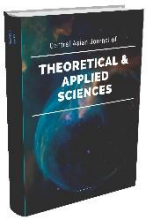

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Spectroscopy: An Important Tool for Structural Illustration of Heterocyclic Drugs

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Abstract: *Spectroscopy is the investigation and measurement of spectra produced by matter interacting with or emitting electromagnetic radiation. Originally, spectroscopy was defined as the study of the interaction between radiation and matter as a function of wavelength. Now, spectroscopy is defined as any measurement of a quantity as a function of wavelength or frequency. During a spectroscopy experiment, electromagnetic radiation of a specified wavelength range passes from a source through a sample containing compounds of interest, resulting in absorption or emission. During absorption, the sample absorbs energy from the light source. During emission, the sample emits light of a different wavelength than the source's wavelength.*

In absorption spectroscopy, the sample's compounds are excited by the electromagnetic radiation provided by a light source. Their molecules absorb energy from the electromagnetic radiation, become excited, and jump from a low energy ground state to a higher energy state of excitation. A detector, usually a photodiode, on the opposite side of the sample records the sample's absorption of wavelengths, and determines the extent of their absorption. The spectrum of a sample's absorbed wavelengths is known as its absorption spectrum, and the quantity of light absorbed by a sample is its absorbance.

Each molecule within a sample will only absorb wavelengths with energies corresponding to the energy difference of the present transition. In simpler terms, this means that a molecule that jumps from ground state 1 to excited state 2, with an energy difference of ΔE, will allow other wavelengths to pass through until it can absorb radiation from a wavelength that corresponds to ΔE. Light that passes through to the photodiode without any absorption is called Stray Radiant Energy, or stray light. Absorption that occurs due to an energy difference between the two states is called an absorption line, and a collection of absorption lines creates an absorption spectra. The frequency of each absorption line in an absorption spectra tells us about the sample's molecular structure, and can be influenced by factors such as stray light, environmental temperature, and electromagnetic fields.

Keywords: *spectroscopy, tool, heterocyclic drugs, absorption, electromagnetic, wavelength, energy.*

Introduction

Heterocyclic compounds play an important role in drug discovery and development and therefore tremendous efforts have been made to develop convenient and green routes for their high yielding synthesis.[1,2] In view of high impact of toxic organic solvents on health, environment, safety, and

moreover, the overall cost of desired products, this study exclusively emphasizes on the solvent-free synthetic methodologies employed for the generation of heterocyclic skeletons of promising pharmacological importance. It mainly includes the synthesis of nitrogen and oxygen containing heterocycles as they represent a major proportion of the bioactive heterocyclic compounds as well as marketed drugs. Several techniques have been used for the quantitation of heterocyclic compounds in pharmaceutical samples such as high-performance liquid chromatography (HPLC) either equipped with UV-visible or fluorescence, in addition to liquid chromatography-mass spectroscopy, UV-visible spectrophotometry, and electrochemical techniques.[3,4]

Spectroscopic methods are widely used for simultaneous determination of different mixtures of drugs without prior separation by some mathematical equations, and these were found to be simple, very rapid, and with low cost compared to LC-MS and LC-GC .Absorption spectroscopy is a powerful tool for quantitative analysis of analyte since there is a relation between the concentration of analyte and amount of light absorbed .Near-infrared and Raman spectroscopy have been increasingly used for real-time measurements of critical process during pharmaceutical processing, as these spectroscopic techniques allow rapid and nondestructive measurements without sample preparations .They are used for quantitative analysis of multicomponent with aid of chemometric tool. Therefore, they are used as Process Analytical Technology (PAT) tool for pharmaceutical industry. [5,6]

Spectrophotometric method is a multicomponent analysis technique where the spectra of drugs overlap and some simultaneous equations can be done to resolve such overlapping where the concentration of each individual analyte can be determined. Spectrophotometric technique depends on the following: (a) The spectrum of the solution: since the spectroscopy is an additive technique, therefore the absorbance of the solution is the sum of the absorbance of its individual components, so the spectrum of the solution is the sum of the spectra of its separate components (b) Beer-Lambert's law must be obeyed,[7,8] as [A = abC] where A is the absorbance, C is the concentration of analyte, a is the absorptivity constant, and b is the path length. There is a direct relation between A and C, so if we construct a calibration curve, it must pass through the origin. Beer-Lambert's law is only applied for the diluted solutions not more than 0.01 M, as the concentration increases, deviation from linearity will occur (c) The absorbance measured is always the difference between the absorbance of the solution of interest present in the sample cell and that of the solution present in reference cell (blank).

