

## Construction of anion binding motifs based on tryptophan

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### Abstract

Two tryptophan-based isophthalamide derivatives [N, N-bis (2-Amino, 3-indolyl methyl propanoate) benzene-1, 3-dicarboxamide and N, N-bis (2-Amino, 3-indolyl methyl propanoate) benzene-1-nitro-3,5-dicarboxamide] are synthesized and characterised them using IR, UV-Visible and NMR spectroscopic techniques. The anion binding abilities of the synthesized motifs are investigated with various anions like, fluoride, acetate, nitrate, chloride, iodide, sulphate, phosphate, etc. in aqueous methanol using fluorescence spectroscopy, and found that these receptors can potentially be more useful for binding to anions such as acetate, nitrate and fluoride.

**Keywords:** Anion binding; Tryptophan; Dicarboxamide; Fluorescence; Receptor

### Introduction

Synthetic receptor molecules that possesses catalytic activity are among the most interesting and challenging molecules to design and synthesize. In view of the importance of anions of various biological processes, medicine and environment, the design of receptors for for anion recognition has become an area of great interest. For instance, simple tweezer-like molecules such as '1' act as receptor for F<sup>-</sup> ion and when coordinated to Re (I) complex shows strong fluorescence quenching activity. The geometry of the tweezers changes (opens-up or compress) with respect to their interaction with the ions [1].

Consequently, receptors containing indole moiety have been designed and synthesized which interact with anions of different size and shape through the formation of N-H X<sup>-</sup> hydrogen bonding interactions.<sup>4</sup> This is exemplified by the compound '2' which has a 2,7-disubstituted indole system as the functional unit of the anion receptor. The indole NH units are located at the centre of the cleft to effectively bind to an anion. As a consequence, these receptors shows high affinity towards carbonylates which has been demonstrated in DMSO- water solution using NMR. But, in the absence of an anion, the NH group of the indole moiety repel each other and they remain apart.

Indole based dicarboxamide derivative such as '4' have been synthesized and these compounds show high selectivity for F<sup>-</sup> in DMSO- water solutions. The binding affinities in this case were determined by NMR and X-ray diffraction techniques. Indole based systems have been found responsible for providing structural rigidity to the receptor and in certain cases exhibit interesting spectroscopic signals which can be monitored. The prominent metal ion receptor property of indole ring of tryptophan has recently been subjected to much investigation due to the newly recognized importance of non-covalent interactions of the metal ions with aromatic rings in chemistry and biology.

Flourescence spectroscopy is a powerful technique in visualising the molecular recognition events, and inherently unique property of the indole chromophore. Therefore, the exploration of the potential of the indole moiety in the fluorescent chromophore and chemo-sensors for metal ions provide promising result. A notable example is provided by the identification of a tryptophan containing protein as a sensor for calcium ion.

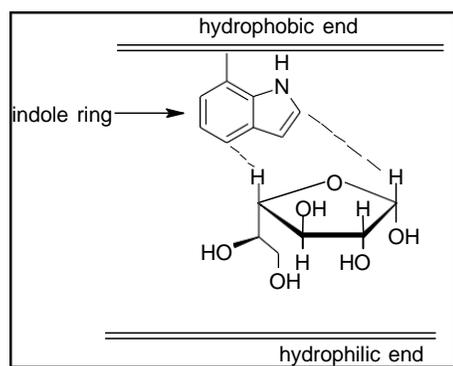
### Chemical receptors have advantages over biological receptors

Chemical hosts as receptor molecules that possesses a cleft or cavity which enables them to bind other molecules. Though much less efficient than biological receptors, these small receptors have potential advantages they can be compartmentalised in order to elucidate and understand the role and influence of H- bonding, anion-pi interaction and electrostatic regulation. Small receptors have a number of potential chemical sensing, separations and catalytic and various biomedical applications.

### Biological role of tryptophan

Tryptophan absorbs UV-light in the region near 280 nm. Indole moiety of tryptophan interact with the carbohydrates in the biological cell membrane. Sugar units such as galactose have a more polar side available to hydrogen bonding with lectin, and a less polar side that can have hydrophobic interactions with non-polar side chain in the proteins, such as indole ring of tryptophan.

