Pharmacochemical Nano-switches

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Abstract

Hydrazones undergo a configurational and/or conformational changes on sequential addition of acid and base. This gives an on/off switching function to the molecule Also dual-controlled nanoparticles exhibiting AND logic can act as a switch. Spectroscopy like ^{1}H NMR is used to study the working and action of these molecules. Such chemical molecular switches help in attaining even more sophisticated levels of controlled release and site targeted drug delivery.

Keywords : Nano-switches, hydrazones, pH,nanoimpellers, nanovalves.

1. Introduction

The name "Pharmacochemical Nano-switches" suggests;

Pharmaco - Related to drugs, Chemical - Chemically operated, Nano - Of nano size

Switches – serves an on/off function.

(Here, it gives output when turned on i.e. release of drug when required and no release on turning off, when the drug is not required.)

Pharmacochemical nano-switches are nanoparticles in which the drug is incorporated or to which the drug is attached and they are chemically operated through a stimulus, for example pH, to release the drug whenever and wherever required and kept intact with no release when not required. Hydrazone based switches are one such example which are controlled by acid/base modulations[1]. And with the help of this prototype , a new switch QPH-E (hydrazone-based rotary switch with quinolinyl stator and pyridine ring as a part of rotar)[2] is discussed with the aim of chemically controlling both the configuration and conformation of a single molecule. Also dual-controlled

nanoparticles which show AND logic can be employed to serve this function[3].

2.The Need

a) To by-pass first pass metabolism in the liver.

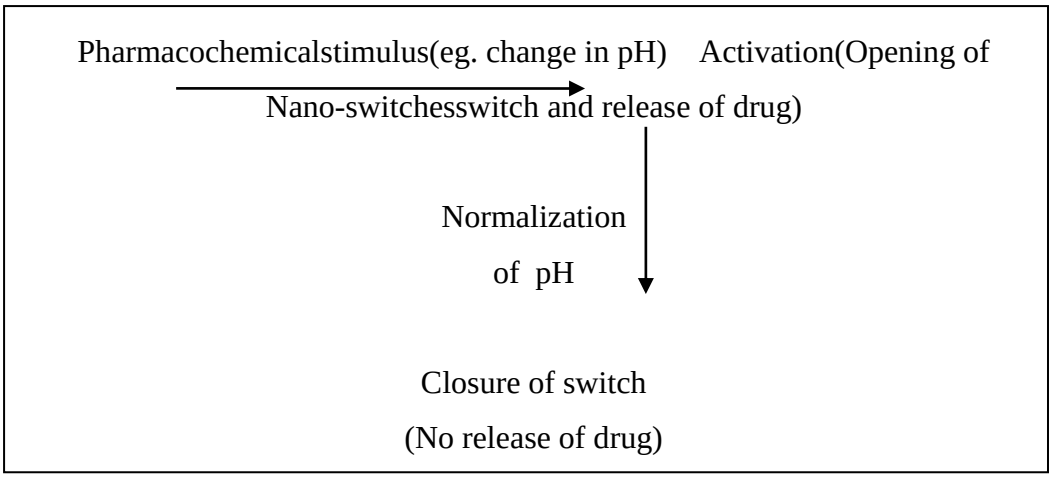
b) To provide site targeted delivery of drugs without affecting healthy cells/ tissues of the body.

c) To prevent systemic toxicities and side-effects with local and systemic tolerance.

d)As a substitute for surgery in certain cases.

3.Working

The working of Pharmacochemical Nano-switches can be explained as follows;



To understand the working of 'Pharmacochemical Nano-switches', the following examples are considered.

