Synthesis, *In silico* studies and *In vitro* evaluation for antioxidant and antibacterial properties of diarylmethylamines: A novel class of structurally simple and highly potent pharmacophore

Sujit Mahato, Anuma Singh, Latha Rangan, Chandan K. Jana

PII: S0928-0987(16)30057-4
DOI: doi: 10.1016/j.ejps.2016.03.004
Reference: PHASCI 3504

To appear in:

Received date: 8 December 2015
Revised date: 11 February 2016
Accepted date: 6 March 2016

Please cite this article as: Mahato, Sujit, Singh, Anuma, Rangan, Latha, Jana, Chandan K., Synthesis, *In silico* studies and *In vitro* evaluation for antioxidant and antibacterial properties of diarylmethylamines: A novel class of structurally simple and highly potent pharmacophore, (2016), doi: 10.1016/j.ejps.2016.03.004

Synthesis, *In silico* studies and *In vitro* evaluation for Antioxidant and Antibacterial Properties of Diarylmethylamines: A Novel Class of Structurally Simple and Highly Potent Pharmacophore

Sujit Mahato[a±]; Anuma Singh[b±]; Latha Rangan[b,*]; Chandan K Jana[a,*]

[a]Department of Chemistry, Indian Institute of Technology, Guwahati, Guwahati 781039, Assam, India
[b]Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
Tel: +91 361 2582328/2214
Fax: +91 361-2582249
*Email: ckjana@iitg.ernet.in; latha_rangan@yahoo.com

[±]Equal contribution
[*]Corresponding authors

Abstract

A series of novel diarylmethylamines were synthesized via simple three component condensation reaction. *In vitro* antibacterial activity of the synthesized compounds was assessed against Gram-positive and Gram-negative bacteria. Compound **1f** containing phenyl and *N*-methyl piperazine moiety was found to be potent against both pathogenic bacteria with MIC value of 31 µg/mL. Diarylmethylamine **1l** containing sesamol and *N*-methyl piperazine units was found to be the most effective against Gram-positive bacteria with MIC value of 15 µg/mL. The compound leads to the damage of the bacterial cell membrane which was demonstrated by flow cytometry (FC) and field emission scanning electron microscopy (FESEM). Radical scavenging activity of compounds **1l** and **1m** was found out to be comparable with that of standard antioxidant BHT. Further, *in silico* studies were carried out to calculate the physico-chemical parameter of the synthesized compounds.

Keywords: Antibacterial activity, Antioxidant activity, Diarylmethylamines/Betti bases, DPPH assay, Flow cytometry

1. Introduction

Since the first observation of drug resistance in 1930s, microbial infection caused due to multi-drug resistance of pathogen is on rise (Wright et al., 2014; Levy et al., 2004; Alanis, 2005). In due course, more than 17 different classes of antibiotics were developed and employed in chemotherapy to fight bacterial infections (Alanis, 2005). However, one or more bacterial strain was found to be resistant to each of these classes of antibiotics. This indicates the importance in developing new class of antibacterial agent. Therefore, efforts are ongoing for the development of new class of antibacterial agents (Chopde et al., 2015; Jogi et al., 2013; Mathew et al., 2010; Krizsan et al., 2014). Many of the known potential antibacterial agents are structurally complex and thus these are difficult to obtain with adequate quantity (Erb et al., 2013; Bedeschi et al., 2014). Hence, identification of new class of safe, highly effective and easily accessible chemotherapeutic agents becomes inevitable. In this context, diarylmethylamines belong to a promising class of molecules because of their important pharmacological profile (Gerlach et al., 2004; Gyémánt et al., 2010), structural simplicity and easy accessibility (Betti, 1900; Mukhopadhyay et al., 2012). Moreover, many of the molecules containing diarylmethylamine scaffold are currently used as pharmaceutical drugs. The examples include Levocetirizine, Zyrtec, Solifenacin, Meclozine, etc (**Scheme 1**).

In quest of the potent and novel class of antimicrobial compounds, we have designed and synthesized a set of structurally diverse diarylmethylamines/Betti bases. Antibacterial action and antioxidant behaviour of all the synthesized compounds were evaluated. Further, the physico-chemical parameters of diarylmethylamines were determined by *in silico* studies.

<Scheme 1>

2. Materials and methods

2.1. General experimental procedures

All reactions were carried out in oven-dried glassware. Commercial grade benzene, benzaldehyde and pyrrolidine were distilled before use. All other solvents and reagents were purified according to standard procedures or were used as received from Aldrich, Acros, Merck and Spectrochem. ^{1}H, ^{13}C NMR spectroscopy: *Varian Mercury plus 400 MHz, Bruker 600 MHz* (at 298 K). The solvents residual proton resonance and carbon resonance ($CHCl_3$, δ (^{1}H) 7.26 ppm, δ (^{13}C) 77.2 ppm; CD_3OD, (1H) 3.31 ppm, δ (^{13}C) 49.0 ppm) were used for calibration. IR spectra were recorded on Perkin Elmer Instrument at normal temperature by grinding the sample with KBr pellet (IR Grade). MS (ESI-HRMS): Mass spectra were recorded on Agilent Accurate-Mass Q-TOF LC/MS 6520, and peaks are given in *m/z* (% of

basis peak). Compounds **1a, 1d, 1e, 1i, 1g, 1h** and **1n** were synthesized following the reported literature procedure (Mahato et al., 2014).

2.2 Biological evaluation

2.2.1. Bacterial strains

The antibacterial activities of synthesized compounds were evaluated against two bacteria: *Listeria monocytogenes*, LM (ATCC 19115) and *Escherichia coli* enterotoxic, ETEC (MTCC 723). All the tested bacteria were grown and maintained on nutrient agar (NA) as described earlier by Kesari et al. (2010).

2.2.2 Determination of MIC

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism. The MIC was determined using the microdilution method, in 96 wells microtitre plates according to protocol described by Ghosh et al. (2013). Compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 2 mg/mL and this concentration was further used to make compound with concentration up to 1.95 µg/mL by two fold dilution method. Test strains were suspended in 0.9% (w/v) NaCl to obtain a cell density of 1×10^8 colony forming unit per millilitre (CFU/mL). These cell suspensions were diluted to obtain a final density of 5×10^5 CFU/mL in each well. Controls (growth, compound and negative control) were also included. Microtitre plates containing different concentrations of compound (1.95 µg/mL - 1 mg/mL) were incubated at 37 ^oC for 24 h and MIC was recorded spectrophotometrically at 600 nm (concentration at which there is sharp decline in the absorbance value). In addition, colour based assay was performed using resazurin (HiMedia) solution prepared in distilled water as described by Sarker et al. (2007). Change in colour from purple to pink and colourless was recorded as positive and the lowest concentration at which colour change occurs was recorded as MIC. Kanamycin (5 µg/mL) was used as standard control for this study. The potency of the compounds was further tested in reducing condition using 10 mM dithiothreitol (DTT) and incubated at 37^oC (Nuding et al., 2006). In order to track the reactive species, one dimension ^1H NMR of the compound (in nutrient broth) was acquired on Bruker 600 MHz after 0, 19 and 38 h.