Several spectrophotometric methods were applied on pharmaceutical compounds including the following: (1) Methods based on the zero order absorption spectra: Like dual wavelength, induced dual wavelength, dual wavelength resolution technique, absorption correction method, absorbance subtraction, advanced absorbance subtraction, absorptivity factor method, area under the curve correction, compensated area under the curve, and spectrum subtraction (2) Methods based on derivative spectra: Like amplitude subtraction, modified amplitude subtraction, amplitude factor, amplitude summation method, simultaneous derivative ratio spectrophotometry,[9,10] modified graphical method via regression equation, differential dual wavelength, differential derivative ratio, successive derivative subtraction coupled with constant multiplication, and derivative transformation (3) Methods based on subtraction of the amplitudes of ratio spectra: Ratio subtraction method, successive ratio subtraction, extended ratio subtraction method, and simultaneous ratio subtraction method (4) Methods based on amplitude difference of ratio spectra: Ratio difference spectrophotometric method, constant center spectrophotometric method,[11,12] constant center coupled with spectrum subtraction, constant value via amplitude difference, constant value and amplitude center method (5) Methods based on modulation of amplitudes of ratio spectra: amplitude modulation advanced amplitude modulation and induced amplitude modulation (6) Methods based on computed geometrical representation: Geometrical amplitude modulation, geometrical induced amplitude modulation, and ratio H-point standard addition method (7)

Methods based on mean centering and wavelet transformation: Mean centering using geometric mean, pure component contribution algorithm, and continuous wavelet transform [13,14]

Discussion

NMR spectroscopy plays a pivotal role in the drug discovery and development process. Here, we discuss current NMR-based screening strategies that are being used in finding hits followed by their validation and further improvement to lead optimization. NMR screening experiments are very efficient and versatile in discovering high-affinity ligands for biologically relevant macromolecules, elucidating ligand-binding sites, identifying small molecules with wide ranges of binding affinity and thus proving to be a valuable and robust tool in the structure-based drug design.[15,16] These NMR screening methodologies are based on the observation of target and ligand resonances as a mode of detection for identifying weak-binding compounds and aid their advancement into potent drug-like inhibitors for use as lead compounds in drug discovery. For exploring the drug discovery and development process, understanding of the structure of biological molecules at the atomic level is essential to use that knowledge to design drug candidates that can target them. Nuclear Magnetic Resonance (NMR) spectroscopy plays a vital role in attaining the desired goal, with a high success rate in screening compounds that can be used as potential drug candidates for curing diseases. During the past decade, NMR spectroscopy has been a very efficient and versatile tool in drug discovery and development as it can shed light on the molecular structure of the biomolecules,[17,18] elucidate and verify the structure of the drugs, and provide structural information on the interaction of the biomolecules (target) with small molecule compounds (ligands); thus NMR spectroscopy proves to be a great tool in pharmaceutical research for example, biocatalytic manufacture of drug islatravir . Since the clinically used drugs are typically natural or synthetic compounds, quantitative analysis by solution-state NMR is quite useful in estimating the contamination profile of the drugs, describing the composition of drug products, and exploring the metabolites of drugs in body fluids, or study the dynamics and kinetics of proteins on solid surfaces ,or enzyme allostery . Solid-state NMR methods can offer knowledge about polymorphism of drugs in powder form and their conformations in tablets or the active site of tryptophan synthase ,or the structural properties of the toxic and non-toxic types of α-synuclein oligomers . Microimaging is useful in studying the dissolution of tablets.[19,20]

