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'Naked-Eye' Colorimetric/Fluorimetric Detection of F^- Ion by Biologically Active 3-((1H-Indol-3-yl)methyl)-4-hydroxy-2H-chromen-2-one Derivatives

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An improved synthetic protocol has been developed for the construction of highly functionalized heterologous alkyl and benzyl indolyl-coumarin derivatives through a rapid, catalyst-free and solvent-free one-pot three component reaction of indole, aldehyde and 4-hydroxy-coumarin. The synthesized compounds display a high selectivity and sensitivity towards F^- ion. The detection of F^- ion was accompanied by fluorescence quenching with noticeable colour change, which is evident from Stern-Volmer plot. The Stern-Volmer plot shows association constant of 6.2625×10^4 M^{-1} with lowest detection limit (LOD) of 0.50 μM for F^- ion. The interaction mechanism was studied by ^{1}H-NMR titration and also supported by advanced TD-DFT calculations. The designed chemosensor has been utilized as a mini colorimetric kit for F^- ion on test paper strips and also capable in detecting F^- ion from commercially available toothpaste and mouth rinser. The chemosensors also exhibited good antifungal and antibacterial activity.

1. Introduction

Synthesis of biologically and pharmaceutically effective sensory molecules remains key objectives of current research in view of their potential applications in biology, medical and sensing material. Coumarin and indole derivatives play an important role in human life due to their valuable biological, medical and optical properties.[1] Therefore, a conjugation of indole and coumarin is expected to be more efficient than individual form because of their several applications such as optical brighterners,[2] photosensitizers,[3] fluorescent and laser dyes[4] as well as additives in food, perfumes, cosmetics and pharmaceuticals.[5] The 4-hydroxycoumarin-indole based receptor brings two fluorescent chromophores into remarkable proximity, possessing an enolic-OH group and indolyl-NH group (hydrogen bond donor position due to which the electronic properties of the receptor is changed allowing the subsequent detection of anions), which leads to a highly sensitive chemosensor.[6] In the past two decade, several reports have been published on the synthesis of α-benzylaminocoumarins and benzylindolyl coumarin derivatives.[7-12] Despite the intrinsic worth of these methods, some of them suffer with several limitations such as long reaction time, use of catalysts, toxic reagents, carcinogenic solvents, limited substrate scope, low to moderate yields, tedious work-up procedure and purification steps. Therefore, it is a challenging task to develop a novel, efficient, economical and eco-friendly approach for the synthesis of relevant heterologous indolyl-coumarin scaffolds.

In spite of the gargantuan potential interest, there is no data available in the literature describing the optical properties of α-benzylaminocoumarins and benzylindolyl coumarin derivatives. Although a very few reports have been published describing the optical behaviour of bis(coumarin)methylene.[13-15] Anions play a noteworthy role in a wide range of biological, environmental, medicinal and chemical processes. Among various anions, F^- ion is one of the most essential ions in the human body with its significant applicability in dental care and potential use for the treatment of osteoporosis, psychiatric and hypnotics.[16] However, excess F^- ion is also harmful to human health. It is easily absorbed and causes acute gastric, fluorosis, cancer and kidney problems.[17, 18] According to World Health Organization (WHO) F^- ion in drinking water is considered beneficial at level of about 5.3×10^{-3} mol L^{-1} but harmful once it exceeds 7.9×10^{-3} mol L^{-1}.[19–21] Thus, it is necessary to add appropriate concentration of F^- ion in drinking water.[22] At present, a large population in the world still drink water containing inappropriate level of F^- ion which leads to several types of pathologies. In India alone about seven regions of West Bengal (WB) are predicted to be endemic for fluorosis and almost 66 million common people in these regions are at high risk of F^- contamination.[23] Therefore, considering these obvious health concerns and to minimize the possibility of excessive F^- ion intake, a simple, economical and selective assay is highly desirable for practical purposes.[24] Several efforts have been made to monitor F^- ion by traditional analytical methods such as Ion chromatography[25] and ion-selective electrodes.[26] However, the major disadvantages of these techniques are that they are time consuming and require costly instruments. Alternatively, the fluorimetric and colorimetric techniques have attracted more attention over other available techniques for detecting cations and anions due to high selectivity, quick response, real-time

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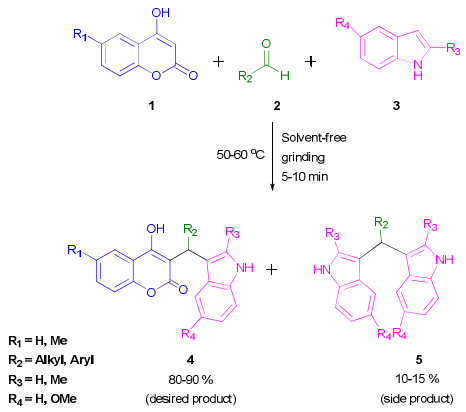
detection and cost effectiveness.[27, 28] In literature, a great deals of excellent colorimetric and fluorescent sensors for F^- ion have been reported,[29-31] while, most of these sensors have complicated structure and synthesized by multi-step procedures.[32–34] The development of chemosensor for the selective and sensitive detection of F^- ions is crucial. Therefore, design and development of synthetic receptors and chemosensor for the selective and sensitive detection of F^- ion have garnered considerable attention for researchers due to toxicity of the F^- ion.