2.2.3 FC analysis

The ability to distinguish different physiological states is very important for assessing and validating the survival and virulence of any pathogenic microorganisms. To determine

the antibacterial action, the mid log phase culture of most susceptible bacteria, i.e., LM was treated with compound **1l** at its MIC and subsequently incubated for 24 h along with the vehicle control (cells treated with 1% DMSO). Bacterial suspensions were centrifuged at 10,000 rpm and cells resuspended in phosphate buffer saline (PBS) to maintain the cell density of approximately 10^4 cells/mL. Heat killed cells (95 ^oC for 15 min) were taken as negative control. Samples were first singly stained by adding 10 µL carboxyfluorescein diacetate (cFDA) (0.25 mM, Sigma Aldrich) or 10 µL propidium iodide (PI) (10 µg/mL, Sigma Aldrich) and incubated at 37 ^oC for 30 min in the dark. All the stained samples were subsequently washed with PBS to remove residual dye. Samples were also double stained following the protocol described by Amor et al. (2002). Multicolour flow analysis was carried out by fully integrated and multiparamatric BD FACSCalibur (Becton Dickinson) system equipped with an air-cooled argon ion laser emitting 15 mW blue light at 488 nm and with standard filter set up. The FC analysis of the cell samples was performed using FACS Flow solution (BD) as the sheath fluid. Samples were kept at low flow rate (12 µL/min ± 3 µL/min) up to a total of 5,000 events per sample. Bacterial cells were analyzed by forward (FSC) and side (SSC) light scatter. Viability in term of membrane integrity and functional cytoplasmic enzymes was examined by staining with cFDA detected by FL1 channel, having 530 nm bandpass filter. Red fluorescence signal of PI was collected in the FL3 channel with >600 nm long pass filter for membrane permeability. The fluorescence signals of individual cells were collected in biexponential mode. Gating was done in dot-plot of FSC-SSC to discriminate bacteria from noise and artefacts. Data were acquired by BD CellQuest Pro software and were analyzed and refined by FloJo software (Tree Star, Stanford, USA).

2.2.4 Statistical analysis

All experiments were set up in a completely randomized design and repeated thrice with a minimum of three replicates. The statistical analysis was carried out using SPSS Statistics 17.0. The MFI values of FC data were subjected to analysis of variance (ANOVA) followed by Tukey's test (Post-hoc analysis) to detect significant difference between the treatments and vehicle control for bacterial strain. Differences were considered significant at a value of $p < 0.05$.

2.2.5 FESEM study

Field emission scanning electron microscopy (FESEM) was used to visualize the changes in the morphology of the LM cells before and after treatment with compound **1l**. Untreated bacterial cells were used as negative control. The bacterial samples were gently

washed with freshly prepared 50 mM phosphate buffer solution (pH 7.2), fixed with 2.5% glutaraldehyde in PBS and rinsed with the same buffer solution. The specimen was dehydrated using sequential exposure for each ethanol concentrations ranging from 30% to 100%. Finally, the specimens were coated with gold and analyzed through FESEM (Carl Zeiss, Ultra 55).

2.3 Antioxidant activity

The free radical scavenging efficacy of all the compounds **1a-1n** were estimated using DPPH (2,2- diphenyl-1-picrylhydrazyl) assay according to the method described by Park et al. (2011). DPPH is known as a stable free radical and strong scavenger for other radicals, which loses its purple colour on accepting an electron from an antioxidant molecule available in a reaction system (Zou et al., 2004). DPPH free radical scavenging activity of the compounds was determined using colorimetric assay. First 80 µL of DPPH solution (0.2 mM DPPH in absolute ethanol) was mixed thoroughly with 20 µL of the compound (concentrations ranging from 1.95 µg/mL to 1 mg/mL), and incubated for 30 min in dark at 25 °C. Butylated hydroxyl toluene (BHT) (Sigma Aldrich, USA) and ascorbic acid were used as positive controls and ethanol as solvent control. The absorbance was recorded at 517 nm in multimode microplate reader (Tecan, Infinite M-200, Switzerland). The ability to scavenge the DPPH radical was calculated as % radical scavenging activity (RSA) using the following equation:

$$RSA\ (\%) = (1 - A/B) \times 100$$

where, A was the absorbance of test compound and B was the absorbance of the DPPH dye. Linear graph of concentration and percentage inhibition was prepared and inhibitory concentration 50% (IC_{50}) values were calculated.

2.4 In silico studies

2.4.1 OSIRIS prediction

OSIRIS Property Explorer was used to estimate the risks of side effects, such as mutagenic, tumorigenic, irritant and reproductive effects, as well as drug-relevant properties including cLogP (lipophilicity), LogS (solubility), drug-likeness and overall drug-score (Ayati et al., 2012).

2.4.2 Lipinski's rule

The molecular properties and drug likeliness of synthesized compounds was examined on basis of Lipinski's rule of five using Molinspiration server

(http://www.molinspiration.com/). Lipinski's rule of five states that for a drug to be orally active if it fulfils all of the following criteria: not more than 10 hydrogen bond acceptors, not more than 5 hydrogen bond donors, molecular weight to be below 500 Dalton and partition co-efficient logP should be less than 5 (Lipinski et al., 1997).

3. Results and discussions

3.1 Chemistry

Various diarylmethylamines were synthesized via three component condensation reaction. Suitable aldehydes were condensed with different secondary amines in the presence of electron rich phenolic compounds to obtain the desired tertiary amines (**Scheme 2**) (Mahato et al., 2014). 2-Naphthol was employed as the nucleophile for syntheses of diarylmethylamines **1a-h,** having either *N*-heterocycle or *N*,*O*- and *N*,*N*-diheterocycle, with very good to excellent yields (up to 96%). Similarly, diarylmethylamine **1i** containing acyclic amine was synthesized from 2-naphthol, benzaldehyde and *N*-benzylmethylamine. On the other hand, phenol and 2,4-di-tert-butyl phenol provided diarylmethylamines **1j** and **1k** respectively. Sesamol is a naturally occurring compound and present in many bioactive molecules (Lavecchia et al., 2013; Singh et al., 2014). Therefore, we anticipated for a superior bioactivity of the diarylmethylamine containing sesamol moiety. Accordingly, to incorporate sesamol moiety, sesamol was selected as the nucleophile in place of 2-naphthol. The reaction of sesamol and *N*-methyl piperazine with salicylaldehyde and vanillin in refluxing ethanol provided the desire diarylmethylamines **1l** and **1m**, respectively, with very good isolated yields. An interesting antioxidant property was also anticipated for compounds **1l** and **1m** due to the presence of electron rich aromatic units. Arylmethylamine **1n** was synthesized from 3,5-di-tert-butylsalicyldehyde via a hydride free formal reductive benzylation of piperidine under microwave heating (Mahato et al., 2014). The structures of all the synthesized molecules were confirmed by spectral (IR, ^{1}H NMR, ^{13}C NMR and HRMS) analyses.

<Scheme 2>

3.2 Biological studies

3.2.1 Antibacterial studies

The present study revealed that the antibacterial activity of compound varies with different functional group(s) present in the compound. The *in vitro* studies for all the synthetic compounds have been carried out against two pathogenic strains of bacteria. Most of the compounds were showing MIC values between 0.015 and 1 mg/mL. The activity data

