04. Found: C, 64.82; H, 5.80; Cl, 12.78; N, 5.05.

4.7.1.14. *N*-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-4-chloroaniline (17). White powder, yield 71%, mp 112–114 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 4.15 (d, J = 5.9 Hz, 2H); 6.09 (s, 2H); 6.31 (t, J = 5.8 Hz, 1H); 6.57 (d, J = 8.9 Hz, 2H); 6.84 (d, J = 8.2 Hz, 1H); 6.88 (d, J = 8.4 Hz, 1H); 7.05 (d, J = 8.9 Hz, 2H); EIMS m/z 261 (M^+). Anal. Calcd for $C_{14}H_{12}ClNO_2$: C, 64.25; H, 4.62; Cl, 13.55; N, 5.35. Found: C, 64.19; H, 4.61; Cl, 13.57; N, 5.36.

4.7.1.15. 4-((4-Chlorophenylamino)methyl)-*N*,*N*-dimethylaniline (18). White powder, yield 46%, mp 93–95 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 2.84 (s, 6H); 4.09 (d, J = 5.9 Hz, 2H); 6.23 (t, J = 5.9 Hz, 1H); 6.56 (d, J = 8.8 Hz, 2H); 6.68 (d, J = 8.6 Hz, 2H); 7.01 (d, J = 8.8 Hz, 2H); 7.15 (d, J = 8.4 Hz, 2H); EIMS m/z 260 (M^+). Anal. Calcd for $C_{15}H_{17}ClN_2$: C, 69.09; H, 6.57; Cl, 13.60; N, 10.74. Found: C, 69.14; H, 6.55; Cl, 13.59; N, 10.75.

4.7.1.16. 4-Chloro-*N*-(2-nitrobenzyl)aniline (19). Yellow powder, yield 72%, mp 83–84 °C, ^{1}H NMR (300 MHz, DMSO-d_6): 4.59 (d, J = 6.0 Hz, 2H); 6.48–6.59 (m, 3H); 7.05 (d, J = 8.8 Hz, 2H); 7.52 (t, J = 7.5 Hz, 1H); 7.58 (d, J = 7.7 Hz, 1H); 7.69 (d, J = 7.5 Hz, 1H); 8.05 (d, J = 8.0 Hz, 1H); EIMS m/z 262 (M^+). Anal. Calcd for $C_{13}H_{11}ClN_2O_2$: C, 59.44; H, 4.22; Cl, 13.50; N, 10.66. Found: C, 59.48; H, 4.21; Cl, 13.48; N, 10.67.

4.7.1.17. 3-Chloro-*N*-(4-nitrobenzyl)aniline (20). Light yellow powder, yield 51%, mp 64–65 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 4.41 (d, J = 5.2 Hz, 2H); 6.20 (t, J = 4.8 Hz, 1H); 6.42 (d, J = 8.0 Hz, 1H); 6.51 (s, 2H); 6.97 (t, J = 8.4 Hz, 1H); 7.53 (d, J = 8.4 Hz, 2H); 8.14 (d, J = 8.4 Hz, 2H); EIMS m/z 262 (M^+). Anal. Calcd for $C_{13}H_{11}ClN_2O_2$: C, 59.44; H, 4.22; Cl, 13.50; N, 10.66. Found: C, 59.49; H, 4.21; Cl, 13.49; N, 10.64.

4.7.1.18. 3-Chloro-*N*-(3-nitrobenzyl)aniline (21). Light yellow powder, yield 43%, mp 87–89 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 4.31 (s, 1H); 4.46 (d, J = 5.7 Hz, 2H); 6.47 (dd, J = 8.2 Hz, J = 2.0 Hz, 1H); 6.58 (t, J = 2.0 Hz, 1H); 6.71 (d, J = 6.8 Hz, 1H); 7.08 (t, J = 8.0 Hz, 1H); 7.53 (t, J = 7.9 Hz, 1H); 7.69 (d, J = 7.2 Hz, 1H); 8.15 (d, J = 8.0 Hz, 1H); 8.23 (s, 1H); EIMS m/z 262 (M^+). Anal. Calcd for $C_{13}H_{11}ClN_2O_2$: C, 59.44; H, 4.22; Cl, 13.50; N, 10.66. Found: C, 59.47; H, 4.22; Cl, 13.48; N, 10.65.

4.7.1.19. 4-((4-Methoxyphenylamino)methyl)-*N*,*N*-dimethylaniline (22). White powder, yield 64%, mp 86–87 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 2.84 (s, 6H); 3.60 (s, 3H); 4.05 (d, J = 6.1 Hz, 2H); 5.56 (t, J = 5.9 Hz, 1H); 6.52 (d, J = 9.0 Hz, 2H); 6.66 (d, J = 9.0 Hz, 2H); 6.67 (d, J = 8.8 Hz, 2H); 7.16 (d, J = 8.8 Hz, 2H); EIMS m/z 256 (M^+). Anal. Calcd for $C_{16}H_{20}N_2O$: C, 74.97; H, 7.86; N, 10.93. Found: C, 74.89; H, 7.87; N, 10.95.

4.7.1.20. *N*-(4-Chlorobenzyl)-4-methoxyaniline (23). White powder, yield 75%, mp 70–72 °C, ^{1}H NMR (400 MHz, DMSO-d_6): 3.74 (s, 3H); 3.84 (bs, 1H); 4.26 (s, 2H); 6.57 (d, J = 8.6 Hz, 2H); 6.70 (d, J = 8.6 Hz, 2H); 7.30 (s, 4H); EIMS m/z 247 (M^+). Anal. Calcd for $C_{14}H_{14}ClNO$: C, 67.88; H, 5.70; Cl, 14.31; N, 5.65. Found: C, 67.82; H, 5.71; Cl, 14.33; N, 5.65.

4.7.1.21. 4-Methoxy-*N*-(3-nitrobenzyl)aniline (24). Light yellow powder, yield 49%, mp 76–78 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 3.76 (s, 3H); 4.44 (s, 2H);6.61 (d, J = 9.0 Hz, 2H); 6.80 (d, J = 8.9 Hz, 2H); 7.52 (t, J = 7.9 Hz, 1H); 7.74 (d, J = 7.7 Hz, 1H); 8.14 (d, J = 8.2 Hz, 1H); 8.27 (s, 1H); EIMS m/z 258 (M^+). Anal. Calcd for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85. Found: C, 65.17; H, 5.45; N, 10.83.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.06.014.

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