Synthesis and Preliminary Biological Activity Evaluation of New N-Substituted Phthalimide Derivatives

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Abstract

A new series of bases of Schiff (H3- H4) derived from phthalic anhydride was synthesized. These Schiff bases were prepared by the reaction of different amines (tyrosine methyl ester, phenylalanine methyl ester, and isoniazid) with the phthalimide derived aldehyde with the aid of glacial acetic acid or triethylamine as catalysts. All the synthesized compounds were characterized by (FT-IR and 1HNMR) analyses and were in vitro evaluated for their antimicrobial activity against six various kinds of microorganisms. All the synthesized compounds had been screened for their antimicrobial activity against two Gram-positive bacteria “Staph. Aureus, and Bacillus subtilis”, two Gram-negative bacteria “Escherichia coli, and Klebsiella pneumoniae”, and two fungi species “Candida tropicalis and Candida albicans” using concentrations of 62.5, 125 and 250 µg/mL of derivative in dimethyl sulfoxide (DMSO). All the synthesized compounds showed no activity at all against Gram-positive bacteria, for Gram-negative bacteria and fungi they showed moderate or no activity except compound H4 which revealed high antifungal activity against Candida tropicalis and Candida albicans at concentrations 125 and 250 µg/mL.

Keywords: Schiff base, phthalic anhydride, antimicrobial.

Introduction

The development of multidrug microbial resistance is the main challenge that modern scientists have so far been facing in the recent decades. The fact that many pathogenic microorganisms taking charge of numerous human and animal diseases have caused resistance mechanisms to the conventional therapies have encouraged hard work investigations in the fields of natural and synthetic chemistry, to discover new drug classes having many efficient therapeutic profiles.

Phthalimide derivatives have a structural core (–CO-N(R)-CO) and an imide ring which confer a biological activity to them. Phthalimides have wide range uses that include as anti-inflammatory agents, antidiabetic agents, antioxidant, Anticonvulsant, HIV-1 Reverse Transcriptase Inhibitor, as protective agents, as an antimicrobial agent using Schiff base principle. The structural feature of the five-membered ring shows they are hydrophobic and this enhances their passage across the biological membranes.
As a result, the Phthalimide subunit has been designed as a hybrid with other molecules to give either synergistic, additive or new biological activity\(^{11,12}\). On another hand, it was found that the Schiff bases are of great benefit for the synthesis of various bioactive medicinal compounds from the primary amine. They possess antimalarial, anticancer, antimicrobial, antioxidant, anticonvulsant and anti-inflammatory characters\(^{13}\). Schiff bases are the compounds own imine or azomethine (\(\text{C}=\text{N}\)) functional group. The nitrogen atom of azomethine may participate in the hydrogen bonding with the active sites of cell components and intervene with normal cell functions\(^{14}\).

Amino acids Schiff base is of high interest in the research world. Numerous scientists developed new strategies due to the special bioactivity of this design. Zahrara Salim et al\(^{15}\), have studied the anti-acute phosphatase activity of amino acid Schiff base. While Yan Zhang et al\(^{16}\), have investigated the increased herring sperm DNA intercalating of tryptophan vanillin Schiff base. Saroji Kumar et al\(^{17}\), in two separate pieces of research work, have proved the beta-lactamase activity of tryptophan and phenylalanine amino acids Schiff bases with substituted benzaldehyde. Some researchers have linked phthalimide moiety with some amino acid either directly proving antimicrobial activity\(^{18}\) or using a spacer, having no Schiff base structure, with antiviral activity as a result\(^{18}\).

Similarly, Isoniazid Schiff bases had been of wide attention in medicinal chemistry. Many researchers condensed isoniazid with various aldehydes to produce Schiff bases with enhanced anti-tubercular and antimicrobial activities. For example, S. Syed Tajudeen et al\(^{19}\), produced Schiff base complexes of isoniazid with significantly enhanced antibacterial activity against microbial strains. As well as Joseph N. Yonge et al\(^{20}\), prepare disoniazid’s Schiff bases by the reaction of isoniazid with pyridine carboxaldehyde which showed good activity against thacteria compared to isoniazid alone. No research work linked isoniazid with phthalimide moiety through Schiff base has been reported.

