Section A-Research paper
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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF INDOLINE BASED NOVEL HETEROCYCLIC COMPOUNDS.

Senthilkumar T [1], ChickiyanSivakumar[2]

[1]Centre for Research and Evaluation, Bharathiar University, Coimbatore, Tamilnadu, India-641 046.

[2]JCT College of Engineering and Technology, Pichanur, Coimbatore, Tamilnadu, India-641 105.

Corresponding Author: Chickiyan Sivakumar[2]

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Abstract

Novel Heterocyclic compounds based on Indoline moiety were synthesized and characterized.1'-Methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3"-indoline] 2,2"-dione compound was characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectrum, UV-Visible spectrum. Alsomolecular docking studies of the best compound were analyzed on anti-bacterial evaluation. The1'-Methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3"-indoline]-2,2"-dioneshowed good binding affinity with the protein.Additionally, in vitro antibacterial activity evaluated for all the synthesized compounds and shows good anti-bacterial activity.

Keyword: Nitrophenyl,Indoline, Acenaphthylene, Antibacterial

Introduction

Indole is a benzoprulle fusing in place of 3 and 4 with benzene and pyroleum rings. There are a wide number of natural compounds produced and bacteria can create them. It reacts to a range of physiological features, including the development of spore and biofilms, plasmid stabilisation, medicine protection, virulence, as intercellular signal molecules [9]. The precursor of the neurotransmitter serotonin is amino acid tryptopen, as an indolederivative [10].

Indole is the heterocyclic nucleus, and its extensive biologic function, such as anti-microbial, anti-convective, analgesic, anti-HIV and anti-oxidative, anti-inflammative, is gaining popularity as a medicinal chemist. The research in recent decades focuses on the new heterocyclic molecules and their improvements to decrease their side effects and discover other pharmacological and biological effects. Most anti-inflammatory drugs express their significant effect by limiting lysosomal enzymes release or by making lysosomal membrane intact, one of the key inflammatory events. Lysosomal membranes are preserved by minimizing lysosomal

components produced by activated neutrophils, such as bactericidal enzymes and proteases, inducing tissue inflammation and damage after extracellular release.

Substance Indole has an intense faecal scent in humans that also exists in carbon tar. Indole is a dense substance It has a floral smell in low concentrations and is a part of many floral scentages such as orange flowers and fragrances. Bacteria may also be turned into the amino acid tryptophane by degradation product. The current part is referred to as indolyl. Structural elements (and for certain substances synthetic prerequisites for) tryptophane-derived tryptamine alkaloids including neurotransmitter serotonin and melatonin, are the electrophilic alternative for the indole compounds, often in place 3.Additional indole compounds include plant hormone auxins (indolyl 3 acetic acid, AAI), tryptophol, antiblockerpindolol (ABI) and natural hallucinogen dimethyltryptamine. Other indole compounds include plant hormone auxins. The name Indole was derived as the first isolated compound for the treatment of indigo teeth was an indigenous compound.

With the study of the dye indigo, indole chemistry started to evolve. The Indoline-2-3-dione and then the oxindole can be converted from indigo. Adolf von Baeyer subsequently reduced oxindole to indole with zinc dust in 1866 [11]. In 1869 a formula was suggested for indole [12]. Some indole derivatives were important dyestuffs until the end of the 19th century. In the 1930s, an interest in Indole arose, when a variety of basic alkaloids, tryptophanes, and auxins contained the indolenucleus[13]. Indoline-2-3-dione is the indole derivative of the other name known as 1H-indole-2,3-dion. In 1841 Erdmann and Laurent found this compound. The result is a mixture of nitric acid and chromic acid from oxidation of indigo colouring [14].

Oxindole is an organic compound heterocyclic. It is a bi-cyclic structure that contains a six-part benzene fusioned with a nitrogen-containing ring with a five-part ring. Oxindole means modified indoline in second place on the 5-member indoline ring with carbonil substitution. Indoline-2-3-dione ship bases for their medicinal properties are studied. When Indoline-2-3-dione is applied to the crude benzene and sulfuric acid, a blue dye (indophenin) is observed. Victor Mayer's isolated product was thiophene, the new heterocyclic drug. Indoline-2-3-dione and its derivatives have different pharmacological characteristics and biological characteristics. This is primarily used for a wide variety of heterocyclic compounds as a beginning material for the synthesis.