In continuation of our research interest on the synthesis of various functionalized molecules, acid catalysis.[35-37] Furthermore, suitable supramolecular sensing probe for cations have been developed and recently published.[38] Influenced by the results of these studies, herein we synthesized heterologous indole-4-hydroxycoumarin derivatives *via* one-pot three component reactions of substituted 4-hydroxycoumarin, aldehyde and indole at 50–60 ^oC under grinding and solvent-free conditions (scheme 1). The current method is significantly improved from earlier described protocols in terms of applicability of wide range of aldehyde, better yield, shorter reaction time, simplistic workup and essentially no need of column chromatography. The synthesized compounds selectively detect F^- ion by naked eye, exhibit intense hypochromic shift in absorption and significant fluorescence quenching in emission spectra upon interaction with F^- ion in acetonitrile. Interestingly, synthesized series of compounds also exhibited significantly antifungal and antibacterial response.



Scheme 1: One pot green synthesis of alkyl/benzylindolylcoumarin derivatives.

2. Experimental details

2.1 Reagents and instrumentation:

All common reagents and solvents were of AR grade, purchased from Sigma Aldrich and Himedia, India and used as received otherwise mentioned. ^{1}H-NMR and ^{13}C-NMR spectra of compounds were recorded in DMSO-d_6 on a Brüker (500 MHz) spectrometer and chemical shifts were reported as part per million (ppm) in δ scale downfield from TMS (as internal standard). The following abbreviations were used to explain the multiplicities: br = broad, s = singlet, d = doublet, dd = double of doublet, dt = doublet of triplet, t = triplet, q = quartet, m = multiplet. The UV–vis. absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer and the fluorescence emission spectra were recorded on a Shimadzu RF-5301 PC Spectrofluorophotometer with a 3.0 cm standard quartz cell. Melting points were recorded on Optimelt automated melting point system. Quantum yield were obtained by using FLS 980 Fluorescence spectrometer (Edinburgh Instruments). The IR spectra were recorded on a Nicolet 6700 FTIR Thermoscientific in the range 4000–400 cm^{-1} with KBr pellet. Fluorescence life time spectra were obtained by using HORIBA Jobin Yvon, Fluorocube Fluorescence Lifetime System.

2.2 General procedure for the synthesis compounds 4a–4n:

A mixture of 4-hydroxycoumarin 1 (1.0 mmol), aldehyde 2 (1.0 mmol) and indole 3 (1.0 mmol) was taken in mortar and mixed thoroughly with pestle and ground at 50–60 °C. The reaction was monitored by thin layer chromatography. After completion, the reaction mixture was cooled to room temperature and washed with ethanol and dried to obtain pure desired product.

3. Results and discussion

3.1 Synthesis, substrate scope and characterization of compounds 4a–4n:

In the design of anion sensor, we were interested in developing heterologous scaffold bearing –OH and –NH groups in the same molecule; a 4-hydroxycoumarin moiety as enolic scaffold and an indole as an –NH entity to detect anions through deprotonation mechanism. At the outset 4-hydroxycoumarin **1a**, benzaldehyde **2a** and indole **3a** were chosen as the model substrate to optimize the reaction conditions. In a preliminary attempt, the reaction was performed in the presence of 20 mol% of I_2 at room temperature, the proposed reaction not proceed under these conditions to give the desired product **4a** (Table 1, entry 1). When reaction was carried out at elevated temperature (40-100 ^oC), the desired product **4a** was obtained in 20% yield along with the traces of side product **5a** (Table 1, entry 2–4). Furthermore, when the reaction was carried out in absence of I_2 under solvent free condition at room temperature for 1h, the reaction underwent smoothly to give the desired product **4a** with 50% yield along with a significant amount of **5a** (40%) (Table 1, entry 5). In order to improve the yield of heterodimeric product **4a**, reaction was explored at different temperatures under neat condition, the optimum 88% yield was obtained at 50 °C (Table 1, entry 7). Thus the reaction at 50 °C under solvent-free conditions emerged as the optimum for subsequent studies. After having the optimized reaction conditions, we further investigated the substrate scope and generality of the reaction. A number of structurally diverse aldehydes, substituted indole and 4-hydroxycoumarin are used

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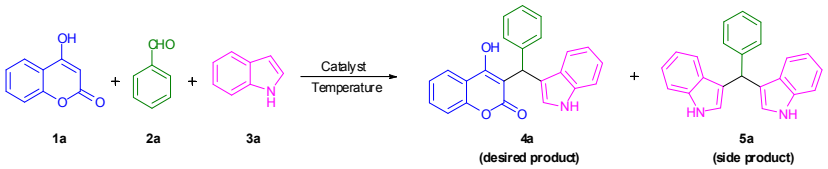
Table 1. Optimization of Reaction Condition for the synthesis of benzylindolylcoumarin **4a**.[a]