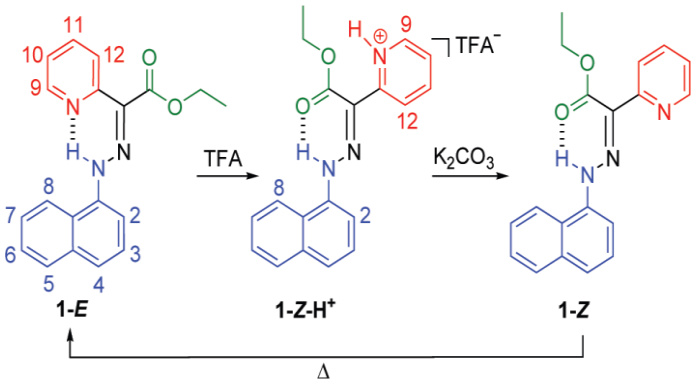
3.1 A pH Activated Configurational Rotary Switch: Controlling the *E*/*Z* Isomerization in Hydrazones

Hydrazones are very easy to synthesize, starting from corresponding ketone and azeridine.

1,2,3-Tricarbonyl-2-arylhydrazones[4] exist in solution as a pair of intramolecularly H-bonded hydrazone isomers[5,6a] that can equilibrate in the presence of catalytic amounts of acid or base[6b]. This process results in the exchange of the relative positions of the substituents around the C=N bond i.e., E/Z isomerization. This original bistable system is based on a hydrazone

building block, and pH[7] is used to control the configuration around the C=N bond. It was observed that replacing one of the carbonyl groups in 1,2,3-tricarbonyl-2-arylhydrazones with a "proton acceptor" group such as pyridine will lead to a system that can be converted fully, effectively and controllably from one isomer to the other by the consecutive addition of acid and base. In order to add another element of nonsymmetry into the system it was decided to use a naphthylhydrazone derivative for the studies. This line of thought led to compound **1-*E*** that upon protonation affords **1-*Z*-H**$^+$, which when treated with base yields the "metastable" **1-*Z*** configuration that thermally equilibrates back to **1-*E***.[8] (See Figure 1).

Figure 1 : Acid/ Base controlled E/Z isomerization of 1-E.



3.1.1Characterization for suitability study

The ^{1}H NMR spectrum of **1-*E*** in CD_3CN shows a characteristic H-bonded N-H resonance at 15.8 ppm, in addition to the expected aromatic and aliphatic signals. Initially the E:Z ratio in the solution is 97:3[9]. The addition of 1.4 equiv of CF_3CO_2H (TFA) to a CD_3CN solution of **1-*E*** results in the protonation of the pyridine subunit[10], which is accompanied by a color change of the solution from light yellow to orange and drastic changes in the ^{1}H NMR spectrum. First of all, the N-H proton signal at 15.8 ppm disappears and a new signal appears at 13.9 ppm. This shift indicates that a rotation around the C=N bond has occurred (*E*/*Z* isomerization) and that the N-H proton is now H-bonded to the

carbonyl group of the ester subunit, yielding **1-*Z*-H**$^+$. The ^{1}H NMR spectrum of **1-*Z*-H**$^+$ shows the presence of a large and broad signal at 4.1 ppm, presumably resulting from excess TFA. Upon passing the CD_3CN solution of **1-*Z*-H**$^+$ over a plug of K_2CO_3 or the addition of 1.4 equiv of triethylamine

(Et_3N), the color of the solution changes back to light yellow. The ^{1}H NMR spectrum , immediately after passing the solution over K_2CO_3, shows the complete disappearance of **1-*Z*-H**$^+$ and the presence of both **1-*E*** and **1-*Z*** in solution respectively. Interestingly, the signals of **1-*Z*** gradually decrease with time and those of the **1-*E*** configuration grow in return[11]. This process is the thermal equilibration between the "metastable" configuration and the stable one, **1-*Z*** and **1-*E***, respectively. This process comes to completion within 2 h at RT, and the system regains its original equilibrium ratio of 97:3. Thus we have a conceptually new type of chemically induced rotary switch using a hydrazone building block.