**Experiment**

**Materials and Methods**

Starting material phthalic anhydride and aldehydes were purchased from (Riedel de Haë n, Germany), tyrosine methyl ester, phenylalanine methyl ester and 4-aminobenzaldehyde from (Hyperchem, China), isoniazid from (Judex, England), acetic acid (BDH, China) ethanol\& methanol (Biosolve, Netherlands), DMSO\& CH\(_2\)CL\(_2\) (Romil, UK) and DMF (Thomas Baker, India). Thin-layer chromatography (TLC) was used to follow up the reaction and to check the purity of synthesized compounds, by using silica gel GF (type 60) pre-coated aluminum sheet from (Merck - Germany), UV-254 lamp was used to visualize the spots, and the elution system used was (Methanol: Ethyl acetate: n-Hexane (0.5: 2: 3)). Stuart SMP3 melting points apparatus was used to measure the melting points, and were uncorrected. IR spectra were made using FT-IR (IR Affinity-1) spectrometer, Shimadzu, Japan at the University of Baghdad - College of Pharmacy. The \(^1\)H-NMR spectra were performed at the College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad. Instrument Model: NMReady-60 spectrometer, 60 Hz by N analysis Corp., Canada. \(^1\)H NMR spectra were obtained on BRUKER model Ultra shield 1\(\cdot\)\(\cdot\)MHz spectrophotometer DMSO-d\(_6\) used as a solvent for samples measurement; it was performed at Central instrumental lab, School of Chemistry, College of Science, the University of Tehran, Iran.

**Chemical synthesis**

**Synthesis of 4-(1,3-dioxoisooindolin-2-yl)benzaldehyde (H\(_1\))**

Compound\(_1\) was synthesized by direct mixing equimolar quantities of “phthalic anhydride” with “4-aminobenzaldehyde” (16.5 mmol) followed by refluxing in acetic acid 60 mL for 4 hours. After cooling, solid particles were separated out then cold water was added to the resultant mixture and was filtered using Buchner, washed with water several times and dried over silica gel under vacuum. The precipitate was collected by filtration and recrystallized from ethanol to obtain a bright yellow powder\(^{21}\).

Yield 68\%; \(m.p.202-204^\circ C; R_f= 0.8; IR(\text{v, cm}^{-1})\): 3012: Ar-(C-H) str., 2754: Ald. (C=O) str., 1697: asym. \& sym. str. of imide (C=O), 1720: aldehyde (C=O) str., 1600-1465: Ar-(C=O) str., 1080, 717: aromatic in plane \& out of plane (C-H) bend; \(^1\)HNMR(\(\delta, ppm\)): 10.06 (1H, s, \(\text{H}_1\)), 7.7-8.07 (8H, m, Ar-H).

**Synthesis of methyl 2-((4-(1,3-dioxoisooindolin-2-yl) benzylidene) amino)-3-(4-hydroxyphenyl) propanoate (H\(_2\))**

A mixture of “L-tyrosine methyl ester” (0.5 g, 2.6 mmol) in 100 ml dichloromethane, compound \(H_1\) (0.643 g, 2.55 mmol), and anhydrous magnesium sulfate (0.75 g, 6.25 mmol) was refluxed for 6 hours), Schiff base formation is indicated by conversion of solution into a yellow-color. The solution allowed cooling at room temperature, the precipitated particles (magnesium sulfate) were discarded by filtration. The filtrate solvent was evaporated to obtain a yellow-colored product and was recrystallized from ethanol\(^{22}\).

Yield =40\%; \(m.p.209-210^\circ C; R_f= 0.58; IR(\text{v, cm}^{-1})\): 3394: phenolic (O-H) str., 3024: Ar-(C-H) str., 2970 & 2873: asym. \& sym. aliphatic (CH\(_2\)) str., 2935 & 2854: asym. \& sym. aliphatic (CH\(_3\)) str., 1782 & 1701: asym. \& sym. of imide (C=O).
str.. 1720: (C=O) str. of ester, 1639: (N=C) Schiff base, 1600-1450: Ar-(C=O) str. 1080 & 721: in and out of plane (C-H) bend.; 1H NMR (δ, ppm): 9.18 (1H, s, phenolic OH). 7.52-8.14 (8H, m, Ar-H). 8.16 (1H, s, CH=N). 6.62 (2H, d) & 6.67 (2H, d) Ar-H of tyrosine part, 4.2 (1H, t, CH3). 6.33 (3H, s, OCH3). 2.88 & 3.14 (2H, 2d, CH2).

