Synthesis of sensitive novel dual Signaling Pyridopyrimidine-Based Fluorescent "Turn off" Chemosensors for Anions determination

Abida Ashraf[1,2], Misbah Khizar[1], Muhammad Islam[2], Abdul Hameed[3], Syed Tarique Moin[3], Muhammad Yaqub[2], Waqar Rauf[4], Muhammad Moazzam Naseer[5], Muhammad Tayyeb Ahsan[2], Zahid Shafiq[2], Javid Hussain[6], Ahmed Al-Harrasi[7], Ricard Boqué[8] and Fazal Mabood[6*]*

[1] *Department of Chemistry, Kutchery Campus, The Women University Multan, Multan 60000, Pakistan.*

[2] *Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan (zahidshafiq@bzu.edu.pk).*

[3] *H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.*

[4] *National Institute for Biotechnology and Genetic Engineering (NIBGE), P. O. Box 577, Faisalabad, Pakistan*

[5] *Department of Chemistry Quaid-i-Azam University Islamabad, 45320, Pakistan*

[6]*Department of Biological Sciences & Chemistry, College of Arts and Sciences, University of Nizwa, Nizwa-616, Oman, (mehboob@unizwa.edu.om,)*

[7]*Natural and Medical Sciences Research Center, University of Nizwa, PC 616, Nizwa, Sultanate of Oman.*

[8]*Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Tarragona, Spain.*

Abstract

The synthesis of 2-hydroxybenzoyl pyridopyrimidines (**M1-M4**) has been carried out with 3-formyl chrome derivatives and 6-amino-1-methyluracil to employ them as anion chemosensors. Pyridopyrimidine-based compounds were studied as colorimetric and fluorescent receptors for anion sensing. The observations with naked eye and the data obtained by UV-visible, fluorescence and ^{1}H NMR spectroscopy demonstrated selectivity for fluoride, acetate and

cyanides among the pool of different anions. However, the spectral data involving fluoride anion showed particularly sharp and strong interactions. In sensing process, the binding of pyridopyrimidine amino (NH) and hydroxyl groups (OH) *via* hydrogen bonding $F^{-}\cdots H-N/-O$ induced the colorimetric and "turn off" fluorescent response. The binding mode of action was further studied by ^{1}H NMR titrations that supported gradual deprotonation mechanism. The fluoride ion detection limit was in the range of 3.51×10^{-8} M to 1.77×10^{-6} M for probes **M1-M4** and were lower than the maximum permissible concentration of F^{-} ion in drinking water (5.3×10^{-3} M) set by World Health Organization (WHO) that was found to be influenced by the nature of aromatic substituents. Theoretical study of receptor interactions with fluoride anion *via* O-H and N-H groups was also carried out at the quantum mechanical level.

Keywords: pyridopyrimidines, colorimetric, fluoride ion sensors, substituent effect, "turn off" fluorescent

1. Introduction

Small molecules have recently seen their increasing utility as anion chemosensors followed by a great interest by the researchers owing to the potential of the area in various analytical applications in drinking water, biological and ecological systems[1-4]. The detection of anions in many agricultural and environmental processes is vital as the released anions, if left unchecked, can have overwhelming effects[5,6]. There are 70% anions of all enzymatic sites which are vital for storage of genetic information. Thus it is mandatory to develop a technique for the recognition and quantification of anions exhibiting discriminate interactions with receptor

sites in chemosensors preferably by hydrogen bonding or by proton transfer modes prevalent in biological and chemical systems[7].

Among the innumerable anions, fluoride ion (F^- ion) is of principal importance because it has been used in preclusion of dental caries and osteoporosis[8-10]. However surplus intake of fluoride ions mainly results in severe health problems like gastric and kidney syndrome, skeletal fluorosis and nephrolithiasis[11-13]. Therefore, there is substantial interest among scientific community to devise methods for monitoring trace level concentrations of target anions[14,15]. For this, various conventional techniques (like neutron activation analysis, hydride generation atomic fluorescence spectrometry) have been well reconnoitered. Those techniques, however, have complicated operational procedures. On the other hand, the colorimetric and fluorometric methods for anion sensing at lower level concentration monitoring are precise, selective and sensitive and are acknowledged in medicine, biology or in the world of chemistry[16-20].

Regarding fluoride anion sensing, many new and innovative chemosensors are still required that has the ability to sense fluoride anion in biochemically challenging solvents like dimethyl formamide (DMF), alcohols and dimethyl sulfoxide (DMSO) where many of the reported chemosensors do not perform well[21-24]. In this context, a number of chemosensors have been synthesized so far, based on fluorescence resonance energy transfer (FRET), proton transfer (PT) or excited state proton transfer (ESPT), photoinduced electron transfer (PET) and intramolecular charge transfer (ICT) mechanisms. However, chemosensors based on proton transfer/hydrogen bonding mechanism are getting increased attention from scientific community[7]. Interestingly, most of these chemosensors contain pyrrole, indole, urea, thiourea, amide and uracil moieties, and a far less attention has been given to the compounds having OH group to be employed as chemosensors despite their higher acidity[25] (Figure 1).

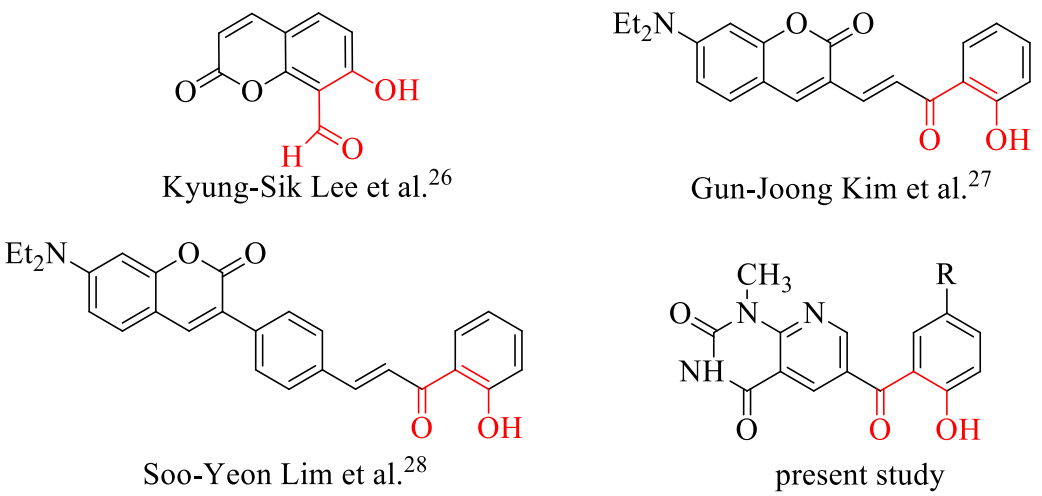


Figure 1. 2-hydroxy benzoyl based receptors and present study.

Herein, in perpetuation of our work on development of novel anion chemosensors, we report 2-hydroxybenzoyl pyridopyrimidine chemosensors for anions especially the fluoride anion. Pyridopyrimidines are well known heterocycles and associated with many pharmacological properties such as CNS disorder, antiviral, anticancer agents[29].

According to literature[26-28] the probes with *o*-hydroy benzoyl group are mostly used for sensing of thiol or cyanide containing amino acids. Related chemosensors of this class like coumarin derived probes known to sense for cyanide and thiol containing amino acids.

To the best of our knowledge, this is the first report in which pyridopyrimidines have been explored as potential chromogenic and fluorogenic chemosensors for fluoride anions. Importantly, the recognition potential of this class of compounds can be tuned by the nature of substituents *i.e.*, by the incorporation of electron donating groups (CH_3) and electron withdrawing groups (F, Cl) which are able to affect anion recognition sensitivity.

2. Experimental

2.1. Materials and Methods. 3-formylchromone (Aldrich), 6-methyl-3-formylchromone (Aldrich), 6-chloro-7-methyl-3-formychromone (Aldrich), 6-fluoro-3-formylchromone (Aldrich), 6-amino-1-methyluracil (Aldrich) and all the tetrabutylammonium salts of analytical grade were used in this study.

Shimadzu 840/Shimadzu prestige-21 and Bruker alpha FT-IR were used for the measurement of FT-IR spectra of the samples. ^{1}HNMR and ^{13}C NMR spectra were recorded using Bruker (Rhenistetten-Forchheim, Germany) AM 300 MHz and 75 MHz spectrometers. UV-Visible and fluorescence spectra were recorded using Shimadzu UV-1800 and spectrofluorophotometer Shimadzu RF-6000 respectively.

2.2. Synthesis of Chemosensors (M1-M4).

General Procedure.

After 5 to 6 hours continues refluxing and stirring of a mixture of (un)substituted 3-formyl chromone (1 mmol), 6-amino-1-methyluracil (1 mmol) and catalytic amount of *p*-toluenesulfonic acid in 10 mL THF at 65 ^{o}C a yellow colored product of 2-hydroxybenzoyl pyridopyrimidines (M1-M4) was formed. The progress of this reaction was monitored by TLC. The reaction mixture was filtered under hot conditions to afford the pure product (Scheme-1).

