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# Simultaneous measurement of hydrogen carbonate and acetate anions using biologically active receptor based on azo derivatives of naphthalene

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# Abstract

A novel receptor based on azo-derivatives of 1-naphthylamine (2-((E)-((4-chloro-3-(trifluoromethyl)phenyl)imino)methyl)-4-((E)-naphthalene-1-yldiazenyl)phenol(2) abbreviated CTNP was successfully designed and synthesized. Its sensing properties were studied deeply. Systematic studies of CTNPwithHCO<sub>3</sub><sup>-</sup> and AcO<sup>-</sup> anions in DMSO disclosed that there is hydrogen-bonding between CTNP and incoming anions. Significant changes in the visible region of the spectrum, as well as a drastic color change of CTNP from pale yellow to red, observed due to interaction as mentioned earlier. The stoichiometry of [CTNP: HCO<sub>3</sub><sup>-</sup>/or AcO<sup>-</sup>] complexes and association constants determined through Job's method and

Benesi-Hildebrand (B-H) plot, respectively. Taking into account the analysis results, CTNP performs the selective recognition of sub-millimolar concentrations of  $HCO_3^-$  and  $AcO^-$  efficiently. The antifungal activity of the receptor was tested against *Aspergillusbrasiliensis* and *Aspergillusniger*. CTNP exhibited excellent antifungal activity against both strains. CTNP also represented antibacterial activity against Gram-positive bacteria: *staphylococcus epidermidies*. It was cleared that designed receptor can be applied under physiological conditions for a long duration.

Keywords: Hydrogen carbonates anion; Acetate anion; Chemosensor; Biological activity

# **1. Introduction**

The design and development of anion detection sensors are one of the most important fields of study in the chemosensor technology. They have several applications [1-4], including in organo-catalysis, biology, medicine, environment and in separating mixtures of anions in industrial systems [5, 6]. Among those common anions, hydrogen carbonate ( $HCO_3^-$ ) and acetate ( $AcO^-$ ) ions have been paid much attention due to their necessity in the field of biology [7,8]. Moreover, physiological acid and base homeostasis are necessary for retaining extracellular and intracellular pH.HCO<sub>3</sub><sup>-</sup> is a natural alkaline buffer that adjusts tissue and blood pH, via the discharge of carbon dioxide ( $CO_2$ ) [9-11].  $CO_2$ , which is produced in eukaryotic cells, in the physiological environment is a conjugate acid according to the equation 1 [11]:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$
 (1)

Carbonic anhydrase (CA) enzymes accelerate the conversion of  $CO_2$  and  $HCO_3^-$  with catalytic rate constant of up to 10<sup>6</sup> S<sup>-1</sup>. Control the equilibrium between CO<sub>2</sub>, H<sub>2</sub>CO<sub>3</sub>, and HCO<sub>3</sub><sup>-</sup> ions is critical factor of a healthy life. Variations in the HCO<sub>3</sub><sup>-/</sup> CO<sub>2</sub> ratio are known to affect the structure and function of proteins in metabolic reactions [12,13]. Low extracellular pH in tumors compared to the healthy tissues induces a physiological obstacle that forbids drugs to penetrate cells, soHCO<sub>3</sub><sup>-</sup> is used as auxiliary to the growth of tumor pH. Hence, determining extracellular pH using hyperpolarized <sup>13</sup>C labeled HCO<sub>3</sub><sup>-</sup> has many usages in basic research of biology and clinical trials [14]. Separate from the physiological importance of  $HCO_3$ , it has a critical role in the bakery and food industry, because it can be used as a leavening agent, pH buffer, and an effervescent agent. Worthy of note that acetate due to its involvement in variety of biochemical functions (enzyme activity and antibody functions) as well as industrial reactions (extend shelf life in foodstuff) is of particular interest. Sodium salts of acetate have been used to production and purification of synthetic peptides, prevent microbial growth, and promote sensory attributes. The elevated level of AcO<sup>-</sup> in blood plasma resulting from oxidation of ethanol is an important factor to cause hangovers [15-18]. Due to ubiquity and practical applications of bicarbonate and acetate ions, it is of utmost importance to develop highly sensitive techniques for monitoring anions. Under laboratory situations, a wide diversity of methods are accessible to quantify the  $HCO_3^$ and AcO<sup>-</sup> content including electrochemical, chromatographic techniques, potentiometric,