Synthesis of methyl 2-((4-(1,3-dioxoisindolin-2-yl) benzylidene) amino)-3-phenylpropanoate (H3).

An ethanolic solution of 2-phenyl alanine methyl ester hydrochloride (2 g, 9.27 mmol, after stirring for half an hour with triethylamine), was added to hot dry ethanolic solution of compound H1 (1.9 g, 7.56 mmol), the mixture refluxed in water bath for 7 hours, a yellow-colored solution indicates Schiff base formation. The mixture was concentrated then allowed to cool. The precipitated Schiff base was filtered, washed with ethanol several times, and dried in the oven at 60 °C. The solid product was then stored under vacuum.

Yield 70%; m.p. 207-209 °C; Rf = 0.41; IR (v, cm⁻¹): 3055: Ar(C-H) str., 2966 & 2889: asym. & sym. aliphatic (CH2) str., 2943 & 2873: asym. & sym. aliphatic (CH2) str., 1782 & 1701: asym. & sym. of imide (C=O) str.. 1728: (C=O) str. of ester, 1639: (N=C) Schiff base str., 1600-1450: Ar-(C=O) str., 1080-721: in and out of plane (C-H) bend.; 1HNMR (δ, ppm): 8.14 (1H, s, CH=N). 7.17-7.90 (13H, m, Ar-H). 4.2 (1H, t, CH3). 3.63 (3H, s, OCH3). 3.02 & 3.14 (2H, 2d, CH2).

Synthesis of N’-(4-(1,3-dioxoisindolin-2-yl) benzylidene) isonicotinohydrazide (H4).

A methanolic solution of isoniazid (1.37 g, 10mmol) was added gradually to a hot methanolic solution of compound H1 (1.66 g, 10 mmol, after stirring with 2 drops of glacial acetic acid), after few minutes solid particles appeared and refluxing was continued for 1 hour. The pale yellowish solid separated was filtered, washed several times with methanol, dried and stored over silica gel in a desiccated jar. It was recrystallized from DMF/Methanol to give a shiny yellow powder.

Yield 81%; m.p. 344-346°C; Rf = 0.25; IR (v, cm⁻¹): 3282: sec.amide (N-H) str., 3059: Ar-(C-H) str., 1789 & 1701: asym. & sym. of imide (C=O) str., 1666: (C=O) amide str., 1604: imine (C=N) str., 1604-1512: Ar-(C=C) str., 1083 & 717cm⁻¹ in and out of plane (C-H) bend.; 1HNMR (δ, ppm): 12.16 (1H, s, NHCO). 7.48-8.80 (12H, m, Ar-H). 8.5 (1H, s, CH=N).

Antimicrobial activity

The Antimicrobial activity was screened using well diffusion methods. Two types of Gram-negative bacteria “Klebsiella pneumoniae and Escherichia Coli” and two types of Gram-positive bacteria “Staphylococcus aureus and Bacillus subtilis” were used for testing them-vitro antibacterial activity, and two fungi species “Candida tropicalis and Candida albicans” for testing the in-vitro antifungal activity isolated from a patient in Nahrain Teaching Hospital, Baghdad, Iraq. Cefotaxime and Miconazole used as standards against antibacterial and antifungal respectively, and DMSO as a solvent. It was done in “The University of Baghdad, College of Education for Pure Sciences Ibn Al-Haitham, central service laboratory”.

Results and Discussion

Chemistry

The pathway for the synthesis of the targeted Schiff bases(H2-H4) is depicted in scheme 1.

Scheme 1. General synthetic pathway of the titled compounds.

The synthetic pathway started by the preparation of aldehyde of phthalimide compound (H1) through imide bond formation using glacial acetic acid as solvent and catalyst. This method showed good yield with ease of production. The Schiff bases were prepared by reaction of compound (H1) with different amines (tyrosine methyl ester, phenylalanine methyl ester and isoniazid with the addition of few drops of “glacial acetic acid or triethylamine”). The reaction involves elimination of one water molecule in order to form a carbon-nitrogen double bond (imine or Schiff base).