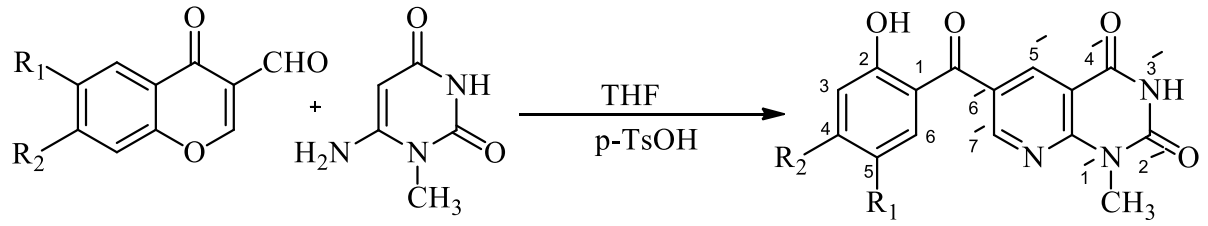
M1 (pale yellow), Yield; 94%, mp 250-254 ^{o}C (244-246 ^{o}C. lit [30]; IR (KBr, cm^{-1}): 3120, 3040 (OH, NH), 1717, 1685, 1620 (C=O), 1595, 1492, 1465 (aromatic C=C), 1320,1280,1240 (C-O), 800, 745 (aromatic out of plane bend) ; ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 3.54 (3H, s, N-CH$_3$), 7.00 (2H, t, $^{3}J_{H,H}$ = 8.1 Hz, benzyl CH), 7.43 (1H, d, $^{3}J_{H,H}$ = 7.5 Hz, benzyl CH), 7.51 (1H, d, $^{3}J_{H,H}$ = 7.5 Hz, benzyl CH), 8.43 (1H, s, pyridyl CH), 8.99 (1H, s, pyridyl CH), 10.38 (1H, $_{S}$, OH), 11.94 (1H, s, NH) ppm; ^{13}C NMR (DMSO- d_6, 75 MHz) δ = 29.1, 111.0, 117.2, 120.0, 124.9, 128.2, 130.9, 134.2, 137.9, 151.0, 154.6, 155.1, 156.8, 161.4,

193.9 ppm; MS (ESI) m/z 296.17 $[M-H]^-$; Anal. Calcd for $C_{15}H_{11}N_3O_4$: C, 60.61; H, 3.73; N, 14.14%; found: C, 60.57; H, 3.70; N, 14.11%.

M2 (light yellow), Yield; 92%, mp 256-258 ^oC; IR (KBr, cm^{-1}): 3212, 3093 (OH, NH), 1705, 1690, 1626 (C=O), 1449 (aromatic C=C), 1328, 1247, 1181 (C-O), 942, 840, 807, 713, 677 (aromatic out of plane bend); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.52 (3H, s, C-CH_3), 3.53 (3H, s, N-CH_3), 6.90 (1H, d, $^3J_{H,H}$ = 8.4 Hz, benzyl CH), 7.21 (1H, s, benzyl CH), 7.29 (1H, dd, $^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 2.1 Hz, benzyl CH), 8.42 (1H, d, $^4J_{H,H}$ = 1.8 Hz, pyridyl CH), 8.97 (1H, d, $^4J_{H,H}$ = 1.8 Hz, pyridyl CH), 10.13 (1H, s, OH), 11.93 (1H, s, NH) ppm; ^{13}C NMR (DMSO- d_6, 75 MHz) δ = 20.3, 29.1, 111.0, 117.1, 124.6, 128.3, 128.7, 130.7, 134.9, 137.9, 151.0, 154.5, 154.6, 155.0, 161.4, 193.9 ppm; MS (ESI) m/z 310.17 $[M-H]^-$; Anal. Calcd for $C_{16}H_{13}N_3O_4$: C, 61.73; H, 4.21; N, 13.50%; found: C, 61.78; H, 4.24; N, 13.48%.

M3 (yellow), Yield; 75%, mp 284-286 ^oC; IR (KBr, cm^{-1}): 3213, 3091 (OH, NH), 1717, 1690, 1625 (C=O), 1570, 1498, 1474, 1450 (aromatic C=C), 1373, 1328, 1247, 1182, 1071 (C-O), 994 (aromatic out of plane bend); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.34 (3H, s, C-CH_3), 3.52 (3H, s, N-CH_3), 6.96 (1H, s, benzyl CH), 7.42 (1H, s, benzyl CH), 8.42 (1H, d, $^4J_{H,H}$ = 2.1 Hz, pyridyl CH), 8.98 (1H, d, $^4J_{H,H}$ = 2.4 Hz, pyridyl CH), 10.53 (1H, s, OH), 11.94 (1H, s, NH) ppm; ^{13}C NMR (DMSO- d_6, 75 MHz) δ = 20.5, 29.1, 111.0, 119.7, 124.1, 124.4, 128.1, 130.4, 137.9, 141.8, 151.0,154.6, 155.2, 155.4, 161.4, 192.1 ppm; MS (ESI) m/z 344.17 $[M-H]^-$; Anal. Calcd for $C_{16}H_{12}N_3O_4Cl$: C, 55.58; H, 3.50; N, 12.15%; found: C, 55.60; H, 3.48; N, 12.13%.

M4 (yellow), Yield; 77%, mp 214-216 ^oC; IR (KBr, cm^{-1}): 3163, 3119, 3026 (OH, NH), 1721, 1675, 1605 (C=O), 1465 (aromatic C=C), 1326, 1280, 1222, 1172, 1069 (C-O), 988, 869, 834, 786, 736, 680 (aromatic out of plane bend); ^1H NMR (DMSO- d_6, 300 MHz): δ = 3.53 (3H, s, N-CH_3), 7.00 (1H, dd, $^3J_{H,H}$ = 9.0 Hz, $^4J_{H,F}$ = 4.5 Hz, benzyl CH), 7.24 (1H, dd, $^3J_{H,F}$ = 8.7 Hz, $^4J_{H,H}$ = 3.3 Hz, benzyl CH), 7.33 (1H, td, $^3J_{H,F}$ = 8.7 Hz, $^4J_{H,F}$ = 3.3 Hz, benzyl CH), 8.43 (1H, d, $^4J_{H,H}$ = 2.4 Hz, pyridyl CH), 8.99 (1H, d, $^4J_{H,H}$ = 2.4 Hz, pyridyl CH), 10.26 (1H, s, OH), 11.95 (1H, s, NH) ppm; MS (ESI) m/z 314.17 $[M-H]^-$; Anal. Calcd for $C_{15}H_{10}FN_3O_4$: C, 57.15; H, 3.20; N, 13.33%; found: C, 57.20; H, 3.17; N, 13.29%.



M1- M4

M1	R_1=H,	R_2=H
M2	R_1=CH_3,	R_2=H
M3	R_1=Cl,	R_2=CH_3
M4	R_1=F,	R_2=H

Scheme 1. One pot synthesis of chemosensors (**M1-M4**).

2.3. UV-visible, Fluorescence and FT-IR Titrations.

For anion sensing the stock solution of probe **M1-M4** of 1 x 10^{-3} M concentration was prepared in DMSO/CH_3CN using (1:9 v/v) solvent ratio. Similarly the stock solutions of 1 x 10^{-2} M concentration of different anion sources like F^-, Cl^-, I^-, ClO_4^-, Br^-, CN^-, SCN^-, HSO_4^- and AcO^-, from TBA salts were also prepared in DMSO/ CH_3CN solvent using (1:9 v/v) solvent ratio. The sensing ability of the new synthesized sensor probe M1-M4 for different anions (F^-, Cl^-, I^-, $ClO4^-$, Br^-, CN^-, SCN^-, $HSO4^-$ and ACO^-) was checked by measuring the absorption as well as emission intensity when titrated with different anions. The overlay FT-IR spectra of probe **M3** has been examined in DMSO.

2.4 ^{1}H-NMR Titrations

Similarly the ^{1}H-NMR titrations of M4 (1 x 10^{-2} M in DMSO-d_6) with different equivalents (0-6) of fluoride ion source of TBAF in DMSO-d_6 was also performed by using Bruker (Rhenistetten-Forchheim, Germany) AM 300 MHz spectrometer to record the^{1}H NMR spectrum of the probes **M1-M4** with different anions .