are presented in **Table 1**. Compound **1a** with 3-nitrophenyl and *N*-methyl piperazine units was effective against Gram-positive bacteria with MIC at 62.5 µg/mL and found less susceptible against Gram-negative bacteria. Compound **1b** having 2-hydroxy phenyl and pyrrolidine moiety was found to have similar activity. On the other hand, compound **1c** with *N*-methyl piperazine showed pronounced activity against Gram-positive bacteria, *L. monocytogenes* (LM) with MIC of 31.2 µg/mL. However, it was ineffective against *E. coli* enterotoxic. Reduced activity of compound **1d** as compared to **1b** was observed by replacing 2-OH-phenyl with phenyl group. Similar reduction in antibacterial activity was found for compound **1e**, when *N*-methyl piperazine in **1a** was replaced by pyrrolidine. An enhanced activity was found for **1f** having *N*-methyl piperazine unit compared to **1d** having pyrrolidine moiety. Interestingly, compound **1f** was found to be very effective against both Gram-positive and Gram-negative bacteria with MIC value of 31.2 µg/mL. A moderate activity was found for compounds **1g**, **1h**, and **1i** containing aromatic heterocycles, morpholine, and acylic amine respectively. Similar activities were found for phenol based compounds **1j**, **1k** and **1n**. These observations indicated that electron rich aromatics (like 2-OH phenyl, 2-napthol) and *N*-methyl piperazine moiety are important for obtaining antibacterial activity. Therefore, in a hope to get more potent candidate, diarylmethylamine **1l** and **1m** were synthesized incorporating naturally occurring electron rich phenol derivative, sesamol, instead of 2-napthol. Pleasingly, compound **1l** was found to be the most effective with MIC value of 15.6 and 31.2 µg/mL against Gram-positive bacteria and Gram-negative bacteria respectively. Its methoxy analogue **1m** also showed similar activity for both Gram-positive and Gram-negative bacteria. The potency of compound **1l** might be contributed by o-quinone methide that is generated by the decomposition of the compound in culture media. From NMR spectral study, the doublet peak at δ 7.24 ppm indicates the presence of qunoidal proton that has also been suggested to act as an anti-bacterial agent (Evans et. al, 1999). Additionally, we observed that DTT has reduced marginally the antibacterial potential of compound **1m** when treated with LM whereas no such effect observed for the compound **1l.** The results further confirm that compound **1l** display an effective antibacterial property in the presence of DTT.

Resazurin based micro broth dilution assay was also employed to study the antibacterial activity of **1l**. The colour change appeared at its MIC (15.6 µg/mL) due to the formation of pink colored resorufin (**Suppl. Fig. 1**). In general, it was observed from current study that compounds except **1f, 1l** and **1m** were less effective against Gram-negative bacteria. This is possibly owing to the fact that Gram-negative bacteria have

lipopolysaccharides (LPS) as outer membrane which renders bacteria to be more resistant to molecules having hydrophilic groups (Nazzaro et al., 2013). However, presence of hydrophobic substituent like 2,4-di-tert-butyl-phenol and piperidine in compound **1k** and **1n** did not exhibit activity against Gram-positive bacteria. Damage to the bacterial tissues through some pro-oxidant effects involving the phenolic hydroxyl group has also been attributed (Hatano et al., 2008; Kumar et al., 2015).

<Table 1>

<Suppl. Fig. 1>

3.2.2 Flow cytometry analysis

Multiparametric FC was carried out to better assess the antibacterial potential of the most promising compound **1l** against LM. Unstained, cFDA-stained, PI-stained, cFDA and PI double stained cells served as controls which enabled to set the FCM detectors. Bacterial cells were exposed to compound **1l** at 7.8, 15.6 (MIC) and 31.2 µg/mL and then simultaneously stained with cFDA and PI. The quadrants of the contour plots were set to ease the illustration of different subpopulations of bacteria and unstained cells appeared in the lower left quadrant. The vehicle control (DMSO) and untreated bacterial cells showed minimum relative fluorescence intensity of PI. But, the positive control (heat killed) showed significant increase ($p < 0.01$, Tukey's post hoc test) in relative fluorescence intensity of PI (98.4%) in tested bacteria with respect to vehicle control. This confirmed that the major cell populations existed as membrane compromised or dead. The dual-parameter contour plots indicate the existence of three main subpopulations of compound-treated LM and showed dynamic changes of the membrane integrity with the increase in concentration of compound (**Fig. 1**). These subpopulations were identified as live, dead and membrane compromised bacteria based on their differential staining characteristics with PI and cFDA.

The cFDA-stained subpopulation ($cFDA^+$ PI^-) decreased with increasing concentrations of compound **1l** and accounted for 81.2, 6.66 and 0.31% of the total population after exposure to compound at 7.8, 15.6 and 31.2 µg/mL, respectively. Whereas, the fraction that stained only with PI ($cFDA^-$ PI^+) increased from 1.99, 52.1 and 87.5% after exposure to compound at 7.8, 15.6 and 31.2 µg/mL respectively, and was scored as the dead population. The partial hydrophilic nature of compound **1l** allows it to penetrate microbial cells and induce alterations in structure and function of the bacterium. This possibly resulted in the loss of microbial viability (Nazzaro et al., 2013). The percent double-stained LM cells ($cFDA^+$ PI^+) showed a subtle fluctuation during the course of the compound stress exposure.

This fraction constituted 2.24, 19.8 and 2.22% of the total population after treatment with compound at 7.8, 15.6 and 31.2 µg/mL and was recognized as "viable but non culturable state" (VBNC). Cells entering the VBNC state often exhibit dwarfing, and a number of major metabolic changes occur, including reductions in nutrient transport, respiration rates, changes in membrane fatty acid composition and macromolecular synthesis (Porter et al., 1995). *L. monocytogenes* is known for its diverse physiological states including viable, dead and membrane compromised population in a heterogeneous sample (Besnard et al., 2000; Oliver, 2005). In the present study, similar behaviour was confirmed with the help of multicolour flow analysis.

Interestingly, it was observed that PI relative fluorescence intensity was maximum when the cells were subjected to heat treatment indicating significant damage and depolarization of the bacterial cell membrane. Stressed population of cells could maintain cell metabolic activity, as determined by the fluorescent dyes. The efficacy of compound **1l** as potential antibacterial agent has been confirmed using flow analysis.

<Figure 1>

3.2.3 FESEM analysis

The most susceptible Gram-positive bacterium (*L. monocytogenes*) was examined by FESEM to observe morphological changes caused by treatment with compound **1l** (**Fig. 2**). FESEM images of untreated LM showed intact, smooth cell surface with defined cell features (**Fig. 2A**). Heat killed and kanamycin treated cells showed complete membrane disruption (**Fig. 2B & 2C**). Shrinking, membrane disintegration and prominent damage of the cell wall was observed in bacterial cells treated with the compound (**Fig. 2D-F**). These findings indicate that compound caused lysis of the bacterium by degrading cell wall and affecting cytoplasmic membrane. Similar phenomenon has also been found in related studies on Gram-positive bacteria (Ghosh et al., 2014).

<Figure 2>

3.3 Antioxidant activity

Antioxidant property of the synthesized compounds has been tested next. A wide variety of methods have been developed for the estimation of antioxidant potential (Prior et al., 2005). Among them, DPPH assay is extensively used due to its stability, and its simple reaction system (Ghosh et al., 2014). The method is based on the reduction of DPPH to the non-radical form DPPH-H by a hydrogen-donating antioxidant (Ghosh et al., 2014). Various concentrations of compounds (1.9 µg/mL - 1 mg/mL) showed radical scavenging activities in

a dose dependent manner (**Fig. 3**). The IC_{50} was determined for the compounds and also for the positive control, BHT and ascorbic acid. As anticipated, we found that both the compounds **1l** (IC_{50} = 370 µg/mL) and **1m** (IC_{50} = 428 µg/mL) containing sesamol moiety showed DPPH radical scavenging activity which are comparable to that of BHT (IC_{50} = 184 µg/mL). Compound **1g** (IC_{50} = 817 µg/mL) and **1i** (IC_{50} = 958 µg/mL) showed lower activity than BHT. Other compounds remain ineffective towards radical scavenging activity. The high radical scavenging capacity of sesamol moiety in compounds **1l** and **1m** is probably due to the presence of free hydroxyl group in its structure (Kumar et al., 2015).