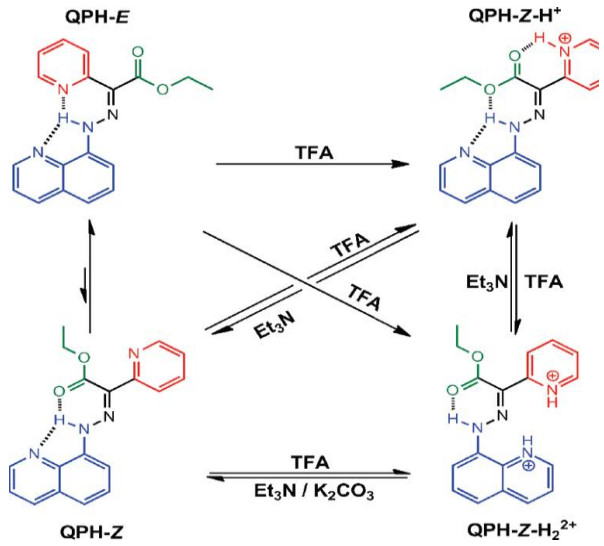
3.2Switching Around Two Axles : Controlling the Configuration and Conformation of a Hydrazone-Based Switch

Previously it is observed how the configuration of a hydrazone-based switch[12] can be controlled by acid/base modulations. Inspired by this prototype, a new switch **QPH-*E*** (hydrazone-based rotary switch with quinolinyl stator and pyridine ring as a part of rotar) is designed with the aim of chemically controlling both the configuration and conformation of a single molecule. The protonation of **QPH-*E*** by trifluoroacetic acid (TFA) leads to **QPH-*Z*-H**$^+$, accompanied by a configurational change originating from the rotation about the C=N double bond[13]. Further protonation leads to rotation about the C-N single bond, generating a new conformational isomer, **QPH-*Z*-H**$_2^{2+}$, that retainsthe *Z* configuration. After deprotonation with triethylamine (Et_3N), both **QPH-*Z*-H**$^+$ and **QPH-*Z*-H**$_2^{2+}$ convert into a neutral metastable species **QPH-*Z***, which eventually equilibrates to give back the thermodynamically more stable **QPH-*E*** isomer. The configuration and conformation of the system can be modulated based on the sequence at which the acid and base are added, leading to a switch that can be prompted to rotate around two

different axles (See Figure 2). This has been validated using ^{1}H NMR andUV-visible spectroscopy.

Hydrazone QPH was also synthesized easily by the coupling of the diazonium salt derived from 8-aminoquinoline with ethyl-2-pyridylacetate.

Figure 2: Schematic Illustration of the Switching Process.



3.3Dual-Controlled Nanoparticles Exhibiting AND Logic

Here, dual-controlled nanoparticles (DCNPs) are discussed in which two different types of machines, namely, nanoimpellers[14] and nanovalves[15], are brought together in and around mesoporous silica nanoparticles supports[16] . The molecular machines are designed to operate in tandem with one another in such a way that the DCNP systems function as AND logic gates and provide sophisticated control of the contents of the pores. In the DCNP systems discussed here, light-responsive nanoimpellers[17] and pH-responsive[18a,b] nanovalves are operated in tandem with one another in such a way that the release of encapsulated guest molecules (the output) requires activation of both the nanoimpellers using 448 nm light (input 1) and the nanovalves using pH changes (input 2). Two different pH responsive nanovalve systems have been employed in this work, resulting in the formation of two different DCNPs: DCNP-**1**, which employs base-responsive nanovalves, and DCNP-**2**, which features acid-responsive nanovalves. Nanoimpellers are based on photoresponsiveazobenzene derivatives that are tethered to the inner pore walls of the mesoporous silica nanoparticles supports. Azobenzene exists in two configurations (trans and cis) and can be interconverted between the two upon absorption of light. When azobenzene derivatives attached to the nanopore interiors are exposed to a wavelength of light that is absorbed by both the trans and cis isomers, a dynamic wagging motion[19], which can be used to impel unbound guest