3. Results and Discussion

One step synthesis of the compounds **M1-M4** as probes (receptors) were achieved (Scheme 1) in good yields. The structures of all the probes were characterized by different spectroscopic techniques including FTIR and NMR spectroscopy. The IR spectrum of the compounds **M1-M4** showed characteristic OH and NH stretching frequencies at 3213-3026 cm^{-1} and the C=O stretching bands were observed at 1721-1589 cm^{-1} respectively. The ^{1}H NMR spectrum of the compounds **M1-M4** showed two characteristic broad singlets (brs) at δ_H 11.93-11.95 ppm and δ_H 10.13-10.53 ppm for NH of pyridopyrimidine and phenolic OH groups, respectively. The pyridine aromatic ring protons appeared around δ_H 8.42-8.99 ppm. The aromatic phenyl ring protons appeared at δ_H 6.90-7.50 ppm. The ^{13}C NMR spectrum of probes **M1-M4** showed peaks at δ_C 192.09-193.96 ppm for carbonyl (C=O) carbon and methyl (N-CH_3) peaks appeared at 29.10-29.11 ppm (Figure-S1).

3.1. Colorimetric Analysis and UV-visible Spectral Studies.

The sensing ability of probes **M1-M4** with different anion anions (F^-, Cl^-, I^-, ClO_4^-, Br^-, CN^-, SCN^-, HSO_4^- and ACO^-) were first observed *via* naked eye to depict colour changes when titrated with different anions. An intense colour change, form colourless to yellow, was observed upon the addition of fluoride anion while the changes were less prominent with acetate and cyanide. However, upon the addition of other anions, no colour change was observed (Figure-2/S2). The color changes of pyridopyrimidine based receptors **M1-M4** on addition of fluoride anion may be attributed to intramolecular charge transfer (ICT) process between phenolic –OH, imine –NH and carbonyl groups of probes *via* hydrogen bond interaction with anions (F^-, OAc^- or CN^-)[31-32]. These observations suggest a host-guest complex formation between probes and anions.

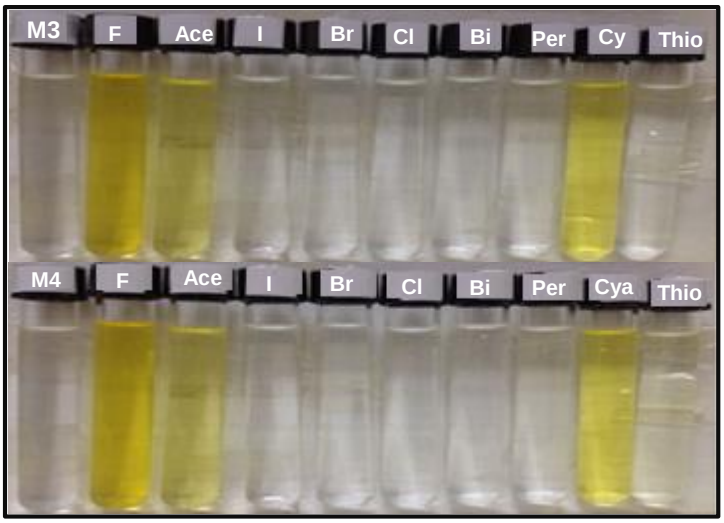


Figure 2. Visual colorimetric responses of chemosensors with different anions.

The interaction/binding ability of receptors **M3** and **M4** with various anions (F^-, Cl^-, I^-, ClO_4^-, Br^-, CN^-, SCN^-, HSO_4^- and ACO^-) was further studied by using UV-visible spectroscopic technique (Figure-3). The UV-Visible spectral profiles of probes **M1-M4** on addition of different anions (10 equiv.) have been shown in the Table-1.

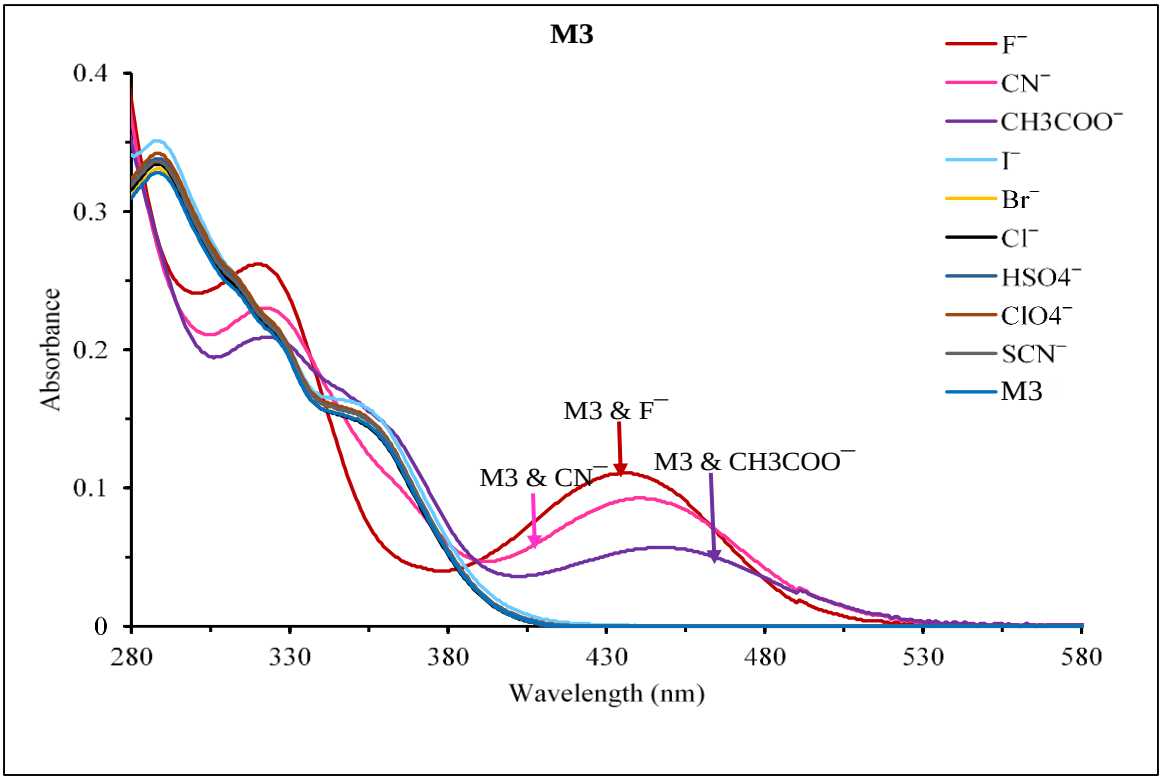
Table1. UV-visible bands for probes **M1-M4.**

Probes	UV-visible spectral bands		Fluoride addition*	Cyanide addition*	Acetate addition*
M1	287 nm	318 nm	422 nm	429 nm	-
M2	287 nm	357 nm	434 nm	441 nm	-
M3	288 nm	353 nm	436 nm	440 nm	445 nm
M4	290 nm	350 nm	437 nm	443 nm	447 nm

* New spectral band

The UV-visible spectra of model receptors **M3** and **M4** without TBAF showed bands at 288 nm, 353 nm and 290 nm, 350 nm, respectively, which are assigned to $\pi-\pi^*$ and $n-\pi^*$ transitions. Upon addition of fluoride anion source (TBAF, 10 equiv.) to model receptor **M4**, the absorbance band at 350 nm was slightly decreased and a new charge transfer band (bathochromic

shift) appeared at 437 nm which indicate the formation of host-guest complex between ligands and anion *i.e.* F^- ion. Similar changes in the UV-visible spectrum of other probes **M1**, **M2** and **M3** have been observed (Table-1). The bathochromic shift may be attributed to ICT transition (between keto-enol tautomeric forms) and hydrogen bonding broken by F^- ion thereby producing deprotonated imine and phenoxide anions. Similar pattern, though slightly less intense, was observed on addition of acetate and cyanide anions (Figure-3/S3).