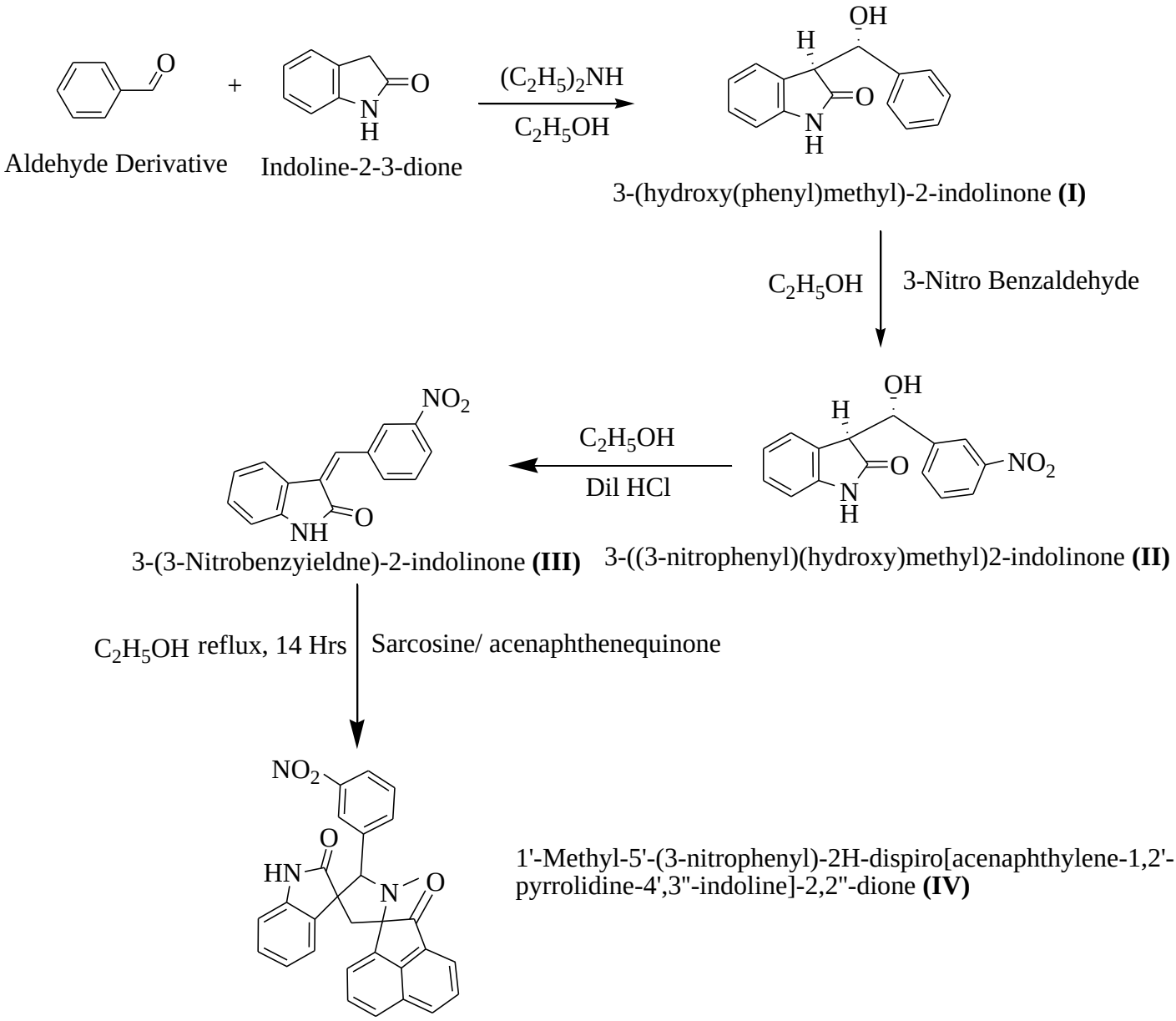
Experimental

Purification of Solvents

The solvents were purified and dried before being used according to the standard procedure [1] and stored over Type 4A molecular sieves. The commercial grade ethanol and methanol were refluxed for six hours with lime and distilled. The middle fraction was collected and used.