molecules out of the nanopores, is generated within these derivatives. On the other hand, the nanovalves employed here[20,21] are based on pH-switchable [2]pseudorotaxanes in which cucurbit[6]uril(CB[6]) rings encircle bisammonium stalks that are tethered to the outer surfaces of the nanoparticles supports. The base-responsive nanovalves used in DCNP-**1** consist of CB [6] /bisalkylammonium [2]pseudorotaxanes. At neutral pH, the bulky CB [6] rings interact tightly with the tethered stalks through ion-dipole interactions, blocking the nanopore orifices and trapping the guest molecules. When the pH is increased and the stalks become deprotonated, the binding interactions are disrupted, and the CB [6] rings dissociate from the stalks, thereby opening the nanovalves and allowing the contents to be released. The acid-responsive nanovalves used in DCNP-**2** consist of bi-stable CB[6]/trisammonium pseudorotaxanes. At neutral pH, the anilinium nitrogen atom remains unprotonated and the CB[6] ring resides on the tetramethylenediammonium recognition unit close to the nanopore orifices. When the pH is decreased and the anilinium nitrogen atom becomes protonated, the CB [6] ring shuttles to the more distal hexamethylenediammonium recognition unit, and the nanovalves are opened.

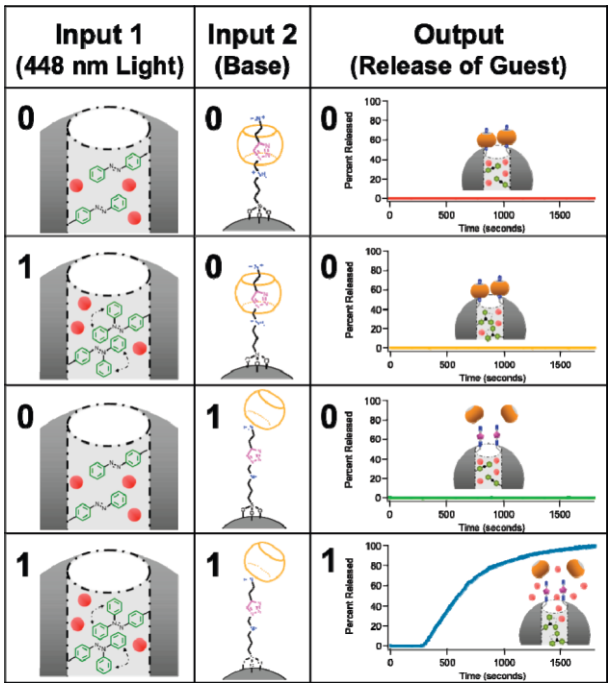
Mesoporous silica is an ideal support for synthetic molecular machines because it is optically transparent (allowing for activation by light and spectroscopic monitoring) and relatively easy to functionalize on both the insides of the pores and the outer surface[22].

3.3.1Testing the operation of dual-controlled systems

In order to test the operation of the dual-controlled systems, the DCNPs were loaded with the fluorescent probe ClRe-$(CO)_3$-2,2′-bipyridine as the guest molecules and luminescence spectroscopy was used to follow their fate. For the nanoimpellers, a 36 mW, 448 nm excitation beam was directed at the nanoparticles and used to activate the dynamic wagging of the azobenzenes. The nanovalves were opened by adjusting the pH appropriately. For DCNP-**1**, the solution was adjusted to pH 10 by the addition of 2 M NaOH (See Table 1), and for DCNP-**2**, the solution was adjusted to pH 4 by the addition of 0.01 M HCl. The release of ClRe$(CO)_3$-2,2′-bipyridine required activation of both the nanoimpellers

and the nanovalves. When light or pH activation alone was used, activating just one machine, the unactivatedmachine was able to keep guest molecules constrained, and no release of guest molecules occurred. Only when both controlled release mechanisms were activated simultaneously, the release was observed.

Table 1: Truth table for an AND gate based on DCNP-1.



4. Formulation

The Pharmacochemical nano-switches can be formulated as tablets or capsules on using suitable excipients or can also be administered parenterally by formulating suitable injectable solutions or suspensions.

5.Disease conditions and pH

The Pharmacochemical nano-switches can be implemented to provide site targeted drug delivery in disease conditions which are characterized by a distinct pH value. Few ofits applications are discussed as follows.