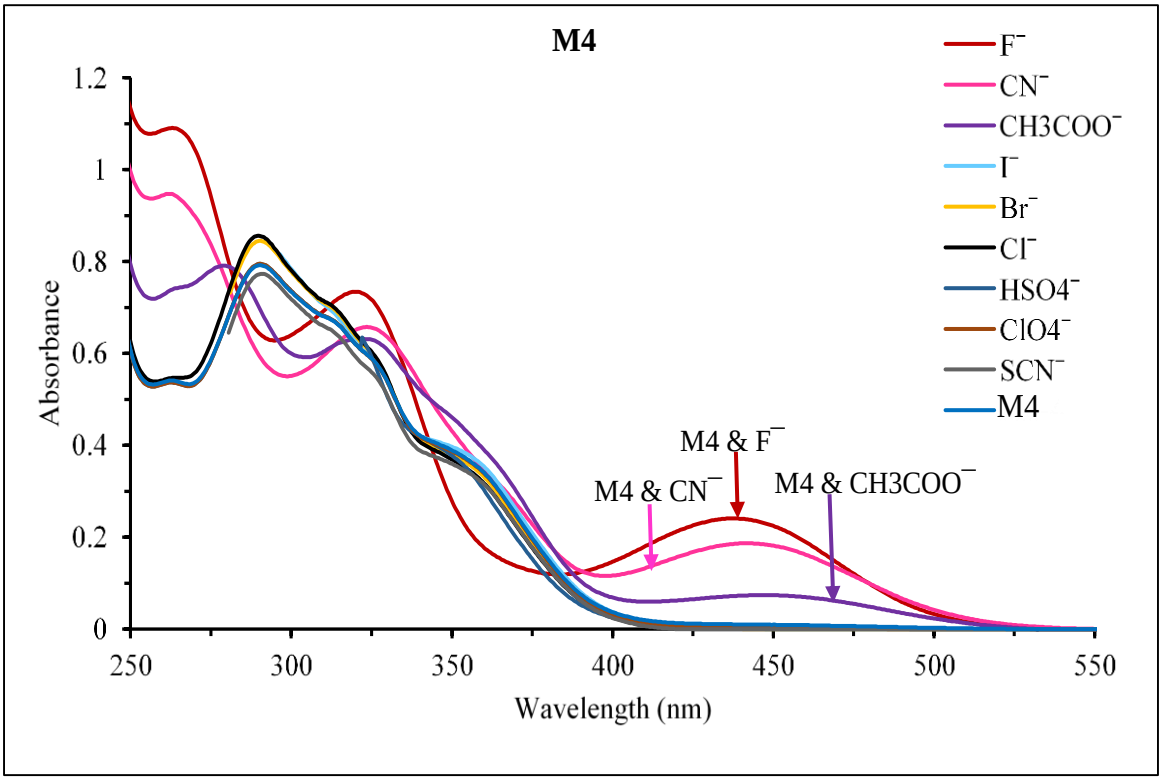


Figure 3. Absorption spectra for **M3** ($1x10^{-3}$ mol L^{-1}) and **M4** ($1x10^{-3}$ mol L^{-1}) in DMSO/ CH_3CN (1:9 v/v) solution with addition of different anions ($1x10^{-2}$ mol L^{-1}).

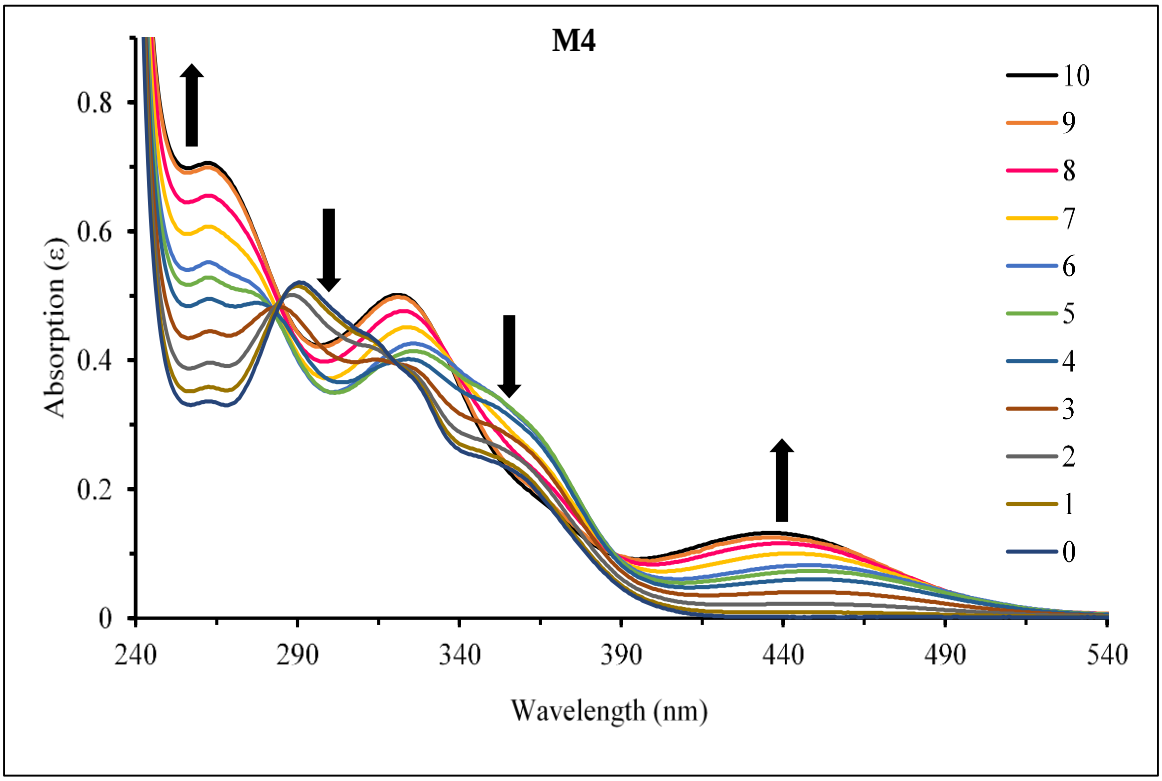


Figure 4. Absorption spectra for **M4** ($1x10^{-3}$ mol L^{-1}) in DMSO/CH_3CN (1:9 v/v) solution with addition of fluoride anion (0-10 equiv.).

To study the ratiometric response of probes with fluoride ion, absorption titrations were carried out with fluoride source (TBAF) against the receptors **M1-M4**. The changes in absorption spectra upon ratiometric addition of F^- ion to the solution of a model receptor **M4** are shown in the (Figure-4). Moreover, a new band with red shift appeared at 437 nm and isosbestic points observed at 286 and 387 nm indicate the formation of complex with F^- ion *via* hydrogen bond interaction with -OH/-NH groups and hydrogen bonding broken by F^- ion thereby producing deprotonated free imine and phenoxide anions. For the other three probes **M1**, **M2** and **M3**, the absorption bands at 318 nm, 357 nm, and 353 nm decreased with the concomitant rise of new bands at 422 nm, 434 nm, and 436 nm and the isosbestic points for three receptors were observed at 284,311 nm **(M1)**, 277,310 nm **(M2)**, and 285,386 nm **(M3)** (Figure-S4).

The continuous variation method was used to calculate the stoichiometric ratio of the receptors **M1-M4**. The Job's plot of probes **M1-M4** with F^- ion in DMSO/ CH_3CN (1:9 v/v) showed maxima at mole fraction of 0.3 which means receptor (M1-M4) and F^- ion form a 1:2 receptor-anion complex (Figure-S5). The binding stoichiometries of (**M1-M4**) were calculated in accordance with Benesi-Hildebrand equation [33-35] (Figure-S6). The association constants (K) between compounds (**M1-M4**) and F^- ion were found to be $1.49x10^6$ Mol^{-1} **(M1)**, $2.83x10^4$ Mol^{-1} **(M2)**, $1.98x10^6$ Mol^{-1} **(M3)** and $1.96x10^6$ Mol^{-1} **(M4)**.

Further the detection limit from absorption experiment was calculated using the general formula $3\delta/S$[7] (Figure-S7). The LOD of probes **M1-M4** for F^- ion were found to be $2.49x10^{-8}$ M, $1.77x10^{-6}$ M, $3.51x10^{-8}$ M and $3.33x10^{-8}$ M, respectively. The detection limits observed for probes **M1-M4** were lower than the maximum permissible concentration of F^- ion in drinking

water (5.3 x 10^{-3} M) set by World Health Organization[36] (WHO). The association constants (K) and LOD of compounds (**M1-M4**) have been shown in Table 2.

Table 2. Comparison of best reported Fluoride ion chemosensors with the present work.

Sr. No.	Receptors	Association constant (M^{-1})	Detection limit (M)	Reference
1	Diketopyrrolopyrrole based sensor	3.24 x 10^7 M^{-2} (F^{-1})	4.20 x 10^{-8} (F^{-1})	[37]
2	Diketopyrrolopyrrole based sensor	2.69x 10^7 M^{-2} (F^{-1})	4.07 x 10^{-8} (F^{-1})	[38]
3	Bis-thiazol-aminophenol based sensor	1.57 x 10^7 (F^{-1})	4.10 x 10^{-7} (F^{-1})	[7]
4	Anthraimidazoledione Based sensor	4.69 x 10^4 (F^{-1})	5.78 x 10^{-7} (F^{-1})	[39]
5	Coumarin-Thiosemicarbazones based sensor	3.48 x 10^4 (F^{-1})	4.34 x 10^{-6} (F^{-1})	[19]
6	Acridine-based thiosemicarbazones sensor	2.86 x 10^3 (F^{-1})	6.17 x 10^{-5} (F^{-1})	[20]
7	Naphthalene-based Azo-azomethine sensor	1.77 x 10^4 (F^{-1})	2.04 x 10^{-6} (F^{-1})	[40]
8	Uracil based sensor	4.69 x 10^4 (F^{-1})	5.78 x 10^{-7} (F^{-1})	[41]
9	**M1**	1.49x10^6 (F^{-1}) 1.79x10^3 (CN^{-1})	2.49x10^{-8} (F^{-1}) 0.68x10^{-5} (CN^{-1})	The present work
10	**M2**	2.83x10^4 (F^{-1}) 0.69x10^3 (CN^{-1})	1.77x10^{-6} (F^{-1}) 4.09x10^{-5} (CN^{-1})	The present work
11	**M3**	1.98x10^6 (F^{-1}) 2.33x10^4 (CN^{-1}) 3.21x10^3 (AcO^{-1})	3.51x10^{-8} (F^{-1}) 1.29x10^{-6} (CN^{-1}) 0.21x10^{-5} (AcO^{-1})	The present work
12	**M4**	1.96x10^6 (F^{-1}) 3.09x10^4 (CN^{-1}) 1.81x10^3 (AcO^{-1})	3.33x10^{-8} (F^{-1}) 1.79x10^{-6} (CN^{-1}) 3.19x10^{-5} (AcO^{-1})	The present work