All the synthesized compounds were characterized and their structures were confirmed by “FTIR and 1HNMR” spectral analyses.

The IR spectra for the parent nucleus (H1) demonstrated characteristic two absorption bands of asymmetrical & symmetrical imide (C=O) stretching displayed at (1697 &1697) cm⁻¹. In
addition, 1720 and 2754 cm\(^{-1}\) are accounted for (C=O)\&(CH\(_2\)) stretching of aldehyde respectively. For compounds (H\(_2\)) and (H\(_3\)) the disappearance of the characteristic aldehyde band and appearance of the band at 1639 cm\(^{-1}\) is a good indication for Schiff base formation i.e. linkage has occurred between the phthalimide core and (tyrosine or phenylalanine methyl ester moiety). Compound (H\(_2\)) has an obvious band at 3394 cm\(^{-1}\) is attributed to phenolic OH group stretching. While Compound (H\(_4\)) shows three important bands one is at 3282 cm\(^{-1}\) for secondary NH stretching and the second is at 1666 for amide carbonyl stretching, and 1604 cm\(^{-1}\) for imine band stretching.

The \(^1\)HNMR spectra of the synthesized compounds were consistent with the assigned structures. Compound (H\(_1\)) showed characteristic signals recorded at 10.06 due to C\(_2\)OH of the aldehyde, besides, the aromatic protons displayed at their expected region as a multiplet at \(\delta=7.7-8.07\) ppm. Compound (H\(_2\)) displayed C\(_2\)=N peak as a singlet at \(\delta=8.16\), while compounds (H\(_3\)) imine C\(_2\)=N peak appeared at \(\delta=8.14\) ppm. A peak of singlet at \(\delta=9.18\) ppm is due to the phenolic group in compound (H\(_3\)). Two doublet peak in the region of \(\delta=3.01-3.08\) ppm is attributed to the C\(_2\)H\(_2\) group of the amino acid part for (H\(_2\)) and 3.08 is due to (C\(_2\)H\(_2\)) of (H\(_3\)). Finally, a sharp singlet peak integrated for three protons at \(\delta=8.5\) ppm is due to C\(_2\)=N.

**Antibacterial activity**

Tables 1 and 2 show the antimicrobial activity of the synthesized compounds at concentrations of (62.5, 125 and/or 250 µg/mL).

| Compound Name | Conc. µg/mL | S. aureus | B. subtilis | E. coli | K. pneumoniae |
|---------------|-------------|-----------|------------|--------|--------------|
|               |             | Zone of inhibition(mm) |
| H\(_1\)       | 62.5        | -         | -          | 11     | -            |
|               | 125         | -         | -          | 12     | -            |
|               | 250         | -         | -          | 12     | -            |
| H\(_2\)       | 62.5        | -         | -          | 11     | 10           |
|               | 125         | -         | -          | 12     | 10           |
|               | 250         | -         | -          | -      | -            |
| H\(_3\)       | 62.5        | -         | -          | -      | -            |
|               | 125         | -         | -          | -      | -            |
|               | 250         | -         | -          | 10     | -            |
| H\(_4\)       | 62.5        | -         | -          | -      | -            |
|               | 125         | -         | -          | -      | -            |
|               | 250         | -         | -          | 10     | 12           |
| Cefotaxime    | 100         | 49        | 43         | 51     | 25           |
| DMSO          | -           | -         | -          | -      | -            |

**Table 2. antifungal activity of the tested compounds**

| Compound Name | Conc. µg/mL | Candida tropicalis | Candida albicans |
|---------------|-------------|-------------------|-----------------|
|               | Zone of inhibition(mm) |
| H\(_1\)       | 62.5        | 18                | -               |
|               | 125         | 24                | -               |
|               | 250         | 22                | -               |
| H\(_2\)       | 62.5        | 14                | -               |
|               | 125         | 15                | -               |
|               | 250         | -                 | -               |
| H\(_3\)       | 62.5        | -                 | 14              |
|               | 125         | -                 | -               |
|               | 250         | -                 | -               |
| H\(_4\)       | 62.5        | 12                | -               |
|               | 125         | 13                | -               |
|               | 250         | -                 | -               |
| Miconazole    | 100         | 15                | 23              |
| DMSO          | -           | -                 | -               |