electrophoresis, flow injection amperometric, polarography, and simple titrations. Some of them are extraordinarily sensitive but need both tedious sample preparation, expensive instrumentation, and multiple experimental steps, which greatly limit their wide applications [19-21]. Therefore, potential advantages, containing ease of operation, safe and economy induces the development of artificial molecular receptors with chromogenic subunits that enable convenient monitoring of HCO<sub>3</sub><sup>-</sup> and AcO<sup>-</sup> anions by the naked eye [22-28].For practical uses, the development of small molecular receptors that can detect both HCO<sub>3</sub><sup>-</sup> and AcO<sup>-</sup> simultaneously is more favorable [29]. Overall, the development of novel receptors with appropriate functionalities remains a challenging work for chemists. Conventional colorimetric anion chemosensor scaffolds employing -NH/-OH/-SH donors can act as anionic binding sites. In all of these subunits, hydrogen bonding is responsible for  $\pi$ -electron delocalization, which induces the deep red color of the solution immediately [30-32]. Simple Schiff base frameworks are ideal modes to design chemosensors due to their excellent photophysical properties and generally one-step synthesis [31,33]. In contrast, few studies in which 1-Naphthylamine was employed to prepare azobenzene dye as a precursor for the synthesis of naphthalene based Schiff base are reported in the literature [34,35]. With these considerations, a novel molecular receptor, CTNP, for detection of HCO<sub>3</sub> /or AcO<sup>-</sup> contents over other physiologically relevant anions was designed and synthesized. In this molecular design, the  $CF_3$  molety as a robust electron-withdrawing group enhanced the polarity of -OH fragment, and so accelerated the deprotonation of the phenol proton upon the addition of hydrogen carbonate and acetate ions. As expected, an extremely color change was observed due to the redshift in absorption when treated with HCO<sub>3</sub>/or AcO<sup>-</sup> ions. Sensor CTNP displayed fast response for selectivity recognition of HCO<sub>3</sub><sup>-</sup>/or AcO<sup>-</sup> in DMSO solution and revealed excellent biological activity against various fungal and bacterial strains [36,37].

### 2. Experimental

### 2.1. General information

All chemical reagents, solvents, and anions (as sodium salts) are available at analytical grade and used without purification. Ultrapure water (18.2 M $\Omega$ /cm) was used throughout all of the experiments. All measurements were performed under aerated condition. The Fourier transform infrared (FT-IR) spectra of the samples were recorded using Unicom Galaxy Series IR spectrometer using KBr pellets. The spectra were calibrated using the polystyrene bands at

3028, 1601, and 1208 cm<sup>-1</sup>.The UV-Vis absorption spectra were measured using double beam Optizen 3220 spectrometer.<sup>1</sup>H-NMR were recorded on Bruker300 and 250 MHz NMR instruments, using tetramethylsilane (TMS) as the internal standard, and chemical shifts were recorded in ppm.A solution of CTNP ( $4.0 \times 10^{-5}$  M) was prepared in dry DMSO. The solution was titrated with continuous additions of stock solutions of HCO<sub>3</sub><sup>-</sup> and AcO<sup>-</sup> anions( $1.0 \times 10^{-4}$ M). The UV-Vis absorption spectra were measured employing a quartz cell,and the excitation slits were set at 5 nm. To investigate the practical applications of the designed receptor, the antimicrobial activity of CTNP was examined against both fungal and bacterial strains using conventional techniques.