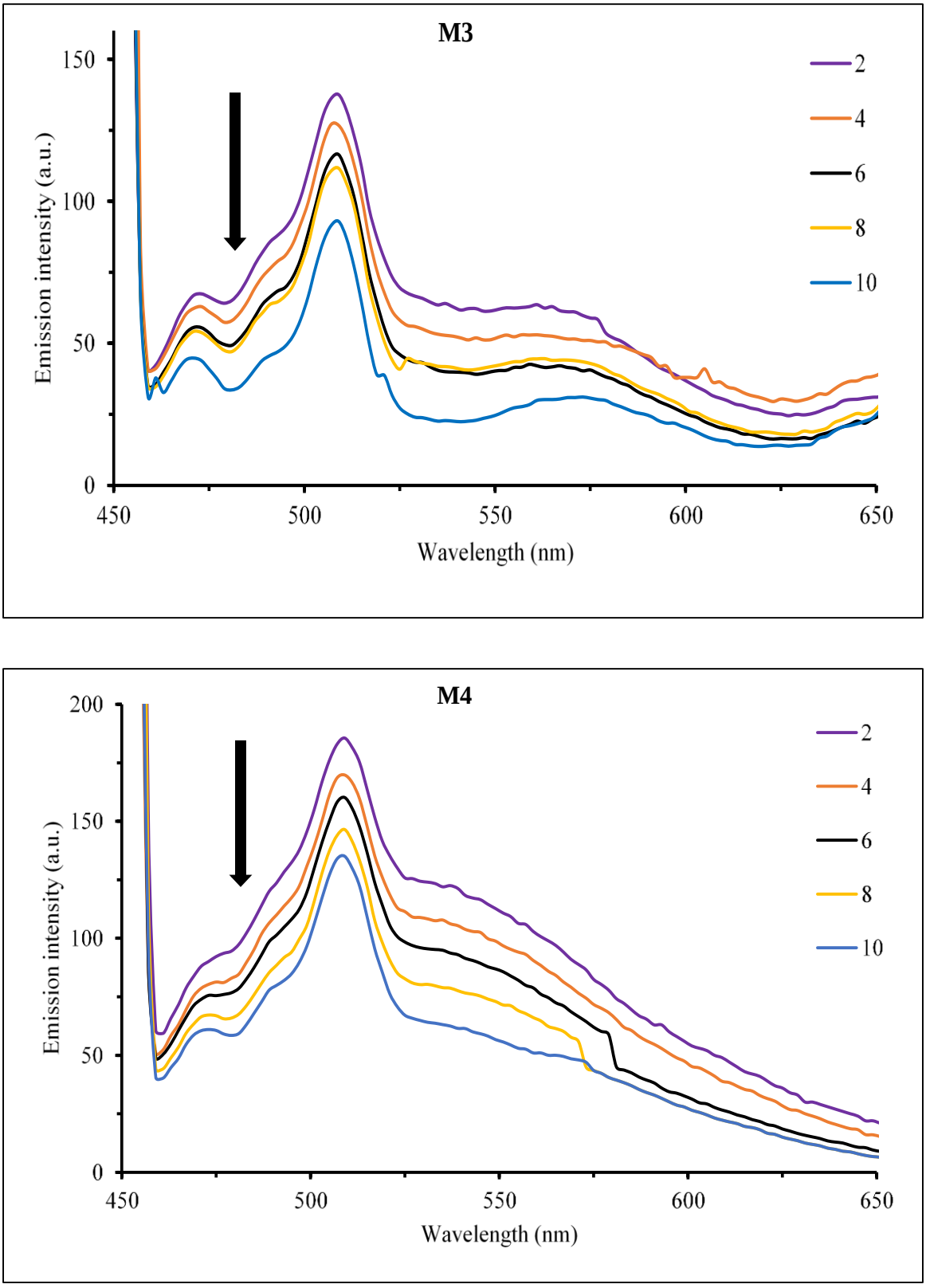
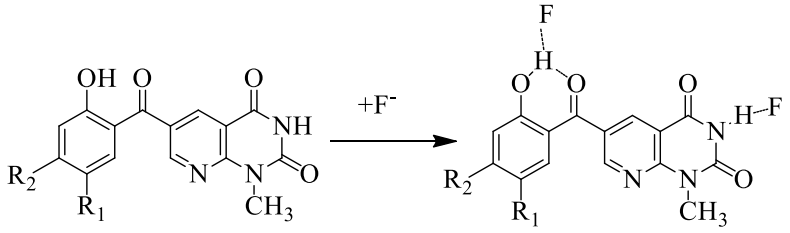


Figure 5. Fluorescence spectra (λ_{ex} = 460 nm) for **M3** and **M4** ($1x10^{-3}$ mol L^{-1}) in DMSO/CH_3CN (1:9 v/v) solution with addition of fluoride anion (10 equiv.).

3.2 Fluorescence Studies.

The anion sensing ability of receptors **M1-M4** was monitored by using Shimadzu RF-6000 spectrofluorophotometer *via* fluorescence titrations in identical conditions that gave bounteous information regarding the "*turn off*" behavior of compounds (**M1-M4**). For the compounds (**M1-M4**) the excitation wavelength was optimized as 440 nm for **M1** and 460 nm for **M2-M4** (Figure-5/S8). The compounds (**M1-M4**) showed emission maxima at 493, 511, 509 and 509 nm, respectively. Upon the addition of anion source (TBAF), the fluorescence spectra of **M3-M4** (Figure-5) showed the emission changes *via* the formation of anion-receptor hydrogen bonding complex. A significant fluorescence quenching was observed in the emission intensity of the receptors **M3** and **M4** with stepwise addition of F^- ion (TBAF 10 equivalents) *via* deprotonation of NH and OH groups following photoinduced electron transfer (PET) mechanism. Moreover, the formation of hydrogen bond between $F^- \cdots H-N/-O$ causes proton transfer *via* ESPT process, which is also supposed to be sensing mechanism involved in fluoride fluorescent chemosensors[42-44].



M1-M4

M1	R_1=H,	R_2=H
M2	R_1=CH_3,	R_2=H
M3	R_1=Cl,	R_2=CH_3
M4	R_1=F,	R_2=H

Figure 6. Plausible mechanism of binding **M1-M4** with F^- ion.

3.3 ^{1}HNMR Titrations

To examine the nature of binding sites of host-guest, ^{1}HNMR titration experiments with a model chemosensor **M4** were carried out in DMSO-d_6 by stepwise addition of TBAF (6 equivalents) as shown in the (Figure-7). ^{1}HNMR spectra of **M4** showed characteristic signal at δ_H 11.95 ppm corresponding to NH protons and the signal at δ_H 10.26 ppm corresponding to OH protons. The peaks at δ_H 11.95 ppm and δ_H 10.26 ppm were observed to disappear completely by the addition of 2 equivalent of fluoride anion.

Upon addition of two equivalent of fluoride anion, the signals of protons on phenyl (H_a, H_b, H_c) and pyridine (H_d, H_e) ring shifted slightly upfield. When 6 equivalents of fluoride anion were introduced compared with free sensor (Figure-7), a theatrical change was observed in signals of phenyl and pyridine protons to upfield (H_a, 0.43; H_b, 0.36; H_c, 0.33; H_d, 0.10; H_e, 0.21 ppm) which suggests the increase of electron distribution in chromophore when the –OH and –NH protons were deprotonated[45-48].