(-) = No activity- slightly active (zone of inhibition between 5-10 mm), moderately active (zone of inhibition between 10-20 mm), highly active (zone of inhibition more Than 20 mm). \(^{24}\)
The data illustrated in Tables 1 and 2 reveals that all the synthesized compounds had been screened for their antimicrobial activity against two Gram-positive bacteria “Staph. aureus, and Bacillus subtilis”, two Gram-negative bacteria “Escherichia coli, and Klebsiella pneumoniae”, and two fungi species “Candida tropicalis and Candida albicans” using concentrations of 62.5, 125 and 250 µg/mL of derivatives in DMSO. For antibacterial activity: moderate activity against G-negative “Escherichia coli, and Klebsiella pneumoniae” appeared at concentrations (62.5 and 125 µg/mL) and 250 µg/mL for compounds (H1) and (H2), respectively. Compounds (H1andH3) were moderately active against only E. coli at concentrations (62.5, 125 and 250 µg/mL) and 250 µg/mL, respectively. However, all of them exhibited no activity against Gram-positive “Staph. aureus, and Bacillus subtilis”.

The zero mm inhibition zone at concentration 250µg/mL while containing inhibitory zone at lower zones at concentrations 62.5 and 125 µg/mL for compound (H2) may be due to at high antimicrobial concentration a small number of bacterial resistant mutants can provide the protection to others by producing signaling molecule to turn on the drug efflux pumps which are transport proteins involved in the extrusion of toxic and antibiotic substrates from within cells to the external environment of bacteria, enhancing the survival capacity of the overall population. (23)

On the other hand, the antifungal activity against Candida tropicalis and Candida albicans showed that compound (H3) has only moderate activity against Candida albicans at a concentration of 62.5 µg/mL. Compounds (H1andH3) were only moderately active against candida tropicalis at concentrations 62.5 and 125µg/mL. Finally, the best activity among all the derivatives was obtained from compound (H1) which proved high activity against Candida tropicalis at concentrations (125, 250 µg/mL).

Conclusion

New Schiff bases derivatives containing phthalimide core were successfully synthesized and their structures were characterized by “IR and 1HNMR spectral” methods. All the synthesized compounds showed no activity at all against Gram-positive bacteria, for Gram-negative bacteria and fungi they showed moderate or no activity except compound (H1) revealed high antifungal activity against Candida tropicalisat concentrations 125 and 250 µg/mL.

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References

1. Tsemeugne J, Fondjo ES, Tamokou J, Rohand T, et al. Activity of a Novel Trisazo Dye from 3-Amino-4H-thieno [ 3, 4-c ][ 1 ] benzopyran-4-one. Int J Med Chem. 2018;2(18):1–8.
2. Bach D, Liu J, Kyung W, et al. Bioorganic & Medicinal Chemistry Synthesis and biological activity of new phthalimides as potential anti-inflammatory agents. Bioorg Med Chem [Internet]. 2017;25(13):3396–3405. Available from: http:// dx.doi.org /10.1016/j.bmc.2017.04.027
3. Lima LM, Castro P, Machado AL, et al. Synthesis and Anti-Inflammatory Activity of Phthalimide Derivatives, Designed as New Thalidomide Analogues. Bioorg Med Chem. 2002;10(9):3067–3073.
4. Jawed HAM, Mohammed MH, Ismeal SH, et al. Synthesis, Characterization and Alpha Glucosidase Inhibition Activity of New Phthalimide Derivatives. Iraqi J Pharm Sci. 2018;27(1):100–108.
5. Nayab PS. New phthalimide - appended Schiff bases : Studies of DNA binding, molecular docking, and antioxidant activities. Luminescence. 2016;32(5):1–10.
6. Abdel-Hafez AA. Synthesis and Anticonvulsant Evaluation of N-Substituted-Isoindolinedione Derivatives. Arch Pharm Res. 2014;27(5):495–501.
7. Vajragupta O, Boonchoong P. New Leads of HIV Integrase Inhibitors New Leads of HIV-1 Integrase Inhibitors. Mahidol Univ J Pharm Sci. 2016;29(3–4):1–10.
8. Behalo MS. Facile Synthesis of Novel Amino Acids Derivatives as Potential Antibacterial Agents using Sustainable Materials. J Chinese Chem Soc. 2017;64(10):1–9.
9. Al-azzawi AM, Shawqi M, Razzak A. Synthesis, Characterization and antibacterial activity of Several New Schiff Bases Linked to Phthalimide Moiety. Kerbala J Pharm Sci Number. 2011;2:124–133.
10. Maheswara CUMA, Jayakar RB, Srinivasan R. International Journal Of Pharma And Bio Sciences Synthesis And Antimicrobial Activity Of A N-Phthalimido And Acetimid Derivatives From Amino Acids And Anhydrides. Int J Pharma Bio Sci. 2010;1(4):81–86.
11. Lamie PF, Philoppe JN, El-gendy AO, et al. Design, Synthesis and Evaluation of Novel Phthalimide Derivatives as in Vitro Anti-Microbial, Anti-Oxidant and Anti-Inflammatory Agents. Molecules. 2015;20(9):16620–16642.
12. Bosquesi PL, Regina T, Melo F, et al. Anti-Inflammatory Drug Design Using a Molecular Hybridization Approach. Pharmaceuticals. 2011;4(11):1450–1474.
13. Muhi-eldeen ZA, Al-kaissi E, Suaifan GARY, et al. Synthesis And Biological Screening of Aminoacylenic Tetrahydrophthalimide Analogues As Novel Cyclooxygenase. Indian J Adv Chem Sci. 2016;4(1):40–48.