2.2. Synthesis of 2-hydroxy-5-(naphthalene-1-yldiazenyl) benzaldehyde (HNDB) Following the general method to createazodyes [38], 1-Naphthylamine (20.0 mmol, 2.86 g) was dissolved in solution of concentrated hydrochloric acid (10 mL) and ultrapure water (5 mL). The homogeneous mixture was heated to 80 °C to form a clear solution. A solution of sodium nitrite(20.0 mmol, 1.4 g) in 10 mL of ultrapure water was added drop wise to the acidic solution of amine during 5 min at 0°C to convert an  $-NH_2$  group to an  $-N_2^+$  (diazo) group. Then, the diazonium salts solution was poured drop wise to a solution of salicylaldehyde (20.0 mmol, 2.5 g), as a coupling reagent which is containing sodium carbonate (70 mmol, 7.4g) and sodium hydroxide (17mmol. 0.7 g) in ultrapure water. The reaction mixture vigorously stirred at 0°C for 3 hours. The obtained precipitate was successively washed with water and air-dried. The product was recrystallized from ethanol solution to obtain pure HNDB (3.67 g, yield:67%) as a deep brown solid. M.P. 153–155°C; <sup>1</sup>H-NMR (250 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 11.37 (s, 1H), 10.10 (s, 1H), 8.29 (d, 1H, J=6.9 Hz), 8.33 (s, 2H), 8.02 (d, 1H, J = 8.4 Hz), 7.96 (d, 1H, J=8.4 Hz), 7.85 (d, 1H, J=6.9 Hz), 7.64 (m, 3H) and 7.20 (d, 1H, J=8.7 Hz). FT-IR (KBr,cm<sup>-1</sup>); v: 1660 (C=O), 1620 (C=C), 1481 (N=N), 1288 (C–O), 3049-3173 (aromatic C-H). For C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>:Anal. Cal.: C, 73.90; N, 10.14; H, 4.38 %. Found: C, 71.99; N, 9.98; H, 4.68 %.

# 2.3. Synthesis of 2-((E)-((4-chloro-3-(trifluoromethyl)phenyl)imino)methyl)-4-((E)naphthalene-1-yldiazenyl)phenol(2)(CTNP)

A solution of 4-chloro-3-(trifluoromethyl)benzylamine (1.0 mmol, 0.195 g) in ethanol was gradually added to a portion of HNDB (1.0 mmol, 0.276 g) in ethanol under stirring, meanwhile 0.5 mL triethylamine was added as catalyst. Then the mixture was refluxed for 6hours at 75 °C. After evaporating the solvent, precipitate was purified by washing with n-

hexane and diethyl ether sequentially, affording brown powders of(0.28 g, yield: 62%), M.P. 140–145°C;<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 13.22 (s, 1H), 8.80 (s, 1H), 8.92 (d, 1H. J=7.5 Hz), 8.20 (t, 2H, J=10 Hz), 7.96 (t, 2H, J=10Hz), 7.83 (d, 1H, J=7.5 Hz), 7.55-7.66 (m, J=5Hz), 7.45 (d, 1H, J=7.5Hz), 7.22 (d, 1H, J=7.5 Hz). FT-IR (KBr,cm<sup>-1</sup>); v: 1620 (C=N), 1475 (N=N), 1281 (C-O), 3055 (aromatic C-H).For C<sub>24</sub>H<sub>15</sub>N<sub>3</sub>OF<sub>3</sub>Cl: Anal. Cal.: C, 63.50; N, 9.26; H, 3.30 %. Found: C, 62.93; N, 9.38; H, 3.42 %.

#### 3. Results and Discussion

#### 3.1. Design and synthesis of CTNP

Our basic idea is to create hydrogen bonding sites for anion recognition through visual color change. Introducing electron-withdrawing group such as  $-CF_3$  to the benzylamine moiety favors the anion binding by increasing molecular polarization. On the other hand, extending the azo-based aromatic ring from benzene to naphthalene causes a pronounced bathochromic shift through lengthening the conjugated  $\pi$ - system [39, 40]. Synthetic procedure for CTNP is outlined in scheme 1. <sup>1</sup>H-NMR and FT-IR spectra confirm the structure. The strong band at 1660 cm<sup>-1</sup> is attributed to the presence of C=O stretching mode completely disappeared in the spectrum of HNDB, while appearing a new characteristic azomethine (C=N) band at 1620 cm<sup>-1</sup> (Fig.1)verify the reaction between HNDB and benzylamine. On the other hand removing of the band at 2890 cm<sup>-1</sup> in CTNP spectrum was observed due to the remove of C-H aldehyde group of HNDB. Meanwhile, the N=N band at 1480 cm<sup>-1</sup> in HNDB spectrum shifted to 1475 cm<sup>-1</sup> in CTNP which confirms the presence of N=N band in the final product. To confirm the Schiff base reaction, <sup>1</sup>H-NMR spectra of HNDB and appearance of newly formed imine signal at 8.8 ppm supports the formation of C=N bond (Figures S1and S2) [22].









Wavenumber (cm<sup>-1</sup>)

Fig. 1:FT-IR spectra of (a) HNDB, (b) CTNP

#### 3.2. Electronic absorption spectra

The absorption spectra of CTNP were measured in DMSO at a concentration of  $4\times10^{-5}$ M at room temperature. The absorption spectra of CTNP exhibit a high energy band centered at 330 nm and a low energy band centered at 380 nm assigned to  $\pi \to \pi^*$  electronic transitions of azo-azomethine and intra-molecular charge transfer transition, respectively [41]. The selectivity of the CTNP receptor was further examined its preference for HCO<sub>3</sub><sup>-</sup> and ACO<sup>-</sup> in the presence of interfering anions such as F<sup>-</sup>, CI<sup>-</sup>, Br<sup>-</sup>,  $\Gamma$ , N<sup>-</sup><sub>3</sub>, SCN<sup>-</sup>, NO<sup>-</sup><sub>3</sub>, NO<sup>-</sup><sub>2</sub>, ClO<sup>-</sup><sub>4</sub>, HSO<sup>-</sup><sub>4</sub> delivered as their sodium salts and dissolved in DMSO/H<sub>2</sub>O (90:10 %; v/v). As anticipated, a noticeable red shift of the main absorption band of free CTNP from 380 to 480 nm upon the addition of HCO<sup>-</sup><sub>3</sub> and ACO<sup>-</sup> anions were observed whereas no other anion induced a significant change in absorption spectra (Fig. 2). More importantly, treatment of CTNP with HCO<sup>-</sup><sub>3</sub> and AcO<sup>-</sup> caused noticeable color change of the solution which revealed that naked-eye selective sensing of HCO<sup>-</sup><sub>3</sub> and AcO<sup>-</sup> became possible (Fig S3). The synthesized ligand (CTNP) is not soluble in H<sub>2</sub>O solvent and therefore cannot be studied in H<sub>2</sub>O. For further

investigation, both the CTNP and anionic salts (HCO<sub>3</sub><sup>-</sup>, AcO<sup>-</sup>) were dissolved in DMSO: H2O (90:10 %; v/v) solvent (Fig S4, S5) and once again the CTNP was dissolved in dry DMSO and the mentioned anions were dissolved in pure H<sub>2</sub>O. (Fig S6, S7) and UV-Vis spectra were obtained. These results indicate that the presence of H<sub>2</sub>O has no effect on the recognition process of HCO<sub>3</sub><sup>-</sup> and AcO<sup>-</sup> ions. But the detection of target anions in dry DMSO is more outstanding in both color and absorbance data.



**Fig. 2:** UV-Vis studies of CTNP ( $4 \times 10^{-5}$  M) in DMSO in the presence of 0.1mM different anions

The sensitivity of CTNP towards  $HCO_3^-$  and  $AcO^-$  was studied by UV-Vis titration tests (Fig. 3, Fig. 4). Addition of  $HCO_3^-$  and  $AcO^-$ anions (0.1 mM) to a solution of CTNP (0.04 mM) in DMSO resulted in a reduction in electronic absorption at 385nm following the build-up of a new absorbance band at 495 and 480 nm, respectively. The presence of well-defined isosbestic points at 425 nm for both anions indicated the formation of receptor-anion complexes (CTNP: $HCO_3^-$ /or  $AcO^-$ ) with no build-up of detectable byproducts or intermediates [42].



**Fig. 3:** Changes in absorption intensity upon titration of CTNP (0.04 mM) with  $HCO_3^-$  (0.1mM). Inset: absorption spectral changes with the incremental addition of  $HCO_3^-$  at selected wavelengths



**Fig. 4:** Changes in absorption intensity upon titration of CTNP (0.04 mM) with AcO<sup>-</sup> (0.1mM). Inset: absorption spectral changes with the incremental addition of AcO<sup>-</sup> at selected wavelengths.

A filling of the absorption spectra data based on modified benesi-Hildebrand equation gave association constants of receptor CTNP with  $HCO_3^-$  and  $AcO^-$  anions of  $2.24 \times 10^4$  M<sup>-1</sup> and  $9.2 \times 10^4$  M<sup>-1</sup>, respectively (Fig S8,S9).To examine the ratio of the receptor interaction with the  $HCO_3^-$  and  $AcO^-$  anions, Job's continuous variation method was employed. The maximum at a mole fraction of 0.5 with both  $HCO_3^-$  and  $AcO^-$  verify that the receptor binds to anions in a 1:1 [CTNP:  $HCO_3^-$ /or  $AcO^-$ ] ratio (Fig. 5) [43].



Fig. 5: Job's plot diagram of receptor CTNP with (a)  $HCO_3^-$  and (b)  $AcO^-$  anions

Based on the calibration curve with the good linear relationship, the detection limit of CTNP towards anions was found as low as  $2.33\mu$ M (HCO<sub>3</sub><sup>-</sup>) and  $0.55 \mu$ M (AcO<sup>-</sup>) by  $3\sigma$  method. The limit of quantitation amounts of CTNP receptor towards anions was also calculated and evaluated (Table 1).The values obtained show that LOD quantities were low enough for the detection of sub-millimolar quantities of HCO<sub>3</sub><sup>-</sup> and AcO<sup>-</sup> ions as found in many chemical systems [44].

**Table 1:** Formation obtained from UV-Vis studies upon titration of CTNP receptor with  $HCO_3^{-}/AcO^{-}$  anions in DMSO

Anion	CTNP λ <sub>ma</sub> (nm)	CTNP +anion λ <sub>ma</sub> (nm)	Isosbestic point (nm)	LOD <sup>a</sup> (mol.L <sup>-1</sup> )	LOQ <sup>b</sup> (mol.L <sup>-1</sup> )	K <sub>a</sub> (mol <sup>-1</sup> .L)
HCO <sub>3</sub> <sup>-</sup>	380	495	425	$2.33 \times 10^{-6}$	$7 \times 10^{-6}$	$2.24 \times 10^4$
AcO <sup>-</sup>	380	480	425	$5.54\times10^{7}$	$1.68  imes 10^{-6}$	$9.2  imes 10^4$

<sup>a</sup>LOD: Limit of Detection, <sup>b</sup>LOQ: Limit of Quantitation

#### 3.3. Competitive binding analysis

The possible interferences of other anions on  $HCO_3^-$  and  $AcO^-$ detection were also analyzed through competitive experiments. The absorbance changes of CTNP receptor (0.04 mM, 3.0 mL) in DMSO were measured in the presence of  $HCO_3^-$  ( 0.1mM, 300 µL) in DMSO:H<sub>2</sub>O (90:10 %; v/v) and 0.1 mM other interfering inorganic anions including  $F^-$ ,  $CI^-$ ,  $Br^-$ ,  $I^-$ ,

 $N_3^-$ , SCN<sup>-</sup>,  $NO_3^-$ ,  $NO_2^-$ , ClO<sub>4</sub><sup>-</sup>, and HSO<sub>4</sub><sup>-</sup>. The results showed no interference with the recognition of HCO<sub>3</sub><sup>-</sup> (Fig. 6). Likewise, the competitive experiments verified that tested anions showed no intervention with the recognition of AcO<sup>-</sup> anion in DMSO: H2O (90:10 %; v/v) (Fig. 7 and Figures S10 and S11) [45,46]. In order to find a suitable way to distinguish HCO<sub>3</sub><sup>-</sup> and AcO<sup>-</sup> anions, competitive experiments were performed in the presence of chloride salts of some transition metals (such as CuCl<sub>2</sub>, NiCl<sub>2</sub> and CoCl<sub>2</sub>). The color of the (CTNP/HCO<sub>3</sub><sup>-</sup>) complex in the presence of Cu<sup>2+</sup>, Ni<sup>2+</sup>or Co<sup>2+</sup> changes from orange to pale yellow (Fig. 8). While the color of the (CTNP/ ACO<sup>-</sup>) complex does not change at the same conditions (Fig. 9). Also substantial change in the absorption spectra of CTNP corresponding to the observed color changes was observed.



**Fig. 6:** Series1(light blue) represent UV-Vis response of CTNP (0.04 mM) in the presence of various anions (0.1 mM), Series 2 (navy blue) represent competitive selectivity of CTNP towards  $HCO_3^-$  (0.1 mM) in the presence of other anions.



**Fig. 7:** Series1(light blue) represent UV-Vis response of CTNP (0.04 mM) in the presence of various anions (0.1 mM), Series 2 (navy blue) represent competitive selectivity of CTNP towards  $AcO^{-}$  (0.1 mM) in the presence of other anions





**Fig. 8:** Changes in intensity and color of CTNP (0.04 mM) with HCO<sub>3</sub><sup>-</sup> (0.1mM) in the presence of transition metals.



**Fig. 9:** Changes in intensity and color of CTNP (0.04 mM) with AcO<sup>-</sup> (0.1mM) in the presence of transition metals.

#### 3.4. Response time and pH dependence studies

To determine the stability of complexes CTNP:  $HCO_3^-/or AcO^-$ , the time-dependent variations in the absorbance spectra of CTNP upon treatment with  $HCO_3^-/or AcO^-$  anions were investigated. The absorbance intensity at corresponding wavelengths reached maximum immediately in the presence of a few drops of  $HCO_3^-$  or  $AcO^-$  ions, implying the fast reaction of CTNP and anions. On the other hand, the absorbance intensities remained constant for several days, indicating that the complexes CTNP:  $HCO_3^-$  and CTNP:  $AcO^-$  are very stable ensembles (Fig. 10).



**Fig. 10:** Time dependence of receptor CTNP in the presence of  $HCO_3^-$  ion (red bar) and  $AcO^-$  ion (green bar).

The absorbance response to  $HCO_3^-/or AcO^-$  anions was also analyzed at various pH values to estimate the working pH range. At acidic pH, receptor CTNP did not display absorbance response to anions due to the protonation of  $HCO_3^-/or AcO^-$  anions. Complexes CTNP:  $HCO_3^-/or AcO^-$  showed remarkable absorbance response at neutral and basic pH values since hydroxide ion concentration of the solution is high. These results confirmed that CTNP could be used for recognition of  $HCO_3^-/or AcO^-$  anions within a pH range of 7.0-10.0 without being affected by the physiological pH [47] (Fig. 11).



Fig. 11: Changes in absorption intensity of receptor CTNP in the presence of  $HCO_3^{-}/AcO^{-}$  with pH variations

#### 3.5. Mechanistic study of CTNP receptor

To further strengthen obtained results, <sup>1</sup>H-NMR spectra of the CTNP receptor were recorded in DMSO (Fig. 12 and Fig. S12). During on addition of both  $HCO_3^-$  as well as AcO<sup>-</sup> anions, significant chemical shifts of proton signals were observed in <sup>1</sup>H-NMR spectra of CTNP. More importantly, complete deprotonation of the phenyl proton signal at 12.9 ppm due to the effect of an electron-withdrawing substituent (-CF<sub>3</sub>) occurred. At the same time, due to the deprotonation process, comparable upfield chemical shifts of phenyl ring protons and imine – CH=N proton through the shielding effect of anionic moiety were observed. The results affirm that  $HCO_3^-$  and  $AcO^-$  ions can acts as a base here and accelerate the deprotonation process during the interaction with the receptor CTNP [32,48-50].



**Fig. 12:** Partial <sup>1</sup>H-NMR (300 MHz) spectra changes of (a) CTNP in DMSO and (b) in the presence of  $HCO_3^-$  (DMSO:D<sub>2</sub>O)

### 4. Biological activity of CTNP receptor

# 4.1. Biological activity

The antimicrobial activity of CTNP was evaluated in vitro against both fungal and bacterial strains by usual techniques.

#### 4.2. Antifungal study

The antifungal test of CTNP was examined against *Aspergillusniger*(ATCC 16404) and *Aspergillusbrasiliensis* (PTCC 5011) strains by agar well diffusion technique. Active fungal were grown on Muller Hinton Agar medium in Petri plates. Three wells of 6 mm diameter were made. After injection of micro-organisms, the CTNP solution (100 $\mu$ L, 300  $\mu$ g/mL) were loaded on Petri plates and plates were incubated at 37 °C for 24 hours. The diameter zone of inhibition (mm) was measured and compared with Nystatin as the reference (Table 2). A

comparative study of the MIC (Minimum inhibitory concentration) and MBC (Minimum bactericide concentration) magnitudes for CTNP and reference indicated that CTNP prevented the fungal growth strains both *Aspergillusniger* and *Aspergillusbrasiliensis* [51,52].