Synthesis of Novel Hetero Compounds

The reaction scheme of this study is shown in Scheme 1



Scheme1: Synthesis of1'-Methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3"-indoline]-2,2"-dione

3-(hydroxy(phenyl)methyl)-2-indolinone

A mixture of (0.01 mole of each) oxindole and benzaldehyde in 100 mL of ethanol, added 1mL of diethyl amine. The whole content was left overnight at normal temperature. The yellow colored needleswere produced, and then recrystallized by using absolute ethanol. Yield: 72%. M.P.: 117-119 ºC.IR data (KBr, ν/cm^{-1}): 1690 (CO), 3405 (NH), 3510 (OH).H^{1} NMR (δ/ppm): 4.45 (s, 2H, CH), 6.21 (b, 1H, OH), 7.62 (m, 9H, Ar-H), 10.16 (s, 1H, NH).

3-((3-Nitrophenyl)(hydroxy)methyl)2-indolinone

A mixture of (0.01 mole of each) oxindole and 3-nitro-benzaldehyde in 100 ml of ethanol, added 1mL of diethyl amine. The whole content was left overnight at normal temperature. The yellow colored needleswere produced, and then recrystallized by using absolute ethanol.Yield: 69%.M.p.: 137-138 ºC.IR data (KBr, ν/cm^{-1}): 1700 (CO), 3398 (NH), 3510 (OH).H^{1} NMR (δ/ppm): 4.42 (s, 2H, CH), 6.21 (b, 1H, OH), 7.63 (m, 8H, Ar-H), 10.11 (s, 1H, NH).

3-(3-Nitrobenzyieldne)-2-indolinone

A mixture of compound (II)(0.01 mole), 1:2 ratio of ethanol and 25% of dilute HCl. Orange needles were formed after allowing the mixture to stand overnight. Yield: 71%.M.P.: 159-160 ºC. IR data (KBr, ν/cm^{-1}): 1604 (C=C), 1684 (CO), 3415 (NH). ^{1}H NMR (δ/ppm): 7.67 (s, 1H, CH), 7.82 (m, 8H, Ar-H), 10.79 (s, 1H, NH).

1'-Methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3''-indoline]-2,2''-dione

To (0.01 mole of each) compound (III), acenaphthenequinone and sarcosine in 50 mL of ethanol added 1 mL of diethyl amine. This was refluxed with stirring for 14 hours followed by the addition of (10 ml) acetic acid. The whole reaction was maintained at ice cold temperature, after concentration yielded the precipitate which was subjected to repeat washing with ethanol (cold) and finally recrystallized from glacial acetic acid to give the required compound.Yield: 1.495 g (73%). M.p.: 152-153 °C.IR data (KBr, ν/cm^{-1}): 1622, 1700, 3371.^{1}H NMR (DMSO/400 MHz, δ/ppm): 2.49 (s, 3H), 3.43 (s, 2H), 4.08 (d, 1H), 7.32-8.38 (m, aromatic), 11.46 (br, NH).^{13}C NMR (DMSO/400 MHz, δ/ppm): δ 22.49, 39.97, 40.47, 52.92, 122.13-173.12.Mass: 479.1608.UV Visible (Ethanol): 246, 342 nm.

Physical Measurements

Perkin–Elmer 240 Essential Analyzer was utilized to gather small scale explanatory information. Room temperature attractive vulnerability was measured by Gouy's strategy. FT–IR information were collected by FT–IR ABB Bomen MB 3000 spectrophotometer. The 1H NMR and ^{13}C NMR were recorded on a BrukerAvance (400 MHz). UV–Vis spectra of the complexes

were recorded on UV-160A UV–Vis spectrophotometer, Shimadzu (Japan). Cleavage of pUC19 DNA was measured by AlphaDigiDoc™ RT. Form V.4.0.0.