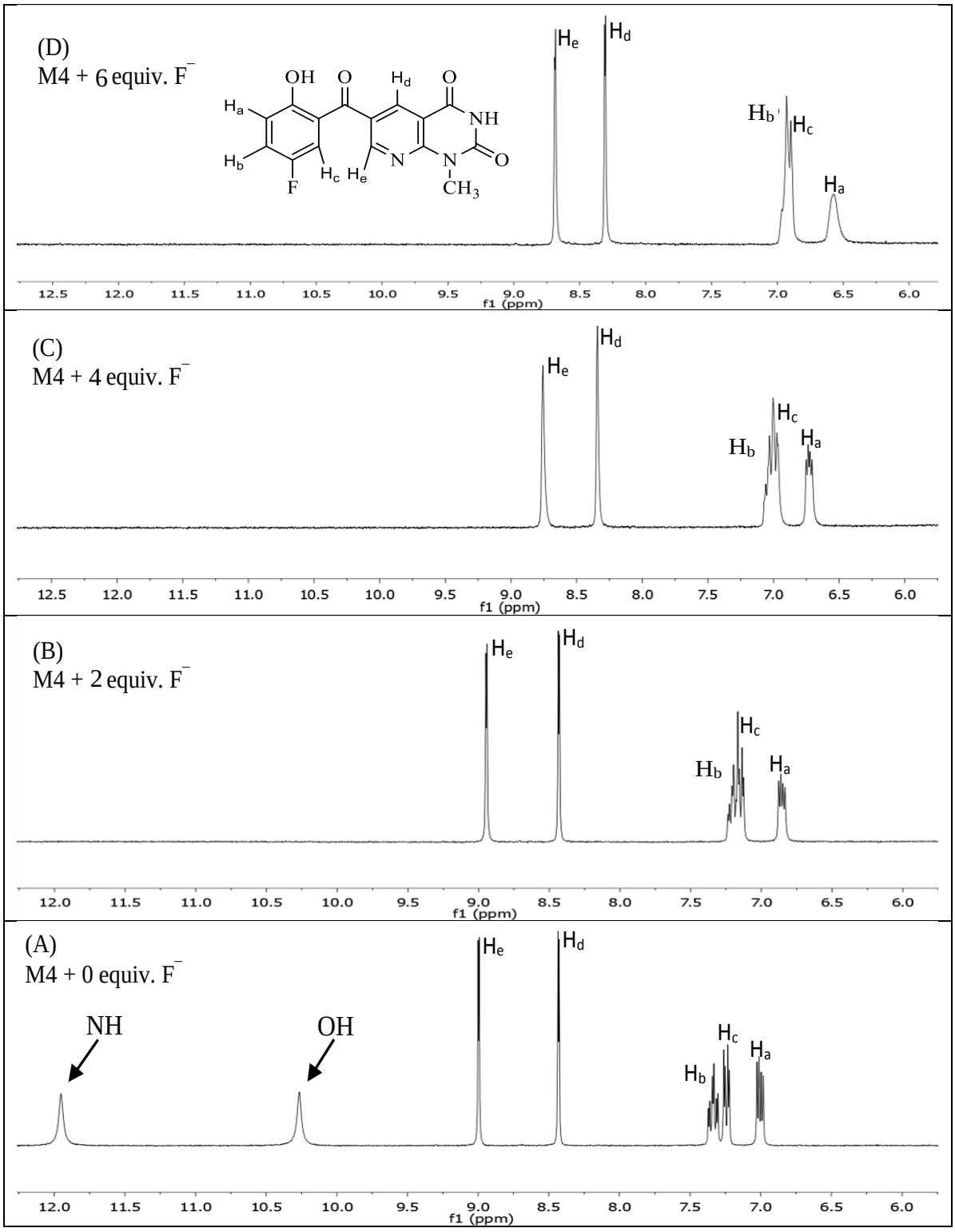


Figure 7. Study of fluoride anion addition to probe **M4** via ^{1}HNMR spectra; (A) without F^- (B) with 2 equiv. of F^- ion (C) with 4 equiv. of F^- ion and (D) with 6 equiv. of F^- ion source (TBAF) in DMSO-*d*$_6$ as solvent.

3.4 FTIR Titrations

The binding discrepancy of F^- ion in probe **M3** has also been examined by using Bruker Alpha FT-IR spectrometer. In case of free receptor **M3**, the N-H, O-H stretching frequencies were observed at 3213, 3091 cm^{-1}, uracyl carbonyl (C=O) bands at 1717 and 1690 cm^{-1} and benzoyl carbonyl (C=O) observed at 1625 cm^{-1}. By adding (2-6 equiv.) of TBAF to receptor **M3**, there was a significant shifting of bands and intensity changes due to binding of F^- ion to the receptor *i.e*. the carbonyl stretching vibrations were observed at 1647, 1603 and 1541 showing considerable shifting of bands relative to free receptor **M3** and the –NH, –OH peaks became significantly broadened and finally disappeared due to strong interaction of F^- ion with the receptor[49,50] (Figure-8), which caused increase in electron distribution due to deprotonation.

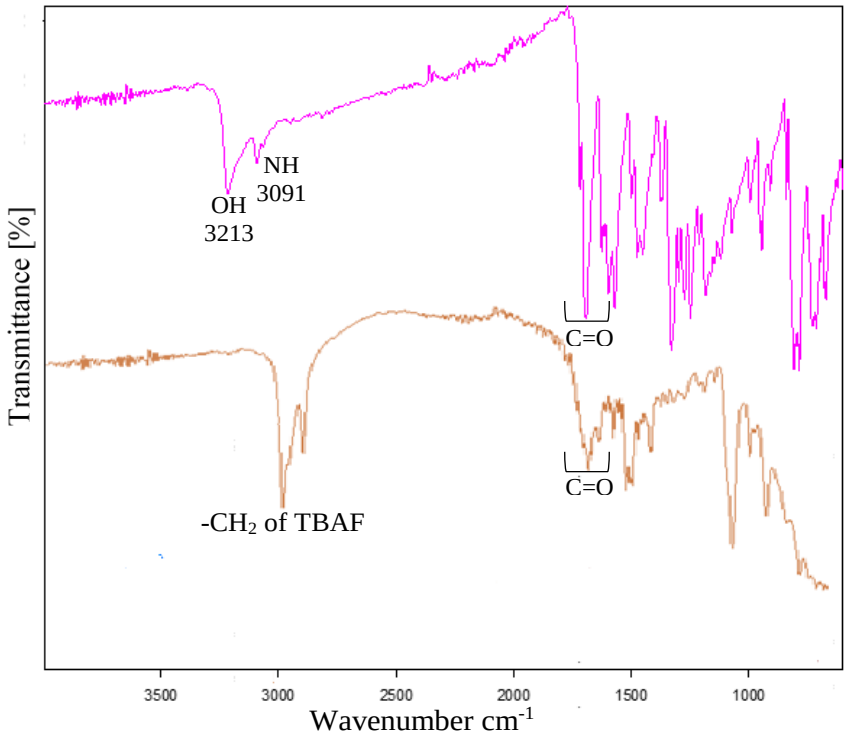


Figure 8. Overlay FT-IR spectra of the free receptor **M3** and complex of **M3**+TBAF.

3.5 Computational Study

The interaction of **M3** and **M4** with $\mathbf{F}^-$ ion was evaluated in terms of interaction energies that were computed on the optimized structures of **M3**, **M4** and its complexes with $\mathbf{F}^-$ ions. These structures were optimized at the density function theory method using B3LYP/6-31+G(d,p) using Gaussian 09[51]. Figure-9 illustrates the optimized structures of **M3**, **M4** and their complex with fluoride ions along with their geometric configurations. Geometry optimizations were followed by the interaction energy calculations using the following general equation.

$$\Delta E_{Complex} = E_{Complex} - E_{Free\ receptor} - E_{Anion}$$

Where E is the interaction energy of the complex consisting of free receptor and anion.

The computed interaction energies of M3 and M4 were found to be -222.57 and -223.64 kcal/mol between free receptor and two fluoride ions. The interaction of **M3** and **M4** with anions resulted in the elongation of the O-H and N-H bond lengths indicating the involvement of these –OH and –NH groups in strong hydrogen bonding with fluoride ions, which ultimately led to abstraction of these hydrogens. It was observed that after the proton abstraction from C-O and C-N, the bond lengths were reduced. Table 3 shows the significant geometric changes after complex formation of receptors **M3** and **M4** with fluoride ions. Frontier orbital analysis was also performed by computing HOMO-LUMO gap energies for both complexes and the values for the gap energy were found to be very close in both complexes thus resulting in very close values of interaction energies between the two complexes (Figure-9). This was also evident from similar values for the association constants. It was thus inferred that the difference in the substitution of halogens on **M3** and **M4** had slight influence on the structural attributes as well as on the

stabilities of the complexes but caused significant effect to determine the sensitivity of F^- ions in chemosensors as demonstrated by different LOD values.