#### 4.3. Antibacterial study

The antibacterial test of CTNP was assessed against bacterial strains by disc diffusion technique. In this technique, blotting paper discs of 8 mm diameter were prepared for the dipping into different levels of concentration solutions of CTNP at room temperature. Nutrient agar medium was used to activate bacterial culture on Petri plates containing mentioned paper discs and incubated overnight at 37°C to monitor growth inhibition of the bacteria. The diameter zone of inhibition (mm) around the discs were measured and compared with that of *Gentamicine* as standard drug (Table 2).The CTNP receptor inhibits the growth of staphylococcus *epidermidies* (ATCC 12228) [53,54].

Micro organism	Diameter of inhabitation (mm)	Inhabitation with	MIC	MBC	•
		reference			_
Staphylococcus epidermidis	12	35 <sup>a</sup>	125	250	•
Aspergillusniger	13	27 <sup>b</sup>	2000	-	
Aspergillusbrasiliensis	13	30 <sup>b</sup>	2000	-	

Table 2: Biological activity results of the CTNP receptor in vitro

<sup>a</sup> Gentamicin as reference for bacterial strains, <sup>b</sup> Nystatin as reference for fungal strains

#### 5. Conclusion

A new Schiff base framework is synthesized starting from naphthalene based azo ligand. The designed structure acts as an efficient receptor for the hydrogen carbonate and acetate ions with high selectivity over other test anions. The sensor system shows a drastic color change upon treatment withanions mentioned above, paving the way for visual recognition ofions. Under conditions of UV-Vis spectral displacement, the excellent binding affinity of receptor towards anions was observed. The synthesized ligand exhibited good potential as and antifungal against antibacterial property vitro Gram-positive in bacteria (staphylococcusepidermidies) and fungal (Aspergillusniger and Aspergillusbrasiliensis) strains.

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#### 7. Conflict of interest

The authors declare no conflict of interest.

#### 8. Appendix

Supplementary data to this article can be found online at

#### 9. References

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# Author Contributions Section

Dear Editor of *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* The manuscript entitled "Simultaneous measurement of hydrogen carbonate and acetate anions using biologically active receptor based on azo derivatives of naphthalene" by Fatemeh Naderi, Masoumeh Orojloo, Mojgan Zendehdel, and Saeid Amani for publication in the *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* as a research article. This submission is approved by all authors for publication in this Journal. I confirm that this manuscript is not under consideration for publication in any other Journal and if accepted, it will not be published elsewhere in the same form in English or in any other language.

Best Wishes and Kindest Regards Saeid Amani, Ph.D. Professor of Inorganic Chemistry, Chemistry Department, Arak University, Dr. Beheshti Ave. Arak 38156-88349, Iran E-mail: **s-amani@araku.ac.ir** and alternative E-mail: amani1331@yahoo.com Phone office: +98 86 34173415; Fax office: +98 86 34173406

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**Declaration of Interest Statement** 

The authors declare no conflict of interest.

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# **Graphical Abstract:**

A novel Schiff base receptor based on azo-derivatives of 1-naphthylamine (2-((E)-((4-chloro-3-(trifluoromethyl)phenyl)imino)methyl)-4-((E)-naphthalene-1-yldiazenyl)phenol(2) abbreviated CTNP was synthesized. Systematic studies of CTNP with HCO<sub>3</sub><sup>-</sup> and AcO<sup>-</sup> anions in DMSO disclosed that there is hydrogen-bonding between CTNP and incoming anions. The antifungal activity of the receptor was tested against*Aspergillusbrasiliensis*and*Aspergillusniger*. CTNP exhibited excellent antifungal activity against both strains. CTNP also represented antibacterial activity against Gram-positive bacteria:*staphylococcus epidermidies*.



### **Highlights:**

- 1. A new molecular receptor (CTNP), based on azo-derivatives of 1-naphthylamine synthesized.
- 2. CTNP exhibited a colorimetric and optical dual-mode response.
- 3. Excellent detection of HCO<sub>3</sub><sup>-</sup> and ACO<sup>-</sup> with low detection limit could be obtained with CTNP receptor.
- 4. The designed receptor also represented antimicrobial activity in vitro against both fungal and bacterial strains.