Molecular Docking

Docking Studies

Molecular docking study was performed, with the aim of evaluating the most preferred geometry of protein-ligand complex. Possible binding modes between the ligands and this target protein were studied by CDOCKER (CHARMm-based DOCKER) protocol incorporated within DS. The algorithm offers full ligand flexibly and employs CHARMm force fields. Ligand binding affinity was calculated using CDOCKER energy, CDOCKER Interaction energy, Hydrogen bonds, binding energies, protein energy and ligand protein complex energy. The CDOCKER energy mentioned in negative values. More negative value energy indicated as higher binding affinity of the ligands with target protein.

ANTIBACTERIAL ASSAY

Test organism

Acceptable Gram positive: *E .coli, Staphylococcus aureus, Salmonella spp.,., Vibrio parahaemolytics, Aeromonas spp., Klebsiella spp., Vibrio spp Proteus Spp Pseudomonas aerogotés, and Bacilus spp*. The major techniques of bacterial analysis were: Gram positive. The Department of Microbiology, Colleges of Christian Medicine, Tamil Nadu, India and IMPTECH, Chandigarh got all of the data and cultures sampled. In Mueller Hinton Broth (3 mL), each vaccine was vaccinated and accompanied by a 24-hour incubator at 37oC. After that the society was diluted.

PREPARATION OF INOCULUMS

Developing cells in Mueller Hinton Broth (Himedia) at 37 °C for 24 hours was used to prepare Bacteria inoculums. The above cell suspensions were dissp;ved with sterile MHB to give initial cell counts of about 10^{-4} CFU/millilitre. Sabouraud dextrose (SDB) yeast grown for 48 hours at 28°C.

DISC DIFFUSION METHOD

20 mL of Mueller Hinton Agar (MHA) acquired from (Hi-media, Mumbai) was used to prepare Petri plates. Antibacterial method was processed by disc-diffusion process. 100 microlitre of suspension having 10^{-8} CFU/mL fungal was used as test culture after becomes famous in the solidified media followed by drying for 10 min. Different concentrations were calculated for anti-hungary behavior tests (500 microgram/disk). Ciprofloxacin (10 micro grams/disks) were used as a positive(+) control disc for a duration of 30 min for a diffusion of 27°C and the surface of the medium, respective solvents were used to prepare negative controls.

RESULT AND DISCUSSION

Spectral characterization of 1'-Methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3''-indoline]-2,2''-dione.The novel Hetro 1'-Methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3''-indoline]-2,2''-dionewas synthesized from the

reaction of oxindole, benzldehyde, and followed by acenaphthenequinone and sarcosine. This compound (IV) received in good Yield (88%). The melting point of the compound is 136-140^{o}C. The IR spectrum of the compound (IV) was confirmed from the peaks corresponding to carbonyl (1719 cm^{-1}) and NH (3245 cm^{-1}) groups and other frequencies.

The ^{1}H NMR spectrum shows further confirmation of the formation of compound (IV). The proton signals obtained for aliphatic (1.91, 2.50 and 4.28 ppm), aromatic (7.23-7.54 ppm) and amine (11.58 ppm) protons. The obtained ^{13}C NMR spectrum of the compound (IV) gives the corresponding carbon signals of the spiro compound. The mass spectrum shows the molecular ion peak at 430.1254. The electronic spectrum shows the bands in the UV region are 245, 338 nm.

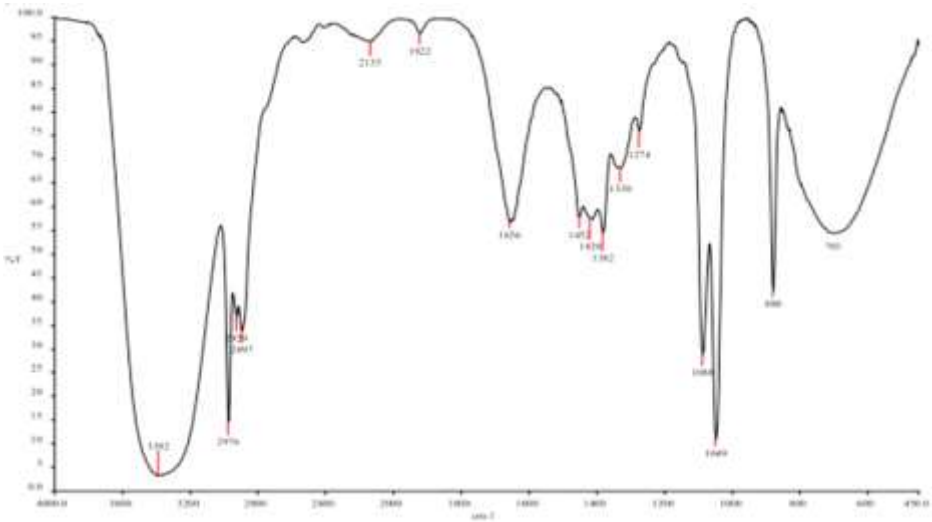


Figure1. IR Spectrum of 1'-Methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3"-indoline]-2,2"-dione.

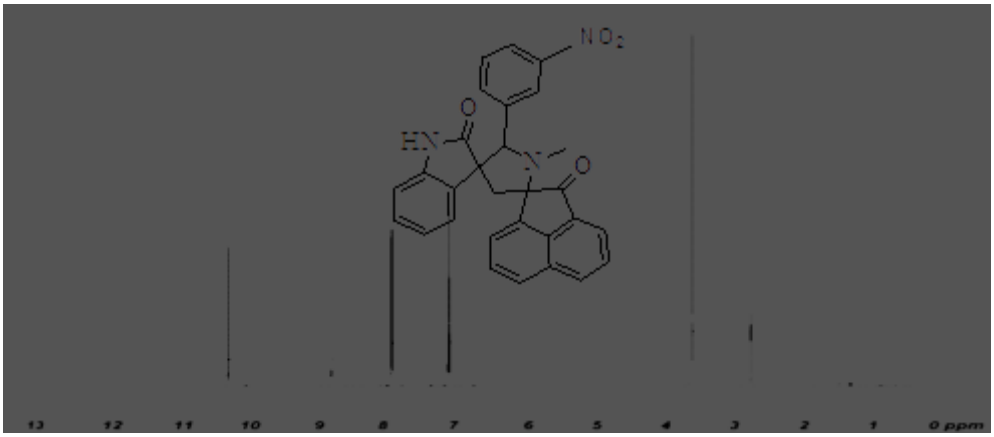


Figure 2.[1]H NMR Spectrum of 1'-methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3"-indoline]-2,2"-dione.

