

## Evaluation of Synthesis of Heterocyclic Compounds

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### ABSTRACT

The octahedral ruthenium(II) complexes  $[Ru(Ln)(PPh_3)_2Cl_2]$  [ $Ln =$  biphenyl furanyl pyridine derivatives] were synthesized and characterized using LC-MS, IR spectroscopy, elemental analysis and magnetic measurements. Complexes show enhancement in antibacterial activity compared to free ligands. From the binding mode investigation by absorption titration and viscosity measurement, it is observed that complexes bind to DNA via intercalation and also complexes promote the cleavage of supercoiled pUC19 plasmid DNA. Cytotoxicity analysis shows 100% mortality of Brine shrimp after 48 h.

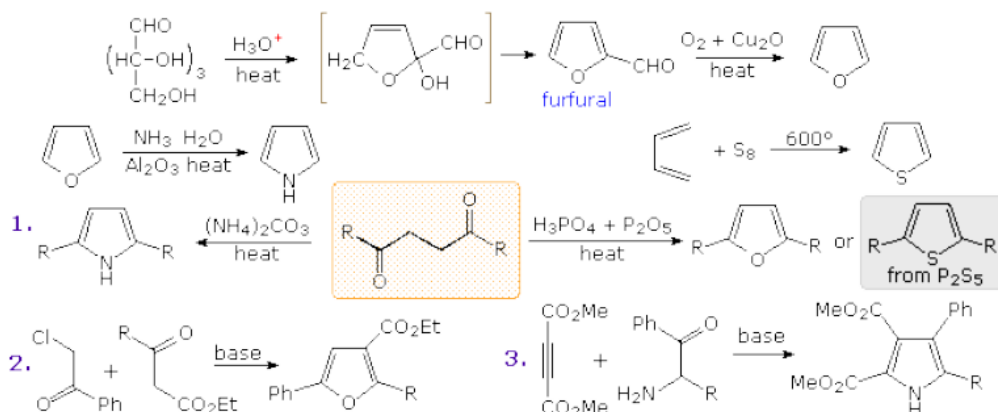
### 1. Introduction

Recently, metal complexes as DNA interacting agents have been talk in medicinal inorganic chemistry<sup>1-6</sup>. DNA intrastrand cross-linking agents are found useful in treatment of cancers, psoriasis and various anemias. Several ruthenium complexes have developed as an alternative to cis-platin as potential anticancer agents with lower toxicity than the platinum counterparts<sup>7-10</sup>. Large interest has been drawn towards the interaction of Ru(II) complexes containing planar polycyclic hetero aromatic ligands<sup>11</sup>. Studies on DNA-binding of the complexes are crucial in development of nucleic acid interaction, chemotherapy and photodynamic treatment<sup>12-14</sup>. Recently, ruthenium(II) polypyridyl complexes have been found to induce apoptosis in A549 cells<sup>15</sup>. Also, the main ligands of Ru(II)-polypyridyl complexes possess extended conjugated planar aromatic structures can insert and stack between the base pairs of DNA. Considerable effects are observed in the binding mode, sites and affinities upon slight changes in the molecular structures of Ru(II) complexes which provide the opportunity to discover important information on site-specific DNA probes. Therefore, studies on modifying the main ligand is quite significant for understanding the optical

properties of DNA-binding and action mechanism of ruthenium complexes.

### Preparation and Reactions of Heterocyclic:

Commercial preparation of furan proceeds by way of the aldehyde, furfural, which in turn is generated from pentose containing raw materials like corncobs, as shown in the uppermost equation below. Similar preparations of pyrrole and thiophene are depicted in the second row equations. Equation 1 in the third row illustrates a general preparation of substituted furans, pyrroles and thiophenes from 1,4-dicarbonyl compounds, known as the Paal-Knorr synthesis. Many other procedures leading to substituted heterocycles of this kind have been devised. Two of these are shown in reactions 2 and 3. Furan is reduced to tetrahydrofuran by palladium-catalyzed hydrogenation. This cyclic ether is not only a valuable solvent, but it is readily converted to 1,4-dihalobutanes or 4-haloalkylsulfonates, which may be used to prepare pyrrolidine and thiolane. Dipolar cycloaddition reactions often lead to more complex five-membered heterocycles.

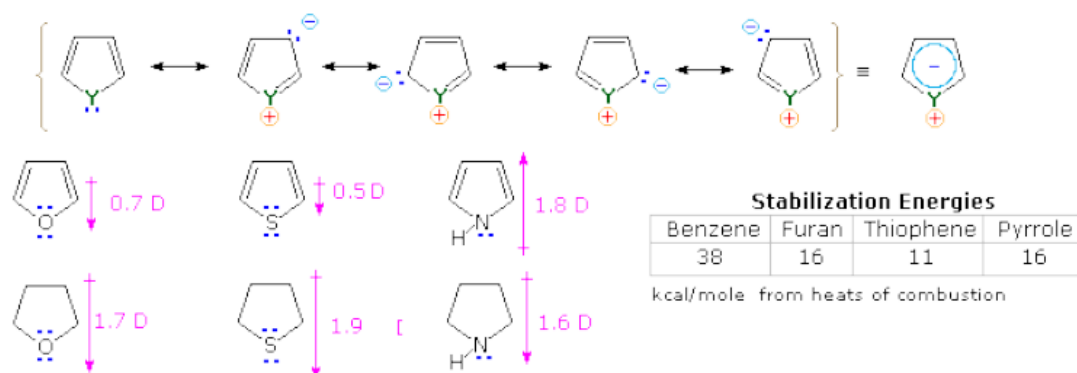


Indole is probably the most important fused ring heterocycle in this class. By clicking on the above diagram three examples of indole synthesis will be displayed. The first proceeds by an electrophilic substitution of a nitrogen-

activated benzene ring. The second presumably takes place by formation of a dianionic species in which the  $ArCH_2(-)$  unit bonds to the deactivated carbonyl group. Finally, the Fischer indole synthesis is a remarkable sequence of tautomerism,

sigmatropic rearrangement, nucleophilic addition, and elimination reactions occurring subsequent to phenylhydrazone formation. This interesting transformation involves the oxidation of two carbon atoms and the reduction of one carbon and both nitrogen atoms.

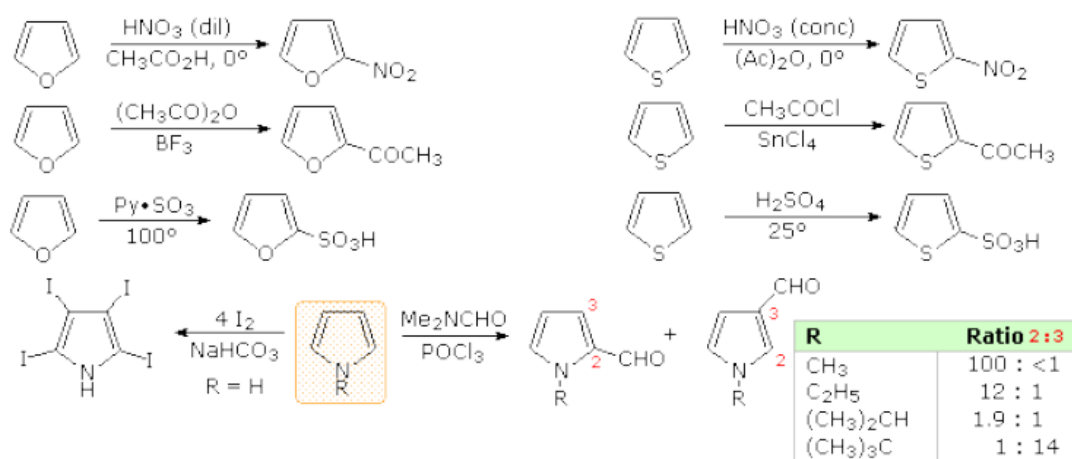
**Reactions:** The chemical reactivity of the saturated members of this class of heterocycles: tetrahydrofuran, thiolane and pyrrolidine, resemble that of acyclic ethers, sulfides, and 2°amines, and will not be described here. 1,3-Dioxolanes and dithiolanes are cyclic acetals and thioacetals. These units are commonly used as protective groups for aldehydes and ketones, and may be hydrolyzed by the action of aqueous acid. It is the "aromatic" unsaturated compounds, furan, thiophene and pyrrole that require our attention. In each case the heteroatom has at least one pair of non-bonding electrons that may combine with the four  $\pi$ -electrons of the double bonds to produce an annulene having an aromatic sextet of electrons. This is illustrated by the resonance



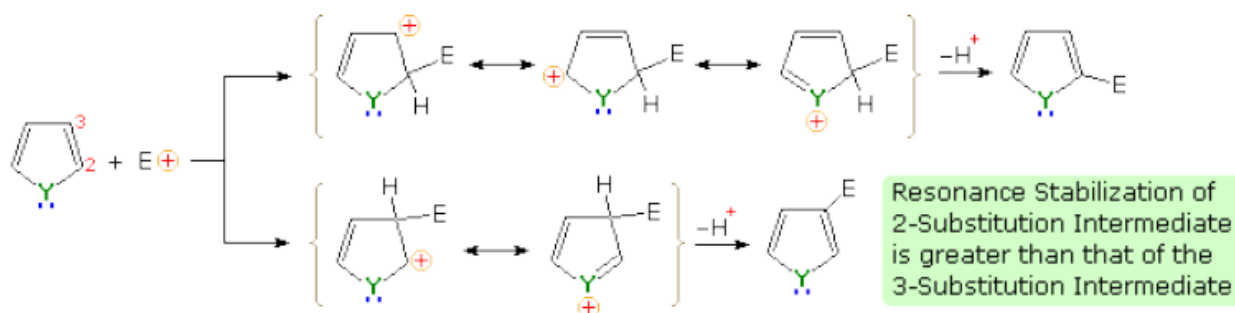
Another characteristic of aromatic systems, of particular importance to chemists, is their pattern of reactivity with electrophilic reagents. Whereas simple cycloalkenes generally give addition reactions, aromatic compounds tend to react by substitution. As noted for benzene and its derivatives, these substitutions take place by an initial electrophile addition, followed by a proton loss from the "onium" intermediate to regenerate the aromatic ring. The aromatic five-membered heterocycles all undergo electrophilic substitution, with a general reactivity order: pyrrole >> furan > thiophene >

description at the top of the following diagram. The heteroatom Y becomes  $sp^2$ -hybridized and acquires a positive charge as its electron pair is delocalized around the ring. An easily observed consequence of this delocalization is a change in dipole moment compared with the analogous saturated heterocycles, which all have strong dipoles with the heteroatom at the negative end. As expected, the aromatic heterocycles have much smaller dipole moments, or in the case of pyrrole a large dipole in the opposite direction. An important characteristic of aromaticity is enhanced thermodynamic stability, and this is usually demonstrated by relative heats of hydrogenation or heats of combustion measurements. By this standard, the three aromatic heterocycles under examination are stabilized, but to a lesser degree than benzene. Additional evidence for the aromatic character of pyrrole is found in its exceptionally weak basicity ( $pK_a$  ca. 0) and strong acidity ( $pK_a = 15$ ) for a 2°-amine. The corresponding values for the saturated amine pyrrolidine are: basicity 11.2 and acidity 32.

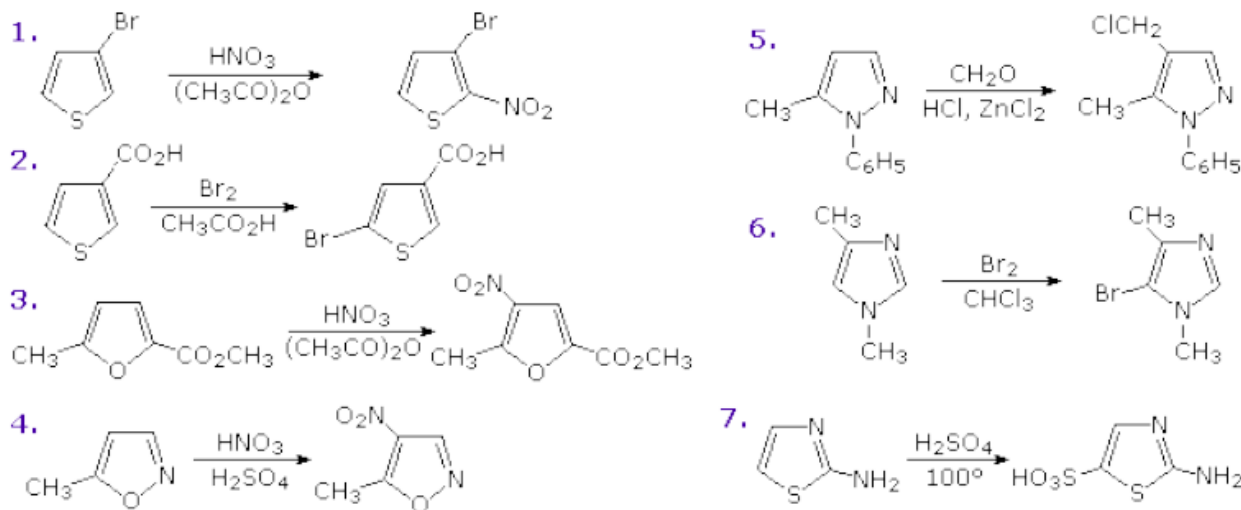
benzene. Some examples are given in the following diagram. The reaction conditions show clearly the greater reactivity of furan compared with thiophene. All these aromatic heterocycles react vigorously with chlorine and bromine, often forming polyhalogenated products together with polymers. The exceptional reactivity of pyrrole is evidenced by its reaction with iodine (bottom left equation), and formation of 2-acetylpyrrole by simply warming it with acetic anhydride (no catalyst).



There is a clear preference for substitution at the 2-position ( $\alpha$ ) of the ring, especially for furan and thiophene. Reactions of pyrrole require careful evaluation, since N-protonation destroys its aromatic character. Indeed, N-substitution of this 2°-amine is often carried out prior to subsequent reactions. For example, pyrrole reacts with acetic anhydride or acetyl chloride and triethyl amine to give N-acetylpyrrole. Consequently, the regioselectivity of pyrrole substitution is variable, as noted by the bottom right equation.



Functional substituents influence the substitution reactions of these heterocycles in much the same fashion as they do for benzene. Indeed, once one understands the ortho-para and meta-directing character of these substituents, their directing influence on heterocyclic ring substitution is not difficult to predict. The following diagram shows seven such reactions. Reactions 1 & 2 are 3-substituted thiophenes, the first by an electron donating substituent and the second by an



Other possible reactions are suggested by the structural features of these heterocycles. For example, furan could be considered an enol ether and pyrrole an enamine. Such functions are known to undergo acid-catalyzed hydrolysis to carbonyl compounds and alcohols or amines. Since these compounds are also heteroatom substituted dienes, we might anticipate Diels-Alder cycloaddition reactions with appropriate dienophiles. These possibilities will be illustrated above by clicking on the diagram. As noted in the upper example, furans may indeed be hydrolyzed to 1,4-dicarbonyl compounds, but pyrroles and thiophenes behave differently. The second two examples, shown in the middle, demonstrate typical reactions of furan and pyrrole with the strong

An explanation for the general  $\alpha$ -selectivity of these substitution reactions is apparent from the mechanism outlined below. The intermediate formed by electrophile attack at C-2 is stabilized by charge delocalization to a greater degree than the intermediate from C-3 attack. From the Hammond postulate we may then infer that the activation energy for substitution at the former position is less than the latter substitution.

electron withdrawing group. The third reaction has two substituents of different types in the 2 and 5-positions. Finally, examples 4 through 7 illustrate reactions of 1,2- and 1,3-oxazole, thiazole and diazole. Note that the basicity of the  $sp^2$ -hybridized nitrogen in the diazoles is over a million times greater than that of the apparent  $sp^3$ -hybridized nitrogen, the electron pair of which is part of the aromatic electron sextet.

dienophile maleic anhydride. The former participates in a cycloaddition reaction; however, the pyrrole simply undergoes electrophilic substitution at C-2. Thiophene does not easily react with this dienophile.

#### Broth dilution method - an in vitro antibacterial study:

The MIC informs about the degree of resistance of certain bacterial species towards the test compounds. MIC was performed by serially two fold dilution of the test compound added to two Gram(+ve) (*Bacillus subtilis* and *Serratiamarcescens*) and three Gram(-ve) (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and incubated at optimum temperature for 24 h. If after 24 h

bacterial growth is observed double of the previously added concentration was taken for the test compounds in tube and incubated for 24 h again. A concentration at which no visible growth of bacteria is observed in the tubes is regarded as minimum inhibitory concentration (MIC) at which bacteria cannot defend against the test compounds.

**DNA binding mode study** Binding mode of complex on interacting to DNA can be investigated by the alteration occurred in the absorption spectral titration. This titrimetric analysis was performed by varying the concentration of the HS–DNA with the addition of 100  $\mu$ L of its aliquots in each successive addition in reference cell and sample cell, keeping the complex concentration constant, with the due correction for the absorbance of HS–DNA alone. Spectra were recorded after 10 minutes of incubation. Further the confirmation of binding mode was done from the viscosity measurement technique. An increase in viscosity of native DNA upon binding of complexes is regarded as diagnostic feature of an intercalation process. The binding mode of present compounds was investigated by taking the HS–DNA solution of 200  $\mu$ M in Ubbelohde viscometer maintained in constant thermometric bath. The time ( $t_0$ ) for DNA alone and ( $t$ ) for successive addition of the complex were monitored.

**BSLA- In vitro cytotoxicity assay** All the complexes were screened for in vitro toxicity using the protocol of Mayer et al., as an initial step for the discovering new drug. This method allows the use of fewer amounts of test compounds and permits a large number of samples and dilutions within a shorter time for analysis. From these stock solutions of 1000  $\mu$ g mL<sup>-1</sup>; a set of 2, 4, 8, 12, 16 and 20  $\mu$ g mL<sup>-1</sup> was prepared. Three such replicates for each test compounds along with the control containing DMSO alone were prepared. After 24 h the numbers of survivals were counted. A graph of log of concentration of samples was plotted against percentage of

mortality of nauplii. The LC<sub>50</sub> value is the antilogarithm of [complex] corresponding to the 50 % mortality.

## 2. Conclusion

The result of various physicochemical techniques matches well with the theoretically proposed structure of complexes. C, H and N elemental analysis of the complexes matches well with the theoretically proposed structure. Also the result of TGA shows that the decomposition of complex taking place in various steps, supports the percentage data of ligands and PPh<sub>3</sub>, indicating the coordination of these ligands to the central metal atom. The different fragment peaks observed in LC-MS spectra supports the proof for the theoretically proposed structure of the complexes. MIC of Ru(II) complexes are compared with their respective ligands and it has been observed that the MIC values of complexes are found higher than the corresponding ligands, which may be due to the chelate effect causing increase in the lipophilicity of complexes compared to ligands and hence complexes can easily penetrate the lipid layer of bacterial cell wall and inhibit the bacterial growth. Complex-I binds more efficiently to the DNA than rest of 16 others and result of viscosity measurement reveals that complexes bind to DNA via classical intercalation mode. From the cytotoxicity study, it is found that LC<sub>50</sub> values of the complexes (10.93-16.78  $\mu$ g/mL) are lower as compared to reference standard potassium dichromate (32  $\mu$ g/mL) suggesting that complexes are significantly toxic at low concentration as compared to reference compound and their lower dose can inhibit the 50 % of population of test species as compared to the higher dose required for the potassium dichromate. The DNA cleavage study of pUC19 shows that all the complexes shows higher % cleavage data (61.09 - 88.26 %) of supercoiled form of pUC19 DNA compared to the reference compound metal salt (21.97 %) suggesting an efficient cleavage of pUC19 DNA by the synthesized complexes as compared to the reference compounds metal salt.

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