<Figure 3>

3.4 In silico studies

3.4.1 Physico-chemical properties by OSIRIS

Physico-chemical properties like lipophilicity, aqueous solubility, molecular weight, etc of a bioactive molecule are important parameters to be considered for the drug discovery and development. Therefore, *in silico* studies were carried out to calculate those parameter for all the synthesized compounds and the results were summarized in **Table 1S**. For most of the compounds, the parameters found are within the standard range. The calculated logP has been shown to be one of the key parameters in quantitative structure activity/property relationship (QSAR/QSPR) studies (Alves et al., 2000; Bayat and Nassab, 2010). Potential antibacterial agents **1f, 1l** and **1m** showed to have logP value of 3.93, 2.5 and 2.43, respectively, which are well below the maximum acceptable value (5). The aqueous solubility is also an important parameter which significantly affects the absorption and distribution characteristics of bioactive molecules. More than 80% of the drugs in the market have aqueous solubility values greater than -4 (Sander et al., 2009). It is also known that the total sum of all polar regions of a molecule's surface correlates well with various bioavailability related properties, such as intestinal absorption and blood brain barrier penetration. The solubility parameters for compounds **1f** (-3.27), **1l** (-2.08) and **1m** (-2.10) were found to be in the acceptable range. Positive value of drug likeness and drug score state that **1l** and **1m** contains predominant fragments which are frequently present in commercial drugs. The analysis also suggested that compound **1l** may have low risk to human health considering the four main parameters (mutagenic, tumorigenic, irritant, and reproductive effectiveness). Overall, with highly desirable physico-chemical parameters and nontoxic behaviour, the structure of compound **1l** disclosed its potential as a promising therapeutic agent.

3.4.2 Lipinski's rule

Lipinski's rule of 5 evaluates whether a given compound can be administered as orally active drug (Lipinski et al., 1997). The analysed data showed that most of the synthesized compounds (**1a-1c** and **1f-1h**, **1j**, **1l**, **1m**) meet the Lipinski's rules of the five, suggesting that the compound theoretically would not have problems with oral bioavailability (**Table 2S**). Compounds **1d**, **1e**, **1i**, **1k** and **1n** with high partition coefficient log P are violating the Lipinski's rule where permeability could be a barrier for their transport in biological system. The methyl groups of **1k** and **1n** contributes to its high lipophilicity, therefore unsuitable for oral consumption.

4. Conclusion

In summary, various diarylmethylamines/Betti bases have been synthesized using simple and efficient synthetic protocol. These compounds were evaluated for their antibacterial activity against Gram-positive and Gram-negative bacteria. The present study revealed that diarylmethylamine derived from sesamol to be more potent than one derived from 2- naphthol and phenol based compounds. Compound **1l** containing sesamol and *N*-methyl piperazine was found to be the most potent against Gram-positive (*L. monocytogenes*) bacteria. It showed antibacterial effect through the damage of bacterial cell membrane which was confirmed by FC and FESEM analyses. Other compounds showed moderate or no activity against Gram-negative bacteria. Compounds **1l** and **1m** also showed strong free radical scavenging activity similar to BHT in DPPH assay. Interesting antibacterial activity and promising drug likeness properties, obtained from *in silico* studies, signified the compound **1l** to be considered as a potential antibacterial agent. Further studies on this novel class of antibacterial agents are ongoing in our laboratory to find more potent candidate.

5. Experimental section

5.1 General procedure (GP) for the syntheses of diarylmethylamines

2-Naphthol or sesamol was added to a solution of secondary amine and aldehyde in benzene or ethanol and the mixture was refluxed for 16 h. After the disappearance of the starting material indicated by TLC, the solvents were allowed to evaporate under ambient condition to obtain the solid residue. The solid was washed with hexane and binary solvent mixture (EtOAc and hexane) to afford analytically pure diarylmethylamine derivatives.

1-((2-hydroxyphenyl)(pyrrolidin-1-yl)methyl)naphthalen-2-ol (1b): According to GP: 2-napthol (0.28 g, 1.96 mmol), salicylaldehyde (0.25 mL, 2.35 mmol), pyrrolidine (0.24 mL, 2.59 mmol) in benzene 4 mL in reflux condition for 16 h, slow evaporation under ambient

condition and washing (hexane, 15 mL) the resulting solid gave **1b** (0.59 g, 96%) as white solid. FTIR (KBr): $\tilde{\nu}$ = 3461, 3056, 2950, 1838, 1615, 1584, 1559, 1057, 1451, 1365, 1275, 1237, 1142, 957, 824, 753, 693, 667, 630, 559, 549, 544, 453 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ = 7.80 (d, *J* = 8.7 Hz, 1H), 7.52 (t, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.16 (t, *J* = 7.7 Hz, 1H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.94 – 6.89 (m, 2H), 6.72 (d, *J* = 8.0 Hz, 1H), 6.56 (t, *J* = 7.5 Hz, 1H), 5.80 (s, 1H), 3.21 – 2.86 (m, 2H), 2.61 – 2.31 (m, 2H), 1.82 – 1.68 (m, 4H).^{13}C NMR (101 MHz, DMSO-d_6) δ = 156.4, 154.6, 132.4, 129.4, 129.3, 129.2, 129.0, 128.3, 127.8, 126.9, 122.8, 121.7, 120.3, 120.2, 117.7, 116.0, 61.3, 51.6 (br. 2C) 23.5 (2C). HRMS (ESI) exact mass calculated for $C_{21}H_{22}NO_2$ ($[M + H]^+$): 320.1645; found: 320.1641.
<**Suppl. Fig. 2 & 3**>

1-((2-hydroxyphenyl)(4-methylpiperazin-1-yl)methyl)naphthalen-2-ol (1c): According to GP: 2-napthol (0.20 g, 1.39 mmol), salicylaldehyde (0.17 mL, 1.66 mmol), *N*-methyl piperazine (0.18 mL, 1.66 mmol) in benzene 2 mL in reflux condition for 16 h, slow evaporation under ambient condition and washing (50 mL; EtOAc : hexane; 1 : 15) the resulting solid gave **1c** (0.42 g, 90%) as white powder. FTIR (KBr): $\tilde{\nu}$ = 3458, 3061, 3045, 2956, 2806, 1622, 1601, 1577, 1517, 1459, 1332, 1283, 1239, 1152, 1139, 1096, 997, 949, 832, 813, 760, 754, 746, 636, 533 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 13.87 (s, 1H), 7.83 (d, *J* = 8.6 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.37 – 7.31 (m, 1H), 7.30 – 7.26 (m, 1H), 7.19 (d, *J* = 7.6 Hz, 1H), 7.17 – 7.15 (m, 1H), 7.04 – 7.00 (m, 1H), 6.68 (t, *J* = 8 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 6.05 (s, 1H), 3.32 – 3.29 (m, 1H), 3.12 – 3.09 (m, 1H), 3.04 – 2.91 (m, 3H), 2.69 – 2.66 (m, 1H), 2.60 – 2.56 (m, 1H), 2.53 (s, 3H), 2.38 – 2.32 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 156.2, 155.2, 133.2, 130.6, 129.5, 129.3, 128.8, 128.7, 126.8, 125.3, 122.7, 121.9, 120.3, 119.9, 116.1, 114.9, 62.5, 55.9, 55.3, 52.7, 47.1, 45.8. HRMS (ESI) exact mass calculated for $C_{22}H_{25}N_2O_2$ ($[M + H]^+$): 349.1911; found: 349.1904.
<**Suppl. Fig. 4 & 5**>

1-((4-methylpiperazin-1-yl)(phenyl)methyl)naphthalen-2-ol (1f): According to GP: 2-napthol (1.0 g, 6.94 mmol), benzaldehyde (0.84 mL, 8.32 mmol), *N*-methylpiperazine (0.923 mL, 8.32 mmol) in benzene 2 mL in reflux condition for 18 h, slow evaporation under ambient condition, washing the solid residue (60 mL; EtOAc : hexane; 1 : 10), gave **1f** as yellowish solid (1.82 g, 80%). FTIR (KBr): $\tilde{\nu}$ = 3439, 3061, 2970, 2839, 2798, 1620, 1598, 1581, 1520, 1473, 1452, 1415, 1265, 1238, 1154, 1136, 1083, 1016, 999, 949, 834, 817, 748, 698, 637, 514, 497 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 13.34 (s, 1H), 7.82 (d, *J* = 8.6 Hz, 1H), 7.64 (t, *J* = 8.6 Hz, 1H), 7.55 – 7.53 (m, 2H), 7.35 – 7.31 (m, 1H), 7.24 – 7.20 (m, 3H),

7.18 – 7.14 (m, 3H), 5.10 (s,1H), 3.62 – 2.43 (br. m, 8H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.0, 139.2, 132.3, 129.6, 128.9, 128.8, 128.1, 126.5, 122.6, 121.1, 119.9, 115.6, 71.6, 55.1, 53.3, 51.0, 45.7. HRMS (APCI) exact mass calculated for $C_{22}H_{25}N_2O^+$ ($[M + H]^+$): 333.1961; Found: 333.1954.