At pH 7.0, tryptophan crosses a lipid bilayer at about 1000-times that of the closely related substance indole.5-hydroxy tryptophan is a precursor for neurotransmitter serotonin. It is also a precursor of the plant growth hormone indole-3-acetate or auxin. Again, the proportion of transcription declines as tryptophan concentration declines [2]. Pellagra is a disease caused by the deficiency of the tryptophan and niacin. The alkaloid harmine can be obtained from tryptophan and acetaldehyde (Figure 1).



**Figure 1:** Hydrphobic interaction of the indole with sugars in cell membrane.

### **Importance of fluorescent study**

Although the fluorescence of tryptophan is widely studied, its photophysics is not fully understood. Numerous explanations have been proposed for the complex fluorescent decay of the tryptophan zwitterions as well as individual tryptophan in peptides and proteins. Two non-radiative decay processes, intermolecular proton and electron transfer account for the different life times of various rotamers. The fluorescent life time and the fluorescent quantum yields of tryptophan containing compounds decrease with increasing temperature. The presence or absence of non-exponential fluorescence decay and the relative fluorescent life-times of the tryptophyl compounds are occurred via charge transfer mode. The relative solvent accessibility of the fluorescent tryptophan residue can be learned by solute quenching. Thus, intrinsic fluorescence and quenching are powerful tools for analysing structural properties of tryptophan based receptors [3].

### **Objective of the study**

In this project work, our objective was to construct anion binding motifs based on tryptophan. Our choice of tryptophan as the amino acid was based on the following reasons:

1. For a neutral receptor, the proximity and the orientation of the donor with respect to the acceptor,
2. It has an indole ring which may influence the anion binding ability of the receptor.
3. Local structural perturbations and global conformational changes can understood by the spectral characteristics of the tryptophan residues

In order to achieve this, we prepared the bis-amide (1, 3-benzene dicarboxa amide). Subsequently, the interaction of the compounds '6' and '7' with various anions (such as F<sup>-</sup>, -OAc, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>) will be monitored using UV-Visible and fluorescence spectroscopy.

### **Result and Discussion**

In the first set of experiments, we performed UV-Visible studies of receptor compounds (6) and (7). The compounds have distinct absorption signals at 290 nm and 297.2 nm respectively in MeOH solution. The nature of the fluorescence spectra of compound (6) with various anions such as acetate, nitrate, sulphate, chloride, etc. Compound (6) has an emission band at 343 nm when excited at 290 nm in MeOH. From the fluorescence studies in aqueous MeOH, we found that the compound 6 and 7 exhibit moderate fluorescence variation in presence of F<sup>-</sup>, -OAc, and NO<sub>3</sub><sup>-</sup> ( X-axis: concentration (mM) of anion; Y-axis: fluorescence intensity in all the plots).

After completion of the study protocol, it was found that with test and standard treatment, the serum level of glucose improved significantly (P<0.05) as compared to diabetic control. In comparative evaluation all formulations found to be effective. The formulation PD1 was found to be more efficacious as compared to others in lowering the blood glucose level. The significant antidiabetic activity of formulations is due to inhibition of free radical generation and subsequent tissue damage induced by alloxan or potentiation of plasma insulin effect by increase of either pancreatic secretion of insulin from existing beta cells or its release from bound form as indicated by significant improvement in glucose level [4].

### **Preparation of the solution for fluorescent study**

5.6 mg of compound '6' (6.1mg in case of compound '7') was dissolved in 10 ml of methanol to get an 1mM solution of the compound. Again, 1mM solution of each of ammonium acetate, ammonium bifluoride, ammonium nitrate, ammonium chloride, ammonium iodide, and ammonium sulphate and ammonium biphosphate was also made.

Then 1 ml of MeOH, 1ml of water, 300 $\mu$ L of the compound 1 (or 2) was taken in the cell for fluorescent study. Then 20  $\mu$ L of the salt solution was added in each time to achieve a titrimetric experiment. The fluorescent intensity and the maximum wavelength were noted for all the salts. Another study consists of taking 1ml of MeOH, 0.5 ml of water, 0.5 ml of salt solution and 300  $\mu$ L of the compound and the fluorescent graphs were studied [5].

### **Conclusion**

In summary, we have synthesized two Tryptophan-based isophthalamide derivatives and characterised them using IR, UV-Visible and NMR spectroscopic techniques. We have evaluated their anion binding ability in aqueous MeOH using fluorescence spectroscopy, and found that these receptors can potentially be useful for binding to anions such as acetate, nitrate and fluoride, etc. Structural aspects of anion-receptor interactions are, however, not yet established.

### **Acknowledgement**

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