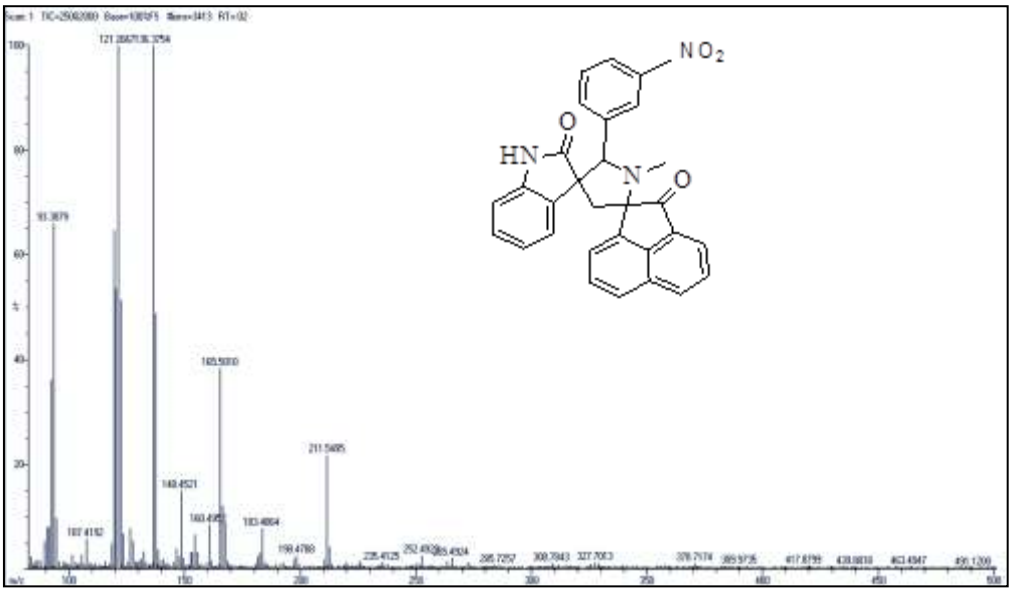


Figure 4. Mass Spectrum of 1'-methyl-5'-(3-nitrophenyl)-2H-dispiro[acenaphthylene-1,2'-pyrrolidine-4',3"-indoline]-2,2"-dione.

Molecular Docking analysis

Molecules A to E were from first group (Indoline-2-3-dionederivatives)and F to J were from second group (oxindole derivatives).Fifth molecule of oxindole derivatives wasfound to bind with stronger affinity than allother molecules with targetproteinaspS(energy -48.89 kcal/mol). The ranking order of molecules based on the binding energy in complex with target protein were observed as following E, H, D, G, A, C, I, F, B and J. As a representative one, the binding interactions between the top ranked molecule and the target proteinwas analyzed.

Totally 16 amino acids were found to fall in the interactions site with that of the ‘molecule E’. Among them three amino acids were found to make hydrogen bond interactions (Gly172, GLN226, and ARG537) with than of molecule E. Further, amino acidsARG217, ARG225, PHE229, GLU482, and ARG537 which were reportedto be present in the active site region wereobserved to be in the interacting region to thatof the chemical ‘molecule E’ (oxiindolederivative); possibly these amino acids would involve in hydrophobic interaction with that of the chemical hit. Sinceadenosine monophosphate (AMP) andaspartyl-adenosine-5'-monophosphate (AMO) were found to be part of the crystalstructure, along with 10 of the research molecules these two were alsodocked as control (Figure 6 and Figure 7).

AMO and proteins complexenergy was calculated to be -50.76 while AMP-protein complex was -38.21. All ofthe ten derivatives docked complex with protein target were found to

havestronger binding affinity (-41.70 to -48.89 kcal/mol) than AMP-protein (-38.21 kcal/mol),though none was foundto be better than the AMO (-50.76 kcal/mol).

However, top twomolecules (5^{th} fromoxindole derivatives and $3^{rd}$from Indoline-2-3-dionederivative) were found to have comparable binding affinity (-48.89 and -48.71 kcal/mol) with tartgetproteinaspS as that of the AMO (-50.76 kcal/mol). Therefore, it may be inferred that these toptwomoleulescould be used as potential leads tofor further improvement and subsequent rationally designof novel drugs against aspartyl-tRNAsynthetasefrom*E. coli*.