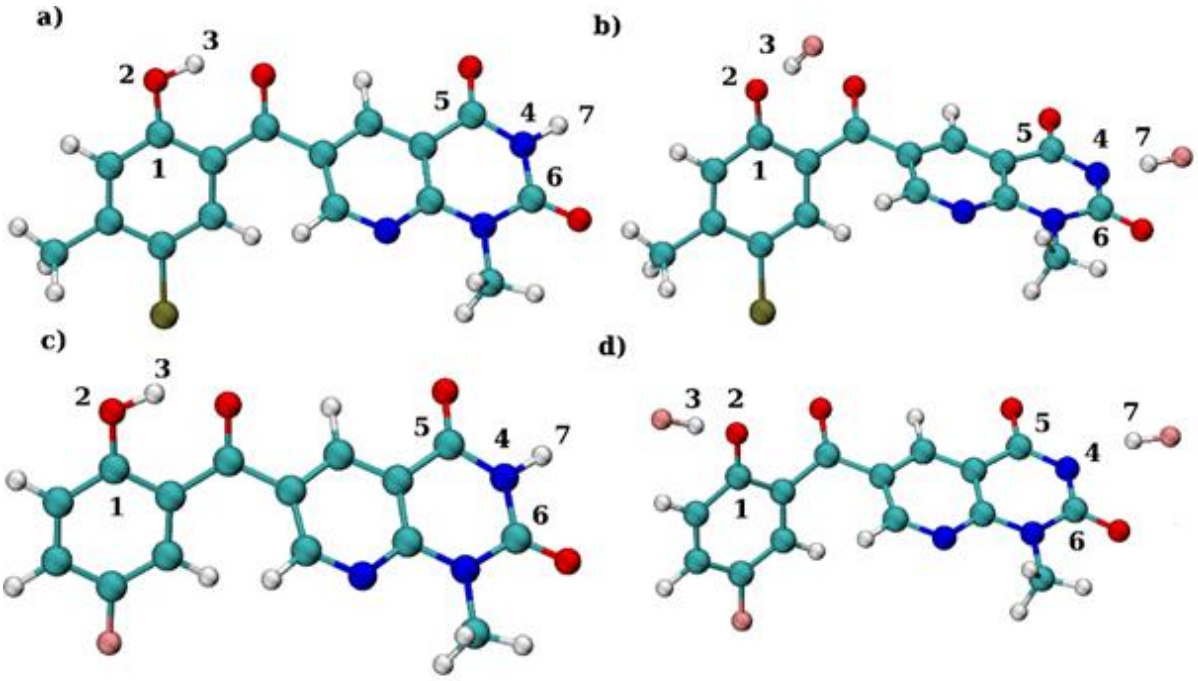


Figure 9. Optimized structures of a) **M3** and b) its complex with fluoride ions, and c) **M4** and d) its complex with fluoride ions obtained from density functional theory (Color representation of atoms : Carbon = Cyan, Chloride = Tan, Fluoride = Pink, Hydrogen = White, Nitrogen = Blue, Oxygen = Red).

Table 3. Optimized bond lengths for **M3** and **M4** and their complexes with fluoride ions at DFT level of theory.

Bond length (Å)	**M3**	**M4**	**M3(2F$^-$)**	**M4(2F$^-$)**
O—H	0.992	0.991	1.436	1.310
N—H	1.013	1.013	1.545	2.921
C—O	1.336	1.338	1.282	1.294
C—N	1.392	1.392	1.369	1.367
C—N	1.392	1.392	1.359	1.332

3.6 Analytical Applications

After predicting the correlation between computational and experimental chemistry, the analytical applications of the sensors were also investigated. The potential application of **M4** as anion sensor in solid state was studied by preparing a test kit of Whatman-40 filter paper coated with DMSO/CH_3CN (1:9) solution of **M4** and dried in air. The colour of test strip changed from colourless to light yellow (Figure-S9) only with F^- ion, which supports the practical applicability of the sensor and ensures its potential to perceive the F^- ion in solid state.

Moreover, the applicability of the 2-hydroxybenzoyl pyridopyrimidine derivatives *i.e.* probe **M4** was explored with toothpaste samples[7] (Figure-S10). The variations in UV-visible spectra clearly indicated the fluoride ion sensing ability of the synthesized 2-hydroxybenzoyl pyridopyrimidines receptors.

4. Conclusion

The new synthesized 2-hydroxybenzoyl pyridopyrimidine chemosensors **M1-M4** containing NH and OH groups as chromogenic units, which were studied as colorimetric and fluorescent receptors for anion sensing and were found to get influenced by the presence of different substituents. Colourimetric, UV-Visible and fluorescence spectral changes were investigated for **M1-M4** in the loop of various anions and the spectral data showed better results for the detection of fluoride ion over acetate and cyanides. The ^1H NMR titrations presented the formation of anionic species and provided proof for the binding mode of interactions of host **M4** with incoming guest fluoride ion. Among the chemosensors **M1-M4,** theoretical study at the quantum mechanical level quantifies the interaction of -OH and -NH groups in the receptors **M3**

and **M4** with fluoride ions that was followed by the deprotonation process in both receptors. The detection limit of fluoride ion was in the range of 3.51 x 10^{-8} M to 1.77 x 10^{-6} M for probes **M1-M4** and were lower than the maximum permissible concentration of F^- ion in drinking water (5.3 x 10^{-3} M) set by World Health Organization (WHO) that was found to be influenced by the nature of aromatic substituents. Moreover, the analytical applications successfully demonstrated the selective quantification of fluoride ion in biological sample analysis.

References:

[1] X. Shang, Z. Yang, J. Fu, P. Zhao, X. Xu, The synthesis and anion recognition property of symmetrical chemosensors involving thiourea groups: Theory and Experiments, Sensors, 15 (2015) 28166-28176.

[2] G. Yan, G. Wu, W. Qu, B. Shi, Q. Lin, H. Yao, Y. Zhang, T. Wei, A reversible dual-channel chemosensor for fluoride anion, Supramolecular Chemistry, 27 (2015) 552-558.

[3] X. Shang, Y. Wang, X. Wei, Z. Fu, J. Zhang, X. Xu, Synthesis and binding ability of molecular probes based on a phenanthroline derivative: Theory and Experiment, Molecules, 18 (2013) 14840-14848.

[4] C. Suksai, T. Tuntulani, Chromogenic Anion Sensors, in: I. Stibor (Ed.) Anion Sensing: -/-, Springer Berlin Heidelberg, Berlin, Heidelberg, 2005, 163-198.

[5] P.A. Gale, Anion and ion-pair receptor chemistry: highlights from 2000 and 2001, Coordination Chemistry Reviews, 240 (2003) 191-221.

[6] H.-W. Rhee, S.J. Choi, S.H. Yoo, Y.O. Jang, H.H. Park, R.M. Pinto, J.C. Cameselle, F.J. Sandoval, S. Roje, K. Han, D.S. Chung, J. Suh, J.-I. Hong, A bifunctional molecule as an

artificial flavin mononucleotide cyclase and a chemosensor for selective fluorescent detection of flavins, Journal of the American Chemical Society, 131 (2009) 10107-10112.

[7] P.B. Thale, P.N. Borase, G.S. Shankarling, A “turn on” fluorescent and chromogenic chemosensor for fluoride anion: experimental and DFT studies, Inorganic Chemistry Frontiers, 3 (2016) 977-984.

[8] K. Tóth, The role of fluorides in the prevention of dental caries, Fogorvosi szemle, 83 (1990) 163-167.

[9] O. Barbier, L. Arreola-Mendoza, L.M. Del Razo, Molecular mechanisms of fluoride toxicity, Chemico-biological interactions, 188 (2010) 319-333.

[10] M. Kleerekoper, The role of fluoride in the prevention of osteoporosis, Endocrinology and Metabolism Clinics, 27 (1998) 441-452.

[11] R.J. Carton, Review of the 2006 United States National Research Council report: fluoride in drinking water, Fluoride, 39 (2006) 163-172.

[12] M.-L. Cittanova, B. Lelongt, M.-C. Verpont, M. Geniteau-Legendre, F. Wahbe, D. Prie, P. Coriat, P.M. Ronco, Fluoride ion toxicity in human kidney collecting duct cells, Anesthesiology: The Journal of the American Society of Anesthesiologists, 84 (1996) 428-435.

[13] P. Singh, M. Barjatiya, S. Dhing, R. Bhatnagar, S. Kothari, V. Dhar, Evidence suggesting that high intake of fluoride provokes nephrolithiasis in tribal populations, Urological research, 29 (2001) 238-244.

[14] Y. Zhou, J.F. Zhang, J. Yoon, Fluorescence and colorimetric chemosensors for fluoride-ion detection, Chemical reviews, 114 (2014) 5511-5571.

[15] N.H. Evans, P.D. Beer, Advances in anion supramolecular chemistry: From recognition to chemical applications, Angewandte Chemie International Edition, 53 (2014) 11716-11754.

[16] B. Dey, R. Saha, P. Mukherjee, A luminescent-water soluble inorganic co-crystal for a selective pico-molar range arsenic (III) sensor in water medium, Chemical Communications, 49 (2013) 7064-7066.

[17] N. Iqbal, S.A. Ali, I. Munir, S. Khan, K. Ayub, M. al-Rashida, M. Islam, Z. Shafiq, R. Ludwig, A. Hameed, Acridinedione as selective flouride ion chemosensor: a detailed spectroscopic and quantum mechanical investigation, RSC Advances, 8 (2018) 1993-2003.