<**Suppl. Fig. 6 & 7**>

2-(phenyl(pyrrolidin-1-yl)methyl)phenol (1j): According to GP: Phenol (0.934 mL, 10.63 mmol), benzaldehyde (1.29 mL, 12.74 mmol), pyrrolidine (1.04 mL, 12.74 mmol) in benzene 2 mL in reflux condition for 18 h. Silica column chromatography (EtOAc : Hexane; 1 : 40) gave product **1j** as yellow oil (1.92 g, 71%). FTIR (KBr): $\tilde{\nu}$ = 3425, 2963, 2924, 2851, 1609, 1591, 1483, 1469, 1453, 1045, 1254, 1125, 1096, 1031, 885, 789, 752, 698, 628 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 12.32 (br, 1H), 7.47 – 7.434(m, 2H), 7.27 – 7.22 (m, 2H), 7.20 – 7.16 (m, 1H), 7.08 (td, *J* = 7.7, 1.7 Hz, 1H), 6.94 (dd, *J* = 7.6, 1.7 Hz, 1H), 6.85 (dd, *J* = 8.2, 1.3 Hz, 1H), 6.68 (td, *J* = 7.4, 1.2 Hz, 1H), 4.36 (s, 1H), 2.61 – 2.43 (m, 4H), 1.82 – 1.75 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 156.7, 142.2, 128.8, 128.4, 128.4, 127.9, 127.8, 126.8, 119.2, 116.9, 75.8, 53.3, 23.6. HRMS (APCI) exact mass calculated for $C_{17}H_{20}NO^+$ ($[M + H]^+$): 254.1539; Found: 254.1535.

<**Suppl. Fig. 8 &9**>

2,4-di-tert-butyl-6-(phenyl(piperidin-1-yl)methyl)phenol (1k): According to GP: 2,4 di-tert-butyl phenol (0.15 g, 0.728 mmol), benzaldehyde (0.088 mL, 0.873 mmol), piperidine (0.086 mL, 0.873 mmol) in benzene 2 mL in reflux condition for 16 h. Silica column chromatography (EtOAc : Hexane; 1 : 200) gave product **1k** as whitish solid (0.20 g, 72%). FTIR (KBr): $\tilde{\nu}$ = 3458, 2964, 2947, 2859, 1483, 1453, 1434, 1357, 1261, 1236, 1199, 1158, 1035, 953, 871, 790, 708, 651 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.56 (s, 1H), 7.43 (br. s, 2H), 7.30 (t, *J* = 7.2 Hz, 2H), 7.25 – 7.23 (m,1H), 7.14 (s, 1H), 6.74 (s, 1H), 4.44 (s, 1H), 3.29 – 1.63 (br. m, 10 H), 1.45 (s, 9H), 1.19 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 153.8, 140.2, 140.1, 136.0, 129.2, 128.7, 127.7, 124.7, 124.2, 122.6, 52.4, 35.2, 34.3, 31.8, 29.8, 26.2, 24.4. HRMS (APCI) exact mass calculated for $C_{26}H_{38}NO^+$ ($[M + H]^+$): 380.2948; Found: 380.2950.

<**Suppl. Fig. 10 & 11**>

6-((2-hydroxyphenyl)(4-methylpiperazin-1-yl)methyl)benzo[d][1,3]dioxol-5-ol (1l): According to GP: Sesamol (0.20 g, 1.45 mmol), salicylaldehyde (0.18 mL, 1.74 mmol), *N*-methyl piperizine (0.19 mL, 1.74 mmol) in ethanol 2 mL in reflux condition for 16 h, slow

evaporation under ambient condition, washing the solid residue (50 mL; EtOAc : hexane; 1 : 10), and recrystallization from ethanol gave **1l** (0.36 g, 72%) as whitish solid. FTIR (KBr): $\tilde{\nu}$ = 3453, 2856, 2809, 1607, 1586, 1500, 1476, 1463, 1282, 1257, 1214, 1183, 1164, 1134, 1041, 987, 936, 875, 759, 571 cm^{-1}.^{1}H NMR (400 MHz, CD_3OD) δ = 7.00 – 6.94 (m, 2H), 6.68 – 6.66 (m, 1H), 6.62 (t, *J* = 7.5 Hz, 1H), 6.51 (s, 1H), 6.25 (s, 1H), 5.68 (s, 2H), 5.01 (s, 1H) 2.81– 2.23 (br. 8H), 2.17 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 155.7, 150.4, 146.6, 139.9, 128.6, 128.0, 126.2, 119.2, 117.3, 116.0, 107.5, 100.7, 97.9, 64.8, 54.8 (2C), 50.4 (2C), 45.4. HRMS (ESI) exact mass calculated for $C_{19}H_{23}N_2O_4$ ($[M + H]^+$): 343.1652; found: 343.1646.

<**Suppl. Fig. 12 & 13**>

6-((2-hydroxy-3-methoxyphenyl)(4-methylpiperazin-1-yl)methyl)benzo[d][1,3]dioxol-5-ol (1m): According to GP: Sesamol (0.10 g, 0.724 mmol), *o*-vanillin (0.13 g, 0.869 mmol), *N*-methyl piperazine (0.096 mL, 0.869 mmol) in ethanol 2 mL in reflux condition for 16 h, slow evaporation under ambient condition, washing the solid residue (30 mL; EtOAc : Hexane; 1 : 10), and recrystallization from ethanol gave **1m** (0.18 g, 67%) as whitish solid. FTIR (KBr): $\tilde{\nu}$ = 3453, 2989, 2961, 1632, 1500, 1480, 1460, 1281, 1139, 1068, 1058, 1037, 994, 934, 864, 789, 730 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 6.70 – 6.67(m, 2H), 6.59 (t, *J* = 7.9 Hz, 1H), 6.44 (s, 1H), 6.21 (s, 1H), 5.66 (d, *J* = 3.0 Hz, 2H), 5.01 (s, 1H), 3.73 (s, 3H), 2.91 – 2.27 (br. m, 8H), 2.17 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 150.4, 147.7, 146.6, 145.0, 139.9, 126.5, 120.2, 118.8, 117.3, 110.5, 107.4, 100.7, 97.8, 64.8, 55.5, 54.8, 50.4, 45.5. LRMS (APCI) exact mass calculated for $C_{20}H_{25}N_2O_5^+$ ($[M + H]^+$): 373.1758; Found: 373.1788.

<**Suppl. Fig. 14 & 15**>

Acknowledgements

SM and AS thank MHRD, Government of India for student fellowships. Thanks to Department of Biosciences and Bioengineering, IIT Guwahati for providing facility related to flow cytometry. Thanks also to Central Instruments Facility (CIF) IIT Guwahati for FESEM, and other analytical facilities. The project was funded by SERB and MHRD for Centre of Excellence in FAST (5-7/2014-TS-VII).

References

Alanis, A.J., 2005. Resistance to antibiotics: are we in the post-antibiotic era? Arch. Med. Res. 36, 697-705.

Alves, C.N., Pinheiro, J.C., Camargo, A.J., Ferreira, M.M.C., Silva, A.B.F., 2000. A structure-activity relationship study of HEPT-analog compounds with anti-HIV activity. J. Mol. Struc-Theochem. 530, 39-47.

Amor, K.B., Breeuwer, P., Verbaarschot, P., Rombouts, F.M., Akkermans, A.D.L., De Vos, W.M., Abee, T., 2002. Multiparametric flow cytometry and cell sorting for the assessment of viable, injured, and dead bifidobacterium cells during bile salt stress. Appl. Environ. Microb. 68, 5209-5216.

Ayati, A., Falahati, M., Irannejad, H., Emami, S., 2012. Synthesis, *in vitro* antifungal evaluation and *in silico* study of 3-azolyl-4-chromanone phenyl hydrazones. DARU J. of Pharm. Sci. doi: 10.1186/2008-2231-20-46.

Bayat, Z., Nassab, S.Q., 2010. Computational approaches to the prediction of the 1-octanol/water partition coefficient of benzimidazole derivatives drugs. J. Chem. Pharm. Res. 2, 306-315.

Bedeschi, A., Fonte, P., Fronza, G., Fuganti, C., Serra, S., 2014. The co-identity of lipiarmycin A3 and tiacumicin B. Nat. Prod. Commun. 9, 237-240.

Besnard, V., Federighi, M., Cappelier, J.M., 2000. Evidence of viable but non-culturable state in *Listeria monocytogenes* by direct viable count and CTC-DAPI double staining. Food Microbiol. 17, 697-704.

Betti, M., 1900. On the addition of amine benzal to the Naphtole. Gazz. Chim. Ital. 30, 301-309.

Chopde, H.N., Meshram, J.S., Pandhurnekar, C.P., Pagadala, R., Jonnalagadda, S.B., 2015. Efficient synthesis, characterization, *in vitro* antibacterial and antifungal activity study and computational tool for prediction of molecular properties of some novel Schiff's base via Betti's protocol and azetidinones. J. Heterocycl. Chem. doi: 10.1002/jhet.2349.

Erb, W., Zhu, J., 2013. From natural product to marketed drug: the tiacumicin odyssey. Nat. Prod. Rep. 30, 161-174.

Evans, G.B., Furneaux, R.H., Gravestock M.B., Lynch G.P., Scott G.K., 1999. The synthesis and antibacterial activity of totarol derivatives. Part 1: Modifications of ring-C and pro-drugs. Bioorg. Med. Chem. 7, 1953-1964.

Gerlach, M., Maul, C., 2004. Substituted 1 and 2-naphthol mannich bases US 20040147570.

Ghosh, S., Indukuri, K., Bondalapati, S., Saikia, A.K., Rangan, L., 2013. Unveiling the mode of action of antibacterial labdane diterpenes from *Alpinia nigra* (Gaertn.) B. L. Burtt seeds. Eur. J. Med. Chem. 66, 101-105.

Ghosh, S., Ozek, T., Tabanca, N., Ali, A., Rehman, J., Khan, I.A., Rangan, L., 2014. Chemical composition and bioactivity studies of *Alpinia nigra* essential oils. Ind. Crops. Prod. 53, 111-119.

Gyémánt, N., Engi, H., Schelz, Z., Szatmári, I., Tóth, D., Fülöp, F., Molnár, J., de Witte, P.A.M., 2010. *In vitro* and *in vivo* multidrug resistance reversal activity by a Betti-base derivative of tylosin. Br. J. Cancer. 103, 178-185.

Hatano, T., Kusuda, M., Tsugawa, M., Taniguchi, S., Shiota, S., Tsuchiya. T., Matsumoto, T., 2008. Chapter II: Effects of natural polyphenols on methicillin-resistant *Staphylococcus aureus* in relation to their chemical and physical properties. ISBN: 978-1-60456-232-3 Phytochemistry Research Progress, 31-47.

Jogi, P.S., Meshram, J.S., Sheikh, J., 2013. Synthesis and evaluation of antimicrobial activity of novel oxazine derivatives using Betti's protocol. Lett. Drug. Des. Disco. 10, 283-288.

Kesari, V., Das, A., Rangan, L., 2010. Physico-chemical characterization and microbial assay from seed oil of *Pongamia pinnata*, potential biofuel crop. Biomass Bioenergy. 34, 108-115.

Krizsan, A., Volke, D., Weinert, S., Sträter, N., Knappe, D., Hoffmann, R., 2014 Insect-derived proline-rich antimicrobial peptides kill bacteria by inhibiting bacterial protein translation at the 70S ribosome. Angew. Chem. Int. Ed. 53, 12236-12239.

Kumar, C.M., Singh, S.A., 2015. Bioactive lignans from sesame (*Sesamum indicum* L.): evaluation of their antioxidant and antibacterial effects for food applications. J. Food Sci. Technol. 52, 2934-2941.

Lavecchia, A., Giovanni, C.D., Cerchia, C., Russo, A., Russo, G., Novellino, E., 2013. Discovery of a novel small molecule inhibitor targeting the frataxin/ubiquitin interaction via structure-based virtual screening and bioassays. J. Med. Chem. 56, 2861-2873.

Levy, S.B., Marshall, B., 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10, s122-s129.

Lipinski C.A., Lombardo F., Dominy B.W., Feeney P.J., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 23, 4-25.

Mahato, S., Haldar, S., Jana, C.K., 2014. Diastereoselective α-C-H functionalization of aliphatic *N*-heterocycles: an efficient route to ring fused oxazines. Chem. Commun. 50, 332-334.

Mahato, S., Haque, M.A., Dwari, S., Jana, C.K., 2014. Divergent reaction: metal & oxidant free direct C-H aryloxylation and hydride free formal reductive *N*-benzylation of *N*-heterocycles. RSC Adv. 4, 46214-46217.

Mathew, B.P., Kumar, A., Sharma, S., Shukla, P.K., Nath, M., 2010. An eco-friendly synthesis and antimicrobial activities of dihydro-2H-benzo- and naphtho-1,3-oxazine derivatives. Eur. J. Med. Chem. 45, 1502-1507.

Mukhopadhyay, C., Rana, S., Butcher, R.J., 2012. Catalyst-free, one-pot, expeditious synthesis of aminoalkylnaphthols at room temperature. Synth. Comm. 42 (20), 3077-3088.

Nazzaro, F., Fratianni, F., Martino, L.D., Coppola, R., Feo, V.D., 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals 6, 1451-1474.

Nuding, S., Fellermann, K., Wehkamp, J., Mueller H.A.G., Stange E.F., 2006. A flow cytometric assay to monitor antimicrobial activity of defensins and cationic tissue extracts. J. Microbiol Methods. 65, 335-345.

Oliver, J.D., 2005. The viable but nonculturable state in bacteria. J. Microbiol. 43, 93-100.

Park, Y.S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Suhaj, M., Cvikrová, M., Martincová, O., Weisz, M., Gorinstein, S., 2011. Comparison of the contents of bioactive compounds and the level of antioxidant activity in different kiwi fruit cultivars. J. Food Comp. Anal. 24, 963-970.

Porter, J., Edwards, C., Pickup, R.W., 1995. Rapid assessment of physiological status in *Escherichia coli* using fluorescent probes. J. Appl. Bacteriol. 79, 399-408.

Prior, L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 3, 4290-4303.

Sander, T., Freyss, J., Korff, M.v., Reich, J.R., Rufener, C., 2009. OSIRIS, an entirely in-house developed drug discovery informatics system. J. Chem. Inf. Model. 49, 232-246.

Sarker, S.D., Nahar, L., Kumarasamy, Y., 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods 42, 321-324.