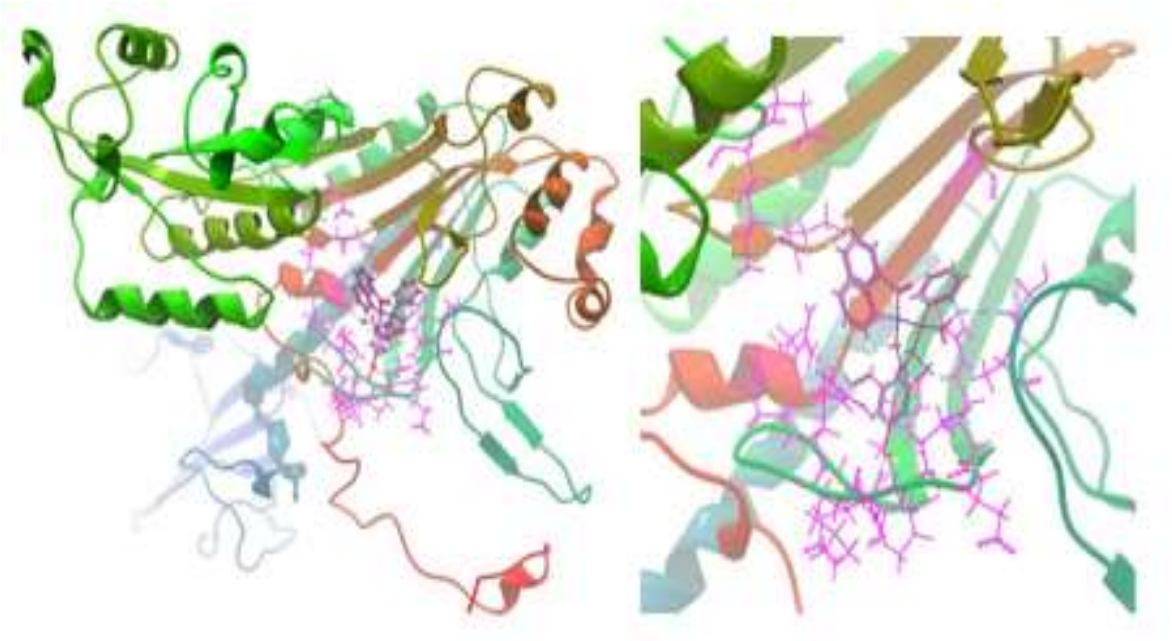


Figure 6.Binding interactions of Aspartyl-tRNAsynthetasesof E. coliandspiro compound. The binding of the 'B' in active site of Aspartyl-tRNAsynthetases

Antibacterial Activity

A number of bacteria and fungi were able to counter antimicrobial behaviors of the synthesized spiro compounds A and F in vitro. The experiments were performed with the tool agar location.

For this study, test cultures of the bacterial strains *Staphylococcus aureus, E .coli, Vibrio spp., Pseudomonas aeroginosa, Aeromonas spp., Klebsiella spp., Salmonella spp., Proteus spp, Vibrio parahaemolytics and Bacillus spp.* were each inoculated into Mueller Hinton broth. Similarly, test cultures of the fungal strains,*AspergillusnigerAspergillus flavors, Pencilliumspp, Trichophyto and Candida albicans. Sabouraud Dextrose Broth* was inoculated.

Check plaques with extracts from fermented cultures were serially detected. Normal antibiotics on the respective plates were also found as positive controls for each culture and for a comparative extract analysis using an antibiotic.

Antimicrobial compounds were isolated at DMSO at 500μg/mL, and each normal antibiotic at DMSO at 10μg/mL. The plates were held at 37 C during the night in an incubator and the inhibition zones are measured at mm.

The literature study revealed that in the spiro compounds mentioned above, compound A showed a characteristic activity against *Candida albicans, Aspergillus flavors, Pencillium spp., Aspergillusniger and Trichophyto spp.* It is interesting to note that numerous indole derivatives gives a large number of biochemical properties. If it is combined to other heterocyclic compounds in between a spiro carbon atom, the result will be greater spectrum of biological activities.

CONCLUSION

To conclude, we had synthesized several spiroheterocycles using 1,3-dipolar addition through the process of heterocyclic compounds like oxindole with it carbonyl compounds such as acetophenone/benzaldehyde and their derivatives with acenaphthenequinone and amino acid (sarcosine). The reactions were found to produce high Yield of novel heterocyclic compounds which were characterized by spectrochemical methods (IR, NMR, UV-Vis and mass spectroscopy). The synthesized ten compounds were subjected to biological activity studies and few compounds were found to be better active. These ten novel compounds exhibit better activity than oxindolespiro compounds, and the results obtained are presented in this thesis.

Molecular docking studies enabled us to propose a possiblemechanism of biological activity of oxindole and Indoline-2-3-dionederivaties.Further research is required to take forward the shortlisted two molecules towards drug designing. In conclusion, the Indoline-2-3-dione and oxindole based compounds display good biological activity than which may helps to develop further biological active derivative compounds.

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