Target Immobilized NMR Screening (TINS) process is rapid, sensitive, and identical for every target irrespective of their size and chemical composition. In this method, the ligands are screened based on their binding capability to the immobilized protein target. The mixture of compounds from a chemical library is applied to the immobilized protein target, and binding is detected by comparing the resulting 1D NMR spectrum with that of a suitable control sample .This method has been validated for a variety of ligands that target proteins and nucleic acids with a K_D from 60 to 5000 μM. For fast characterization of the ligand-binding site, TINS can be used in competition mode. [21,22]This method has several advantages. It requires a smaller amount of target protein in comparison to other fragment-based approaches, as the protein can be reused to screen the library of compounds. It is sensitive to the binding of a ligand to the target with a wide range of affinities and therefore not likely to overlook new hits. Besides this, weak binding interaction and binding of the ligands to other low-affinity allosteric sites can be eradicated. A very high level of specificity can be achieved by selecting the control sample cautiously. It may also be useful for screening of hard targets like membrane proteins, that are difficult to produce or insoluble.[23,24]

Fluorine chemical shift Anisotropy and exchange for Screening (FAXS) is reliable, highly reproducible, large dynamic range, ligand-based ^{19}F NMR screening method . The basic principle of this method is that when the competitive ligand binds to the target protein, it displaces the fluorinated spy molecule that is already bound to it. The signal of the fluorinated spy molecule that was broad initially becomes sharp due to its displacement from the receptor proteins and is detected by ^{19}F NMR. This method has significant

advantages over the original competition-based approaches using ^{1}H NMR. For instance, the screening of a mixture of compounds becomes easier due to the absence of spectral overlap and requires low consumption of protein. A large chemical shift anisotropy of fluorine and a significant exchange contribution helps in selecting a weak-affinity spy molecule, thus resulting in a lower binding affinity threshold for the identified NMR hits.[25,26]

NMR spectroscopy has evolved as a powerful tool in drug discovery and development. It has been widely applied for identifying and validating hits and in the process of lead optimization. NMR binding experiments are very efficient and versatile in discovering high-affinity ligands for biologically relevant macromolecules, elucidating ligand-binding sites and identifying small molecules with wide ranges of binding affinity. Since NMR provides structural information at atomic-level resolution, its interplay with other screening methods, such as HTS, mass spectroscopy, X-ray crystallography and *in silico* screening is quite advantageous in the rapid advancement of the hit-to-lead optimization process.[27,28] Another potential application of NMR is as a tool to monitor protein-ligand interactions within living cells, *i.e.*, 'in vivo' NMR screening, in drug discovery . With the fast-paced progression in NMR methodology development, sensitivity and resolution, NMR-based screening will lead to higher throughput and can be applied to even larger protein targets. NMR will be a robust tool for designing novel therapeutics that target the challenging "undruggable" protein targets. Further progress in innovative NMR screening methods can only add to an arsenal of tools for drug discovery and development.[29,30]

Results

The experimental FT-IR and UVvis (in chloroform, ethanol and N,N-dimethylformamide solvents) spectral results, the molecular geometry, the simulated vibrational and UV-vis (in gase phase and chloroform solvent) spectra, the proton and carbon-13 NMR chemical shift values, HOMO-LUMO, NBO analyses and NLO properties were calculated using DFT/B3LYP and HSEH1PBE with LanL2DZ which stands for "Los Alamos National Laboratory 2-Double-Z" basis set. Furthermore based on the above consideration, we performed molecular docking studies into the active sites of VEGFR-2 kinase and KAS III.[31,32]

The nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for determining the composition, structure and TABLE III. The calculated 1H and 13C NMR chemical shifts (ppm) at the B3LYP and HSEH1PBE/LanL2DZ levels for 5-acetyl-2,4- dimethylthiazole function of complex molecules and to compute the reliable magnetic properties which provide the accurate predictions of molecular geometries and the isotropic chemical shift analysis . In this framework, the 1H and 13C NMR chemical shift values were calculated at the mentioned levels by using GIAO model. The calculated 1H and 13C NMR chemical shift values of the title molecule in gas phase and in the chloroform solvent at the B3LYP and HSEH1PBE /LanL2DZ levels. The present work indicates that the calculations on the 5-acetyl-2,4-dimethylthiazole molecule give us a reliable assignment for the NMR, IR and UV-vis spectra of the molecule. Meanwhile, the results of NBO analysis for title molecule exhibit that the calculated strongest stabilization energy values in $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions were confirmed by the observed data obtained from the experimental UV-vis. spectrum in the chloroform solvent. The study of the HOMO-LUMO analysis at the HSEH1PBE /LanL2DZ level in this work confirms the $\pi \rightarrow \pi^*$ transition for the 5-acetyl-2,4-dimethylthiazole molecule. Furthermore, computer aided ligand binding studies identified possible interactions and binding poses of 5-acetyl-2,4-dimethylthiazole with the VEGFR-2 and KAS III. The docking result revealed that ligand formed more strongly hydrogen bond interaction with active residues of VEGFR-2. The structure of proteins is important for their functions in the biological environment. The conformations of proteins are effective in determining binding molecules and binding force. The region where the ligand will be bound to the protein is closely related to the shape, size, charge, hydrophilic and hydrophobic properties of the ligand. In molecular doking studies, the most stable