5.1 The chemotherapy used in treatment of cancer has serious side-effects. The drugs like Melphalan are mutagenic and can cause leukemia. Its oral absorption

is erratic and intravenous formulation has higher risk of side-effects. Such drugs can be given through the pharmacochemicalnano-switches. The pH is on an average lower in the tumor mass than normal tissue. Most of the solid tumors have lower extracellular pH (6.5) than the surrounding tissues (pH 7.5). The pH is compartmentalized in tumor tissue into an intracellular component (pHi), which is similar in tumor and normal tissue and an extracellular component (pHe), which is relatively acidic in tumors[23]. This acidic nature of the tumors would cause the release of the drug from the Pharmacochemical nano-switches and this switch would be turned off giving no release of drug at the site of normal healthy cells.

5.2 Aneurysm occurs in the arteries as a result of deposition of lipids, cholesterol etc which form a plaque thus narrowing the lumen of the artery. Mainly it occurs in the abdominal artery. Presently aneurysm is treated through surgery where a by-pass is done or the area where the plaque is formed is cut and removed and the artery is again stitched. The Pharmacochemical nano-switches can be used here as a substitute for surgery. Drugs that would dissolve the plaque can be incorporated within these switches and they would release the drug at plaqued area. If the plaqued region of the artery has a different pH than the surrounding area it would help to activate the switch. The switch would turn off on dissolution of the plaque. Also they can be used to treat atherosclerotic plaque in similar manner.

5.3 Also anti-ulcer drugs can be administered through these switches to reduce their adverse effect on gastric acid secretion[24].

5.4 The most simple application of Pharmacochemical nano-switches is in treatment of acidity. The pH of the stomach decreases in hyperacidity. The very simple use of the Pharmacochemical nano-switches can be observed over here. Antacid agents can be given through these switches which would be turned on in the acidic pH of the stomach to release the antacid and as soon as the acidity is lowered the switch would turn off.

5.5 Also if a pH change is detected in the beta cells of islets of langerhans[25] during entry of glucose the Pharmacochemical nano-switches can be implemented here to give release of insulin to treat diabetes.

6. Advantages

1)The Pharmacochemical nano-switches provide a new mode of drug delivery.

2)The side-effects and the foreign body reactions may be prevented with local and systemic tolerance.

3)Being nanoparticles the Pharmacochemical nano-switches do not give anaphylactic reactions.

4)Provide a new way of efficient targeted drug delivery and controlled release.

5)Their synthesis is also not very complicated.

7. Conclusion

There are various modes of targeted drug delivery systems. Also certain new modes are under research. The Pharmacochemical nano-switches is one of the new ways to achieve targeted drug delivery. They rely on the pH of the surrounding environment to give their action. These Pharmacochemical nano-switches can help in attaining even more sophisticated levels of controlled release. They can be synthesized as per the requirement without much efforts and the drug can be incorporated with it. They provide efficient drug action at the desired site with minimized side effects.

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8)No change was observed in ^{1}H NMR spectrum of 1-E upon the addition of 1.0 equiv of Et_3N or K_2CO_3 to the solution. Upon the addition of 0.1 equiv of acid (TFA)only the appropriate amount of isomerization occurred. This rules out the catalytic equilibration process observed in other 1,2,3-tricarbonyl-2-arylhydrazone systems.

9) This isomer ratio remained constant event after heating the solution at 55^0C for 1 h.

10) The addition of 1.4 equiv of TFA yields 98% pyridine protonation. For full protonation 2.0 equiv were required. Only 1.0 equiv of TFA was needed to fully protonate the starting material, ethyl-2-pyridylacetate.The H-bonding with the N-H proton and the conjugation with the aromatic system apparently decrease the basicity of pyridine nitrogen in 1-*E*. Subsequently when CH_3SO_3H was used as the acid, only 1.0 equiv was needed to fully protonate the pyridine subunit.

11) When Et_3N was added to the solution, the fully equilibrated spectrum was generated instantaneously and this process was not observed. The reason for this might be the larger contact time the solution has with Et_3N.

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