14. Kajal A, Bala S, Kamboj S, Sharma N, Saini V. Schiff Bases: A Versatile Pharmacophore. J Catal. 2013;2(13):1–14.

15. Al-garawi ZSM, Tomi IHR, Al-daraji ALIHR. Synthesis and Characterization of New Amino Acid-Schiff Bases and Studies their Effects on the Activity of ACP, PAP and NPA Enzymes (In Vitro). J Chem. 2012;9(2):962–269.

16. Zhang YAN, Wang X, Ding L. Interaction between tryptophan-vanillin Schiff base and herring sperm DNA. J Serb Chem Soc. 2010;75(9):1191–1201.

17. Sah SK, Rasool U, Shanthi B, et al. Antibacterial Potential Of L-Tryptophan On Extended-Spectrum Beta-Lactamase Producing Escherichia Coli Strains: A Pathological Promising Approach. Asian J Pharm Clin Res. 2018;11(7):368-374.

18. Al-masoudi NA, Abood E, Al-maliki ZT, et al. Amino acid derivatives. Part 6. Synthesis, in vitro antiviral activity and molecular docking study of new N-α-amino acid derivatives conjugated spacer phthalimide backbone. Med Chem Res. 2016;25(11):2578–2588.

19. Tajudeen SS, Kannappan G. Indian Journal of Advances in Chemical Science Schiff Base – Copper (II) Complexes: Synthesis, Spectral Studies and Anti-tubercular and Antimicrobial Activity. Indian J Adv Chem Sci. 2016;4(1):40–48.

20. Yong JN, Mainsah EN, Ntum SJ, et al. Synthesis, Characterization and Antibacterial Studies of Some Isoniazid-derived Schiff Bases. International Research Journal of Pure and Applied Chemistry. 2016; 12(1): 1-8.

21. Lima LM, Castro P, Machado AL, et al. Synthesis and Anti-Inflammatory Activity of Phthalimide Derivatives, Designed as New Thalidomide Analogues. Bioorg Med Chem. 2002;10:3067–3073.

22. Albert J, Crespo M, Granell J, Rodríguez J, et al. Cyclopalladation of Schiff bases from methyl esters of α-Amino acids. Unexpected activation of the O-Me bond with formation of a bianionic tridentate metallacycle. Organometallics. 2010;29(1):214–225.

23. Yorur-goreci C, Demir Z, Altas N. Green Synthesis of New Amino Acid Schiff Bases and Their Biological Activities. Turkish Chem Soc. 2016;3(3):15–26.

24. Dabholkar V V, Gavande RP. Synthesis and antimicrobial activities of novel 1, 4-benzothiazine derivatives. Arab J Chem [Internet]. 2016;9(S1):S225–S229. Available from: http://dx.doi.org/10.1016/j.arabjc.2011.03.009.

25. Li J, Xie S, Ahmed S, et al. Antimicrobial activity and resistance: influencing factors. Front. Pharmacol. 2017; 13(8):1-11.