[18] P. Ghosh, N. Kumar, S.K. Mukhopadhyay, P. Banerjee, Sensitive and fluorescent Schiff base chemosensor for pico molar level fluoride detection: In vitro study and mimic of logic gate function, Sensors and Actuators B: Chemical, 224 (2016) 899-906.

[19] M. Islam, A. Hameed, K. Ayub, M.M. Naseer, J. Hussain, R.D. Alharthy, A. Asari, R. Ludwig, M.a. Rashida, Z. Shafiq, Receptor-spacer-fluorophore based coumarin-thiosemicarbazones as anion chemosensors with “turn on” response: spectroscopic and computational (DFT) studies, ChemistrySelect, 3 (2018) 7633-7642.

[20] I.O. Isaac, I. Munir, M. al-Rashida, S.A. Ali, Z. Shafiq, M. Islam, R. Ludwig, K. Ayub, K.M. Khan, A. Hameed, Novel acridine-based thiosemicarbazones as ‘turn-on'chemosensors for selective recognition of fluoride anion: a spectroscopic and theoretical study, Royal Society open science, 5 (2018) 180646.

[21] J.-M. You, H. Jeong, H. Seo, S. Jeon, A new fluoride ion colorimetric sensor based on dipyrrolemethanes, Sensors and Actuators B: Chemical, 146 (2010) 160-164.

[22] A. Aggeli, M. Bell, L.M. Carrick, C.W. Fishwick, R. Harding, P.J. Mawer, S.E. Radford, A.E. Strong, N. Boden, pH as a trigger of peptide β-sheet self-assembly and reversible switching between nematic and isotropic phases, Journal of the American Chemical Society, 125 (2003) 9619-9628.

[23] I.H. Badr, M.E. Meyerhoff, Highly selective optical fluoride ion sensor with submicromolar detection limit based on aluminum (III) octaethylporphyrin in thin polymeric film, Journal of the American Chemical Society, 127 (2005) 5318-5319.

[24] M. Melaimi, F.P. Gabbaï, A heteronuclear bidentate Lewis acid as a phosphorescent fluoride sensor, Journal of the American Chemical Society, 127 (2005) 9680-9681.

[25] G.-Y. Li, T. Chu, TD-DFT study on fluoride-sensing mechanism of 2-(2′-phenylureaphenyl) benzoxazole: the way to inhibit the ESIPT process, Physical Chemistry Chemical Physics, 13 (2011) 20766-20771.

[26] K.-S. Lee, H.-J. Kim, G.-H. Kim, I. Shin, J.-I. Hong, Fluorescent chemodosimeter for selective detection of cyanide in water, Organic letters, 10 (2008) 49-51.

[27] G.-J. Kim, K. Lee, H. Kwon, H.-J. Kim, Ratiometric fluorescence imaging of cellular glutathione, Organic letters, 13 (2011) 2799-2801.

[28] S.-Y. Lim, S. Lee, S.B. Park, H.-J. Kim, Highly selective fluorescence turn-on probe for glutathione, Tetrahedron letters, 52 (2011) 3902-3904.

[29] F. Buron, J. Mérour, M. Akssira, G. Guillaumet, S. Routier, Recent advances in the chemistry and biology of pyridopyrimidines, European journal of medicinal chemistry, 95 (2015) 76-95.

[30] S.V. Ryabukhin, A.S. Plaskon, D.M. Volochnyuk, A.A. Tolmachev, Chlorotrimethylsilane-mediated synthesis of functionalized fused pyridines: Reaction of 3-formylchromones with electron-rich aminoheterocycles, Synthesis, 2007 (2007) 1861-1871.

[31] D.A. Jose, D.K. Kumar, P. Kar, S. Verma, A. Ghosh, B. Ganguly, H.N. Ghosh, A. Das, Role of positional isomers on receptor–anion binding and evidence for resonance energy transfer, Tetrahedron, 63 (2007) 12007-12014.

[32] D. Kumar, K.J. Thomas, 2-Hydroxyarylimidazole-based colorimetric and ratiometric fluoride ion sensors, RSC Advances, 4 (2014) 56466-56474.

[33] S.L. Ashok Kumar, R. Tamilarasan, M.S. Kumar, A. Sreekanth, Bisthiocarbohydrazones as colorimetric and “turn on” fluorescent chemosensors for selective recognition of fluoride, Industrial & Engineering Chemistry Research, 50 (2011) 12379-12383.

[34] S. Bayindir, A simple rhodanine-based fluorescent sensor for mercury and copper: The recognition of Hg2+ in aqueous solution, and Hg2+/Cu2+ in organic solvent, Journal of Photochemistry and Photobiology A: Chemistry, 372 (2019) 235-244.

[35] S. Bayindir, K. Yararli, The easy synthesis of new N-substituted 5-oxindoline-rhodanines and their sensing ability: the recognition of acetate ions in aqueous solution, New Journal of Chemistry, 43 (2019) 8168-8178.

[36] A. Kumar, S. Kumar, N. Ahmed, ‘Naked-eye’colorimetric/fluorimetric detection of F− ions by biologically active 3-((1 H-indol-3-yl) methyl)-4-hydroxy-2 H-chromen-2-one derivatives, RSC Advances, 6 (2016) 108105-108112.

[37] X. Yang, L. Zheng, L. Xie, Z. Liu, Y. Li, R. Ning, G. Zhang, X. Gong, B. Gao, C. Liu, Y. Cui, G. Sun, G. Zhang, Colorimetric and On–Off fluorescent chemosensor for fluoride ion based on diketopyrrolopyrrole, Sensors and Actuators B: Chemical, 207 (2015) 9-24.

[38] X. Yang, G. Zhang, Y. Li, Z. Liu, X. Gong, B. Gao, G. Zhang, Y. Cui, G. Sun, Colorimetric and fluorogenic signalling of fluoride ions by diketopyrrolopyrrole-based chemosensor, RSC Advances, 5 (2015) 22455-22462.

[39] B. Bhattacharyya, A. Kundu, N. Guchhait, K. Dhara, Anthraimidazoledione based reversible and reusable selective chemosensors for fluoride ion: naked-eye, colorimetric and fluorescence “ON-OFF”, Journal of fluorescence, 27 (2017) 1041-1049.

[40] H. Khanmohammadi, K. Rezaeian, H. Radaie, Naphthalene-based Azo-azomethine chemosensor: naked eye detection of fluoride in semi-aqueous media, Inorganic Chemistry Research, 2 (2019) 32-39.

[41] N. Kaur, G. Jindal, "Switch on" fluorescent sensor for the detection of fluoride ions in solution and commercial tooth paste, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 223 (2019) 117361.

[42] G.Y. Li, G.J. Zhao, Y.H. Liu, K.L. Han, G.Z. He, TD-DFT study on the sensing mechanism of a fluorescent chemosensor for fluoride: Excited-state proton transfer, J. Comput. Chem., 31 (2010) 1759-1765.

[43] S. Bayindir, M. Toprak, A novel pyrene-based selective colorimetric and ratiometric turn-on sensing for copper, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 213 (2019) 6-11

[44] S. Bayindir, F. Lafzi, A simple oxindole-based colorimetric $HSO4^{-}$ sensor: Naked-eye detection and spectroscopic analysis, Journal of Photochemistry and Photobiology A: Chemistry, 376 (2019) 146-154.

[45] M. Boiocchi, L. Del Boca, D.E. Gómez, L. Fabbrizzi, M. Licchelli, E. Monzani, Nature of urea−fluoride interaction: incipient and definitive proton transfer, Journal of the American Chemical Society, 126 (2004) 16507-16514.

[46] X. Peng, Y. Wu, J. Fan, M. Tian, K. Han, Colorimetric and ratiometric fluorescence sensing of fluoride: tuning selectivity in proton transfer, The Journal of organic chemistry, 70 (2005) 10524-10531.

[47] M.S. Kumar, S.L.A. Kumar, A. Sreekanth, Highly selective fluorogenic anion chemosensors: naked-eye detection of F^- and AcO^- ions in natural water using a test strip, Analytical Methods, 5 (2013) 6401-6410.

[48] S. Bayindir, N. Saracoglu, A facile one-pot method to synthesise 2-alkylated indole and 2, 2′-bis (indolyl) methane derivatives using ketones as electrophiles and their anion sensing ability, RSC Advances, 6 (2016) 72959-72967.

[49] A.R. Chowdhury, P. Banerjee, A simple hydrazine based molecule for selective detection of Fluoride ion in DMSO, Journal of Chemical Sciences, 129 (2017) 463-470.

[50] S.K. Dey, B.K. Datta, G. Das, Binding discrepancy of fluoride in quaternary ammonium and alkali salts by a tris (amide) receptor in solid and solution states, CrystEngComm, 14 (2012) 5305-5314.

[51] M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. Petersson, Gaussian 09, Gaussian, Inc., Wallingford CT, 2009.

Conflict of Interest

All the authors certify that there is no conflict of interest to be declared.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: