Schiff Bases Derived from 2-Hydroxy and 2-Methoxy Naphthaldehyde: Exploration of In Silico Docking, DNA Cleavage, Antibacterial Activities and SAR

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Abstract

Antibacterial activities, molecular docking and DNA cleavage activities of five structurally related Schiff bases were performed and their structure activity relationship was studied. Antibacterial activities of these compounds against gram-negative and gram-positive bacteria were evaluated by using well diffusion method and the molecule 1-[E]-[4-hydroxyphenyl]iminomethyl]naphthalene-2-ol which carries two hydroxyl functional groups was observed as one of the most dynamic antibacterial agents. The antibacterial activities of this molecule was compared with an earlier research work and concluded that the antimicrobial activities of these Schiff base analogues can be improved by introducing OH groups in their core structure. Docking interactions were investigated against p55bl kinase protein. The compound 1-[E]-[3-nitrophenyl]iminomethyl]naphthalene-2-ol exhibited good docking proficieny with 24 interactions based on statistical potentials and the compound (E)-1-(2-methoxy-1-naphthyl)-N-(3-nitro phenyl)methanimine showed significant docking interaction based on hydrogen bonding. DNA cleavage efficiency of all the Schiff bases was investigated using Lambda DNA by gel electrophoresis method.

Keywords: Schiff base; Antibacterial properties; Docking studies; DNA cleavage

Introduction

The search for new antibacterial agents against pathogenic microorganisms those developed resistance to the existing antibiotic drugs has become an essential area of current medicinal chemistry research. Increasing number of immuno-compromised patients due to chemotherapy, HIV infection and other multi resistant bacterial infections made the research in this field very important [1,2]. Kinase proteins are perceptible targets for anticancer drug design as they play main role in oncogenesis. US Food and Drug Administration has approved 11 kinase inhibitors and several researches are going on to develop specific small molecule inhibitors as host of different kinases which are affianced in cancer and other diseases [3]. Schiff bases are considered as the ‘privileged’ ligands in the field of medicinal chemistry and drug research as they are easy to synthesis and have broad spectrum of biological activities like Anti-inflammatory, Analgesic, Antimicrobial, Anticonvulsant, Anti-tubercular, Anticancer, Antioxidant, Anthelmintic and so forth. The nitrogen atom of the imine functional group of the Schiff base may be involved in the formation of a hydrogen bond with the active centres of cell constituents and interferes in normal cell processes. Succinctly, they are one of important class of compounds having therapeutic potential for the treatment of various diseases. Schiff bases which can bind or cleave DNA are now in great consideration due to their importance in the development of anticancer and antimicrobial agents [1,4-6]. Schiff bases are characterized by imine group –N=CH-, and they explicate the mechanism of transamination and racemization reactions in biological system [7]. Metal complexes of Schiff bases have been widely investigated due to their antitumor and heribical uses [8]. Naphthalene Schiff bases with ortho-hydroxy substituent possess interesting structural characterictic like radiation induced or temperature induced reversible colour changes commonly known as photo chromism and thermo chromism respectively [9]. Similarly, inductive effects produced due to other substituents like methoxy or nitro functional groups play vital role on their biological activity. There are considerable researches devoted to the structural investigations of Schiff bases derived from naphthaldehyde, rather not as much of attention has been paid to investigate their biological activities. Hence our current research is intended to focus on the biological significance of Schiff bases derived from substituted naphthaldehyde. As a continuation of our interest, Schiff bases derived from naphthaldehyde were synthesised. The functional groups were deliberately planned to afford hydrogen bond donor and acceptor sites to ensure stronger interactions with biological receptors. Anti bacterial activity, protein docking interactions and DNA cleavage activities of these Schiff bases were contemplated and their SAR was studied.

Materials and Methods

All the reagents required for the synthesis of Schiff bases, DNA cleavage experiment as well as anti bacterial studies were brought from Aldrich Chemicals, Bangalore, India and were chemically pure. All the solvents were freshly distilled before use.

Experimental

General Procedure for the synthesis of Schiff bases: Aldehyde (1 mmol) was dissolved by stirring in hot ethanol (10 mL). Aniline derivative (1 mmol) was added slowly to the solution of aldehyde. This mixture was refluxed for 3-4 hrs. The resulting precipitates were filtered, washed with cold ethanol and dried over vacuum to give orange to yellow coloured products. Five Schiff bases compounds synthesised will be herein after referred to as L3(a) to L3(e) as follows.

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Docking studies

Docking interactions of L3(a) to L3(c) with p53bkl kinase protein were investigated by using the software PATCHDOCK and PYMOL. The ligand structures (2D) were sketched using CHEMKETCH software and converted to 3D and further refined and optimized Using CHIMERA. Finally, pdb file was generated for docking studies. The essential the rotatable bonds are assigned to allow flexibility in the ligand. In this study all the rotamers were allowed. The pdb file of the protein (The three-dimensional solution structure of the SH2 domain from p53bkl kinase) was downloaded from the database www.rcsb.org/ pdb (pdb id is 1BLJ). Docking of the ligands into receptor was carried out using BETA (1.3 version) set of programs.

Anti bacterial activities

Antibacterial activity of the synthesized compounds was tested against four bacterial strains using agar well diffusion method [10]. Muller Hilton Agar media was used to inoculate the bacteria culture. The test compounds were prepared in DMSO (10 mg/ml). These samples were loaded directly in to 5 different wells in the media, kept in incubator for 17 hours at 300°C and zone of inhibition was observed as diameter. All determinations were done in triplicates. Tetracycline and Gentamycin were used as the standards (0.03 mg/ml), where the zone of Inhibition was 1.1 cm and 0.8 cm respectively. The minimum inhibitory concentration (MIC) was obtained by serial broth-dilution method [11] at different concentrations such as 20, 40, 50, 60 and 80 µl. The results of MIC values of antimicrobial activity are given in Tables 1-3.

DNA cleavage studies

DNA cleavage experiment was conducted using Lambda DNA by gel electrophoresis in presence of H2O2 as oxidant. The reaction mixture was incubated at 35°C for 2 hrs as follows: DNA 20 µM, Schiff base 40 µM, H2O2 40 µM in Tris HCl buffer (PH=7.2). After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using Tetracycline and Ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the experiments were performed at room temperature.

Results and Discussion

Docking studies

Changes within the core structure of a ligand can alter the strength and quality of its interactions with any biological receptor. The first Kinase inhibitor reached in clinic was Gefitinib and it contains a quinazoline fused ring system as well as substituted anilines in their structure. Bosutinib and Lapatinib are also kinase protein inhibitors which restrain aniline ring in their composition [12]. Similarly, a large numbers of naphthalene derivatives are also proved as biologically active [13-15]. Research works published by Kim on a series of molecules containing naphthalene rings are proved as potent and selective JNK3 inhibitor in cell, enormously reducing phosphorylation [16]. Considering these factors, we have introduced ortho substituted naphthyl ring as well as substituted aniline in the structure of each Schiff bases to analyse the importance of these groups in attaining suitable ‘pose’ for inhibiting blk Kinase. Functional groups in these moieties are designed in such a way that molecules should contain an activating group (–OH,–OCH3) and a deactivating group (–NO2) to evaluate the consequences of these groups in different positions. In L3(b), as the nitro group is at the para position of amine precursor; it is expected to have more control on the basicity of the imine nitrogen [17]. Where as in L3(a) and L3(d), this nitro group is at the meta position which have comparatively lesser effect on the imine nitrogen. Similarly, ortho hydroxyl group on the naphthalene ring is expected to act as hydrogen bond donor, and methoxy group as hydrogen bond acceptor [18]. The synthesised compounds L3(a) to L3(e) were tested for their docking, antibacterial as well as DNA cleavage predilection.

Table 1: Docking Interactions of L3(a) with different amino acid residues.

| Total score | Interacting amino acid | Interactions | Bonding |
|-------------|------------------------|--------------|---------|
| -4.334 kcal/mol | ARG21 | 12 | Knowledge-based |
| | HIS66 | 11 | Knowledge-based |
| | LYS68 | 1 | Knowledge-based |

Docking Interactions of L3(a) with different amino acid residues.

Table 2: Docking Interactions of L3(d) with amino acid residues.

| Score | Interacting amino acid | Interactions | Bonding |
|-------|------------------------|--------------|---------|
| -4.334 kcal/mol | LEU52 | 2 | H-OH |

Antibacterial studies

Antibacterial activity of the Schiff bases L3(a) to L3(e) were investigated with E. coli, Pseudomonas aerogenase, Staphylococcus aureus and Bacillus cereus. The antibacterial screening reveals that the compounds L3(c), L3(d) and L3(e) has varying degree of activity against tested microorganisms. The compound L3(c) which carries two...
OH functional groups (Figure 2) was observed as the most successful among all the Schiff bases and was active against all the tested bacterial strains (Tables 3 and 4). This result supports the findings done by Hisaindee whose research demonstrate the broad-spectrum inhibitory activity of Schiff base compounds containing hydroxyl group against microorganisms [13]. Hence it is worth to compare the observation made by Soleiman; that a structurally similar Schiff base derived from 2-hydroxy naphthaldehyde (compound number 10) was inactive against E. coli [13]. But we observed an improved activity by adding a hydroxyl group at the para position of aniline precursor. Similarly, it is interesting to note that the compounds L3(c) and L3(e) which are derived from 2- methoxy naphthaldehyde are found to be active against E. coli [13]. Hence it is worth to compare the observation made by Soleiman; that a structurally similar Schiff base derived from 2-hydroxy naphthaldehyde (compound number 10) was inactive against E. coli [13]. But we observed an improved activity by adding a hydroxyl group at the para position of aniline precursor. Similarly, it is interesting to note that the compounds L3(d) and L3(e) which are derived from 2- methoxy naphthaldehyde are found to be active against

| Compound | Bacteria | Concentration | Zone of inhibition(cm) |
|----------|----------|---------------|------------------------|
| L3(c)    | Bacillus cereus | 80 µl sample  | 1.2                    |
| L3(c)    | E. coli   | 20 µl sample  | 1.2                    |
| L3(c)    | E. coli   | 40 µl sample  | 1.2                    |
| L3(c)    | E. coli   | 60 µl sample  | 1.3                    |
| L3(c)    | E. coli   | 80 µl sample  | 1.0                    |
| L3(c)    | E. coli   | 50 µl sample(DMSO) | 3.5                |
| L3(c)    | Pseudomonas aeruginosa (G-) | 20 µl sample  | 1.2                    |
| L3(c)    | Pseudomonas | 40 µl sample  | 1.2                    |
| L3(c)    | Pseudomonas | 60 µl sample  | 1.3                    |
| L3(c)    | Pseudomonas | 80 µl sample  | 1.0                    |
| L3(c)    | Pseudomonas | 50 µl sample(DMSO) | 3.5                |
| L3(d)    | Bacillus cereus | 20 µl sample  | 1.4                    |
| L3(d)    | Bacillus cereus | 40 µl sample  | 1.2                    |
| L3(d)    | Bacillus cereus | 60 µl sample  | 1.0                    |
| L3(d)    | Bacillus cereus | 80 µl sample  | 1.0                    |
| L3(d)    | Bacillus cereus | 50 µl sample(DMSO) | 0.0                |
| L3(e)    | Staphylococcus aureus | 20 µl sample  | 0.0                    |
| L3(e)    | Staphylococcus | 40 µl sample  | 0.0                    |
| L3(e)    | Staphylococcus | 60 µl sample  | 0.4                    |
| L3(e)    | Staphylococcus | 80 µl sample  | 0.6                    |
| L3(e)    | Staphylococcus | 50 µl sample(DMSO) | 0.0                |

Table 3: Antibacterial activity of L3(c), L3(d) and L3(e).

OH functional groups (Figure 2) was observed as the most successful among all the Schiff bases and was active against all the tested bacterial strains (Tables 3 and 4). This result supports the findings done by Hisaindee whose research demonstrate the broad-spectrum inhibitory activity of Schiff base compounds containing hydroxyl group against microorganisms [13]. Hence it is worth to compare the observation made by Soleiman; that a structurally similar Schiff base derived from 2-hydroxy naphthaldehyde (compound number 10) was inactive against E. coli [13]. But we observed an improved activity by adding a hydroxyl group at the para position of aniline precursor. Similarly, it is interesting to note that the compounds L3(d) and L3(e) which are derived from 2- methoxy naphthaldehyde are found to be active against E. coli [13].

Figure 1: Schematic diagram of Schiff bases L3(a) to L3(e).
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Figure 2: X-Ray crystallography structure of non planar molecule L3(d) [12].

Figure 3: X-Ray crystallography structure of L3(d) to exhibit the ‘C-H...N’ interaction [12].

Table 4: Schiff base ligands those which does not show antibacterial activity.

| Compounds | Bacteria                  |
|-----------|---------------------------|
| L₃(a) L3 a| Bacillus cereus           |
| L₃(a) L3 a| Staphylococcus aureus     |
| L₃(a)     | E. coli                   |
| L₃(b)     | Pseudomonas aeruginosa    |
| L₃(b)     | Bacillus cereus           |
| L₃(b)     | Staphylococcus aureus     |
| L₃(b)     | E. coli                   |
| L₃(c)     | Pseudomonas aeruginosa    |
| L₃(d)     | Staphylococcus aureus     |
| L₃(d)     | E. coli                   |
| L₃(d)     | Pseudomonas aeruginosa    |
| L₃(e)     | Bacillus cereus           |
| L₃(e)     | E. coli                   |
| L₃(e)     | Pseudomonas aeruginosa    |

Figure 4: Interactions of L₃(a) with p55blk kinase protein.

Figure 5: Interactions of L₃(d) with p55blk kinase protein.

Figure 6: Tetracycline zone of Inhibition is 1.1 cm and Gentamycin is 0.8 cm.

Figure 7: Antibacterial activities of L₃(c).

Figure 8: Antibacterial activity of L₃(d); (1) and L₃(e); 2.

gram positive bacteria. Compounds L₃(a) and L₃(b) are inactive against all the tested bacterial strains and they carry an electron withdrawing nitro group at the aniline site in common.

DNA cleavage studies

DNA cleavage activity was monitored by gel electrophoresis, using
observed for the Schiff base L 3(a) (lane 2), L 3(b), and L 3(c). The study is the evidence for the oxidative cleavage.

Presence of smear on the electrophoresis gel reveals that L3(a), L3(b) and L3(c) are able to convert super coiled DNA to open circular DNA more efficiently than other Schiff base ligands. The authors are thankful to The Government Science College Bangalore, India for the constant encouragement.

Pseudomonas aeruginosa and E. coli are Gram-negative antibacterial agents against which possess two electron donating groups was observed as superior protein with 24 docking interactions; while the Schiff base ligand L3(c) was found to be an excellent docking agent for p55blk kinase with potential antimicrobial activity. Ambrosio et al. 2021 22-28. Indeed, it has been shown that the structure of the molecule in developing a target specific potential drug molecule.

Antibacterial activity, protein docking and DNA cleavage activity of structurally related Schiff bases were investigated. Schiffbase Ligand L3(a) was found to be an excellent docking agent for P55bk kinase protein with 24 docking interactions; while the Schiff base ligand L3(c) which possess 2 electron donating groups was observed as superior antibacterial agent against E. coli and Pseudomonas aeruginosa with an inhibition zone of 3.5 cm. Thus, the present work reports the role of hydrogen bond donor and acceptor groups, steric hindrance and effects of substituents in the core structure of the molecule in developing a better, target specific potential drug molecule.

a control sample. Result tells that cleavage obtained for L 3(d) (lane 5), and L 3(e) (lane 6) are insignificant. But a considerable cleavage was observed for the Schiff base L 3(d) (lane 2), L 3(b), and L 3(c). The study reveals that L 3(a), L 3(b) and L 3(c) are able to convert super coiled DNA to open circular DNA more efficiently than other Schiff base ligands in this series (Figures 5-9). Presence of smear on the electrophoresis gel is the evidence for the oxidative cleavage.

Conclusion

Antibacterial activity, protein docking and DNA cleavage activity of structurally related Schiff bases were investigated. Schiffbase Ligand L3(a) was found to be an excellent docking agent for P55bk kinase protein with 24 docking interactions; while the Schiff base ligand L3(c) which possess 2 electron donating groups was observed as superior antibacterial agent against E. coli and Pseudomonas aeruginosa with an inhibition zone of 3.5 cm. Thus, the present work reports the role of hydrogen bond donor and acceptor groups, steric hindrance and effects of substituents in the core structure of the molecule in developing a better, target specific potential drug molecule.

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