geometric structure of the 5-acetyl-2,4-dimethylthiazole ligand obtained by DFT calculations was used and low full fitness scores were obtained. This result showed that the active site of the proteins and the ligand were quite compatible. Furthermore, the small HOMO-LUMO gap of the ligand means that this molecule was soft and reactive in chemical reactions. The reason why the title molecule interacts highly with both proteins used in the molecular docking study may be the high reactivity of the molecule due to its softness. As a result, we can say that 5-acetyl-2,4-dimethylthiazole could be used as potential compound for developing antitumor agents. However, biological tests need to be performed to confirm the molecular docking predictions.[33]

One of the directions developed by the organic synthesis and NMR spectroscopy SRL is the study of the structure of complexes and thermodynamics of the so-called sequence specific intercalation compound of biologically active compounds with bioreceptors, in particular, with DNA.This direction was formed due to the attempt to explain the effect of changes in the biological activity of DNA-binding aromatic compounds during the injection another aromatics as caffeine, vitamin B2 (riboflavin), chlorophyllin, etc.

The main scientific problem, which scientists of NMR spectroscopy SRL try to solve, is to explain the mechanisms of biological synergism in multicomponent of DNA-binding system. The main current hypothesis, which is put forward by the laboratory personnel, is the explanation of such synergistic action of the drug mixture from the point of view of all non-covalent interactions types of aromatic compounds with each other and with DNA. The main types of such interactions are the self-association of aromatic compounds with each other (the formation of complexes of the same type of molecules), heteroassociation (the formation of complexes of the different-type molecules), and competition binding of aromatic compounds with the bioreceptor, i.e. with DNA.

This general direction of scientific activity of the NMR spectroscopy SRL is divided into four main subdirections:

- ✓ study of the energy of small aromatic molecules (ligands) complexation with nucleic acids;
- ✓ study of the structure and thermodynamics of heteroassociation of aromatic biologically active compounds (BACs);
- ✓ development of the theory of interceptor-protector action of aromatic ligands binding with nucleic acids;
- ✓ study of homo- and heterodimeric binding of biologically active compounds with nucleic acids.

The main result of the work of biomolecule NMR-spectroscopy SRL is represented by about two dozen articles published in the world's leading journals (journals with a sufficiently high impact factor), which are indexed simultaneously in two international scientometric databases, Scopus and Web of Science. The final product of the laboratory work is a new scientific knowledge about the mechanism of synergistic interaction of DNA-directed antitumor agents in combination with aromatic biologically active compounds. Theoretical developments will form the basis for fundamentally new regimens for combined chemotherapy of certain types of oncological diseases with a potentially higher suppression efficiency of both leukemia and solid tumors.

Winning of the grant of the Russian Scientific Foundation (RSF) No. 14-14-00328 "Creation of a scientific basis for a new regimen for combined fullerene chemotherapy to treat oncological diseases with DNA-directed drugs" became a stimulus in terms of achievement and overfulfillment of performance indicators for the laboratory staff. The grant was obtained in the middle of last year and was intended for the period up to 2016 inclusive. Professor Maxim Evstigneev, the Doctor of Physical and Mathematical Sciences, is the head of the project and the leading research associate of the SRL. The receipt of this grant was a logical continuation of the work begun in 2013.[34]

As the title implies, the cornerstone of the project is the application of well-known, but currently insufficiently studied from the biological point of view, spherical carbon nanoparticles - fullerenes C60.The unique set of biomedical properties of C60 fullerene has been discovered recently, and this enables to use it as a potential element of the combined therapy of the oncological diseases. The last researches in which a laboratory personnel took part, enabled to prove exceptionally marked strengthening of antitumor effect of the doxorubicin with C60 fullerene in vitro and in vivo levels.

The works on RSF grant projects include two main parts: physico-chemical and biomedical. The physico-chemical part plans to conduct researches in interaction of C 60 fullerene aqueous colloidal solution with different bioactive compounds, it also includes detailed structured thermodynamic analysis of complex formation process, creation of the computing models and analytical approaches to study of such systems. The biomedical part studies the biological effect of fullerenes as well as the anticancer drugs in ex vivo conditions closed to native (leukemia contaminated blood), this enables to obtain the objective information about drug collaborative mechanism, to choose optimal conditions for the synergism monitoring and to formulate launch environment for conducting the first phase tests in vivo for drugs. It is worth to say that the biomedical part of the project is implemented by professors and students of the Ecology, Physiology and Biological Evolution Subdepartment of the Institute of Engineering Technology and Natural Science under the guidance of Marina Skorkina, Doctor of Biology, Associate Professor.[33]

The main result of this project is to understand biological synergism mechanism in terms of fullerene-drug systems, and as a consequence, to create a methodology of a new more efficient process of the combined fullerene chemotherapy of some oncological diseases. This research remains the "know-how" and promises breakthrough results in improving efficiency of the modern chemotherapy. According to our scientists, the success of solving this challenge, that is launching of a new working way of combined chemotherapy in clinical experience in the nearest future, may lead to significant social and economic effect nationwide as well as worldwide and its result cannot be overestimated.[34]

Conclusions

NMR (Nuclear Magnetic Resonance) Spectroscopy has found significant applications in drug discovery based on its capacity to map molecular interactions at the atomic level. Chemical shifts, cross relaxation, and exchange of protons are among the NMR parameters which are highly sensitive to the exact environment of the molecules, and therefore yield information about whether a small molecule (candidate compound) binds to a target protein (receptor) or to other macromolecules. These NMR parameters are also used to exactly map the part of the macromolecular target to which the ligand is bound. Spectacular advances in the use of NMR spectroscopy in drug discovery and development have been triggered by a greater understanding of the disease process at the molecular level. Structure – Activity Relationship Studies in Drug Development by NMR Spectroscopy presents comprehensive reviews on NMR spectroscopic drug development written by leading experts in the field.

Heterocyclic nucleus imparts an important function in medicinal chemistry and serves as a key template for the development of various therapeutic agents. Mostly researchers have maintained their interest in sulphur and nitrogen-containing heterocyclic compounds through decades of historical development of organic synthesis but heterocycles with other heteroatoms such as oxygen, phosphorus and selenium also appear. Here are widespread therapeutic uses of synthetic heterocycles such as antibacterial, antimycobacterial, trypanocidal, anti-HIV activity, genotoxic, herbicidal, analgesic, DntiinflDmmDtory, muscle relaxants, antileishmanial agents, anticonvulsant, anticancer, antimalarial, antifungal and lipid peroxidation inhibitor, antitubercular, hypnotics, antidepressant, antitumoral, anthelmintic and insecticidal agents .Imidazole drugs have broadened scope in remedying various dispositions in clinical medicines. Medicinal properties of imidazole include anticancer, b-lactamase inhibitors, 20- HETE (20- Hydroxy-

5,8,11,14-eicosatetraenoic acid) synthase inhibitors, carboxypeptidase inhibitors, hemeoxygenase inhibitors, antiaging agents, anticoagulants, Dnti-inflDmmDtory, antibacterial, antifungal, antiviral, antitubercular, antidiabetic and antimalarial .Some imidazole drugs, at high concentrations, could exert direct inhibitory action on membranes, without interference with sterols and sterol esters .Imidazole and its derivatives are reported to be physiologically and pharmacologically active and find applications in the treatment of several diseases. It was carried out the in vitro antibacterial activity of newly synthesized compound. *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Salmonella typhimorium. Clostridium profingens* and *Pseudomonas aeruginosa* were used to investigate the activity. He antibacterial screening revealed that some of the tested compounds showed good inhibition against various tested microbial strains. [35]

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