Singh, H., Sindhu, J., Khurana, J.M., Sharma, C., Aneja, K.R., 2014. Syntheses, biological evaluation and photophysical studies of novel 1, 2, 3-triazole linked azo dyes. RSC Adv. 4, 5915-5926.

Wright, P.M., Seiple, I.B., Myers, A.G., 2014. The evolving role of chemical synthesis in antibacterial drug discovery. Angew. Chem. Int. Ed. 53, 8840-8869.

Zou, Y., Lu, Y., Wei, D., 2004. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro* J. Agric. Food. Chem. 52, 5032-5039.

Figure Captions

Fig. 1 Dual parameter contour plot for viability assessment of *L. monocytogenes* against compound **1l** by flow cytometry. Proper control cells stained with cFDA and PI (A) and (B) and bacteria treated with (C) 7.8 µg/mL; (D) 15.6 µg/mL and (E) 31.2 µg/mL. Three main subpopulations, corresponding to viable cFDA-stained cells (upper left quadrant), injured cells double stained with PI and cFDA (upper right quadrant), and dead PI-stained cells (lower right quadrant), can be readily differentiated.

Fig. 2 Field emission scanning electron micrograph of *L. monocytogenes* (LM) (A) untreated bacteria, (B) positive control as heat killed cells, (C) cells treated with antibiotic kanamycin, and (D-F) bacterial cells after treatment with compound **1l** at its MIC showing damage in cell membrane, dwarfism and blebbing.

Fig. 3 DPPH scavenging activity of all compounds. BHT used as positive control at varying concentration ranging 1 mg/mL - 1.95 µg/mL.

Suppl. Fig. 1 Colorimetric microbroth dilution assay of compound **1l** against *L. monocytogenes* CC, compound control; GC, growth control; NE, negative control; SC, sterility control.

Suppl. Fig. 2 ^{1}H NMR spectrum of compound **1b**

Suppl. Fig. 3 ^{13}C NMR spectrum of compound **1b**

Suppl. Fig. 4 ^{1}H NMR spectrum of compound **1c**

Suppl. Fig. 5 ^{13}C NMR spectrum of compound **1c**

Suppl. Fig. 6 ^{1}H NMR spectrum of compound **1f**

Suppl. Fig. 7 ^{13}C NMR spectrum of compound **1f**

Suppl. Fig. 8 ^{1}H NMR spectrum of compound **1j**

Suppl. Fig. 9 ^{13}C NMR spectrum of compound **1j**

Suppl. Fig. 10 ^{1}H NMR spectrum of compound **1k**

Suppl. Fig. 11 ^{13}C NMR spectrum of compound **1k**

Suppl. Fig. 12 ^{1}H NMR spectrum of compound **1l**

Suppl. Fig. 13 ^{13}C NMR spectrum of compound **1l**

Suppl. Fig. 14 ^{1}H NMR spectrum of compound **1m**

Suppl. Fig. 15 ^{13}C NMR spectrum of compound **1m**

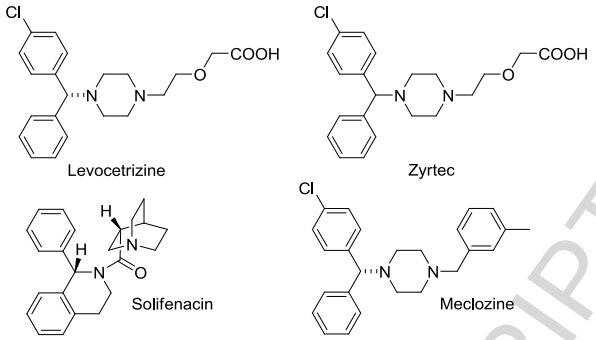
Scheme 1. Important diarylmethylamines.

Scheme 2. Syntheses of diarylmethylamines.

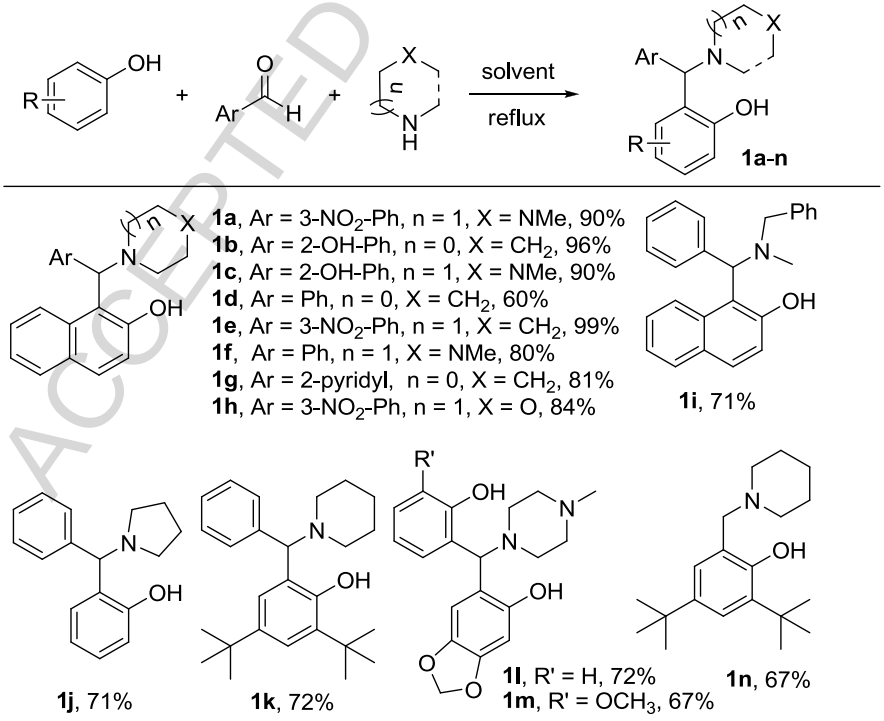
Table 1. The minimum inhibitory concentration (MIC) values (μg/mL) of compound **1a-1n** against selected Gram-positive and Gram-negative bacteria.

Compounds*	Gram (+) ve *L. monocytogenes* (LM)	Gram (-) ve *E. coli* enterotoxic (ETEC)
1a	62.5	250
1b	62.5	250
1c	31.2	-
1d	250	-
1e	500	250
1f	31.2	31.2
1g	250	125
1h	-	250
1i	250	1000
1j	250	250
1k	-	250
1l	15.6	31.2
1m	62.5	31.2
1n	-	250

*Positive control – Kanamycin (5 μg/mL)



Scheme 1



Scheme 2

Table 1S. *In silico* prediction of drug likeness property of synthesized diarylmethylamines (**1a-1n**).

No.	Mutagenic	Tumorigenic	Irritant	Reproductive effective	cLogP	LogS	Molecular weight	TPSA	Drug likeness	Drug score
1a	Red	Red	Green	Green	3.0	-3.73	377	72.53	-1.06	0.17
1b	Red	Red	Green	Green	4.29	-4.09	319	43.7	0.33	0.19
1c	Red	Red	Green	Green	3.58	-2.97	348	46.94	4.15	0.28
1d	Red	Red	Red	Green	4.64	-4.39	303	23.47	0.64	0.15
1e	Red	Red	Green	Green	4.06	-5.12	362	69.29	-7.42	0.1
1f	Red	Red	Green	Red	3.93	-3.27	332	26.71	5.69	0.22
1g	Red	Red	Green	Green	3.69	-3.62	304	36.36	1	0.24
1h	Red	Red	Green	Green	1.95	-3.46	365	91.41	-6.63	0.15
1i	Red	Red	Green	Green	5.27	-5.02	353	23.47	1.4	0.16
1j	Green	Green	Red	Green	3.44	-2.78	253	23.47	3.66	0.67
1k	Green	Green	Red	Green	6.95	-5.37	379	23.47	-9.78	0.11
1l	Green	Green	Green	Green	2.5	-2.08	342	65.4	5.58	0.87
1m	Red	Green	Red	Green	2.43	-2.10	372	74.63	4.45	0.41
1n	Green	Green	Red	Green	5.23	-4.11	303	23.47	-9.99	0.18

Table 2S. *In silico* prediction of drug likeness property of compounds (**1a-1n**) by Lipinski's rule.

Lipinski rule	Log P	Number of hydrogen bonds donors	Number of hydrogen bond acceptors	Molecular weight	Violation
1a	4.45	6	1	377.44	0
1b	4.65	3	2	319.40	0
1c	4.45	4	2	348.45	0
1d	5.02	2	1	303.40	1
1e	5.46	5	1	362.43	1
1f	4.51	3	1	332.45	0
1g	3.85	3	1	304.39	0
1h	4.40	6	1	364.40	0
1i	6.02	2	1	353.46	1
1j	3.86	2	1	253.34	0
1k	7.48	2	1	379.59	1
1l	3.16	6	2	342.39	0
1m	2.76	7	2	372.42	0
1n	5.70	2	1	303.49	1

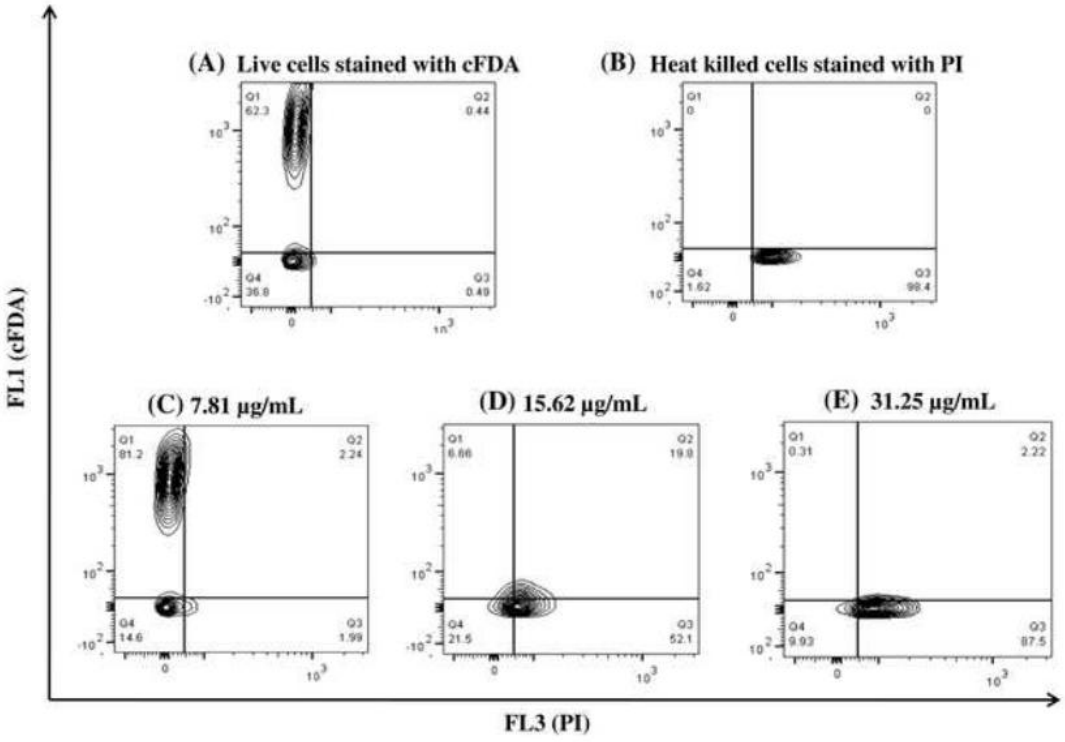


Fig. 1

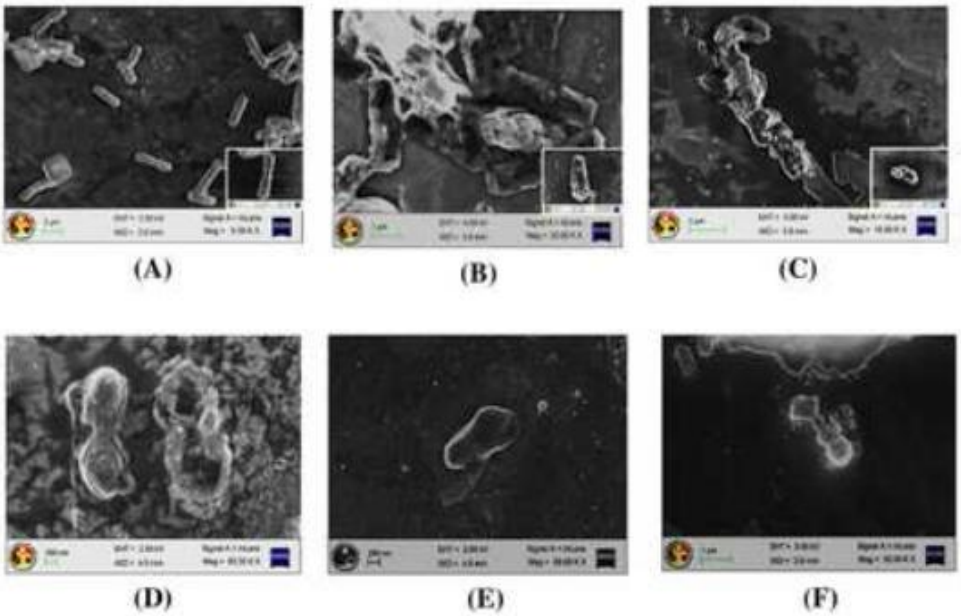
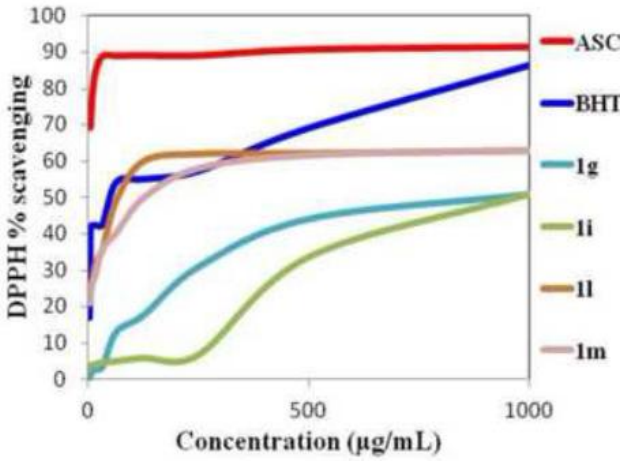
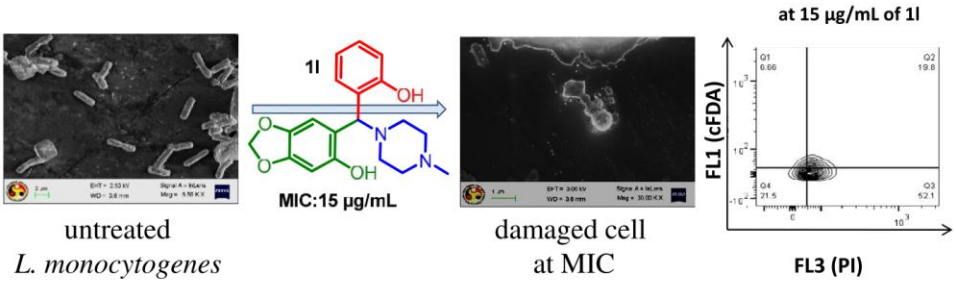


Fig. 2



Compounds	IC_{50} (µg/mL)
1g	817
1i	958
1l	370
1m	428
BHT	184
Ascorbic Acid	112

Fig. 3



Graphical abstract