1a 2a 3a 4a (desired product) 5a (side product)

Entry	Catalyst	Temperature (°C)	Yield[b] (%)	
			4a	5a
1	I_2[c]	RT	--	40
2	I_2[c]	40	Trace	35
3	I_2[c]	50	20	Trace
4	I_2[c]	100	--[d]	--[d]
5	-	RT	50	40
6	-	30	60	10
7	**-**	**50**	**88**	**5**
8	-	60	88	5
9	-	100	--[d]	--[d]

[a] The reaction was carried out using 4-hydroxycoumarin **1** (1.0 mmol), aldehyde **2** (1.0 mmol) and indole **3** (1.0 mmol) in mortar and mixed thoroughly with pestle. [b] isolated yield, [c] catalyst 20 mol % used, [d] reactants decomposed.

Table 2. Summarized results of substrate scope under optimized reaction conditions.[a]

S. No.	Comp.	R_1	R_2	R_3	R_4	Time(min)	Yield[b](%)
1	**4a**	H	Phenyl	H	H	5	88
2	**4b**	H	4-Bromophenyl	H	H	5	85
3	**4c**	H	4-Chlorophenyl	H	H	5	86
4	**4d**	H	2-Chlorophenyl	H	H	5	85
5	**4e**	H	4-Methylphenyl	H	H	8	90
6	**4f**	H	3-Methoxyphenyl	H	H	10	88
7	**4g**	H	4-Nitrophenyl	H	H	5	85
8	**4h**	H	4-Fluorophenyl	H	H	9	88
9	**4i**	H	Ethyl	H	H	5	90
10	**4j**	H	Ethyl	H	OCH_3	6	90
11	**4k**	CH_3	Ethyl	CH_3	H	6	88
12	**4l**	H	4-Bromophenyl	CH_3	H	8	80
13	**4m**	H	4-Chlorophenyl	CH_3	H	8	82
14	**4n**	H	2-Chlorophenyl	CH_3	H	8	81

[a] The reaction was carried out using 4-hydroxycoumarin **1** (1.0 mmol), aldehyde **2** (1.0 mmol) and indole **3** (1.0 mmol) in mortar and mixed thoroughly with pestle and ground at 50–60 °C. [b] isolated yield.

for this purpose. There were no significant effects on the reaction yield by substituents on indole and 4-hydroxycoumarin. However, substituents on aldehydes exhibited marginal effects on both yield as well as in reaction rate. The aldehyde bearing electron withdrawing substituent afforded the product **4g** with 85% yield, whereas aldehyde having electron rich group like 4-methyl and 3-methoxy afforded the product with relatively better yield (Table 2, entry 5 and 6). The assigned structures of the products **4a–4n** were well characterized by using analytical tools such as ^{1}H, ^{13}C-NMR and IR spectroscopy (S1-S27).

3.1.1 3-((1H-indol-3-yl)(phenyl)methyl)-4-hydroxy-2*H*-chromen-2-one (4a):

Yield: 323 mg (88%) as white solid; MP = 191 °C; IR ν_{max} (KBr, cm^{-1}): 3417, 3345, 2940, 2830, 1689, 1621, 1563, 1417, 1278, 943, 758; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 11.6 (s, broad, D_2O exchangeable, 1H), 10.9 (s, D_2O exchangeable, 1H), 8.02 (d, *J* = 7.5 Hz, 1H), 7.60 (t, *J* = 7.0 Hz, 1H), 7.37-7.28 (m, 7H), 7.10–7.03 (m, 4H), 6.90 (t, J = 7.5 Hz, 1H); 6.08 (s, 1H); ^{13}C-NMR [125 MHz (DMSO-d_6)] δ (ppm): 162.2, 160.7, 152.6, 143.1, 136.4, 132.3, 128.6, 128.2, 127.7, 126.1, 124.7, 124.3, 123.9, 121.3, 118.9, 118.7, 116.7, 116.6, 114.6, 111.9, 108.9 37.5.

3.2 Anion sensing: colorimetric and fluorimetric studies:

The UV–vis absorption and fluorescence emission spectra of all of the compounds were investigated in acetonitrile and the results are summarised in Table S2, ESI†. Out of the 14 compounds **4a-4n**, for the complete study of optical behaviour, **4a** was selected. The chemosensor **4a** (1.0×10^{-5} M) exhibited a strong absorption band centred at 485 nm in acetonitrile which can be assigned to the π–π* transition (Fig.

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2a). Upon gradual addition of F^- ion (TBAF, 0–20 equiv.) to the above solution of **4a**, the absorption band at 485 nm regularly decreased and a new absorption band appeared at 415 nm (blue shift of 70 nm) (Fig. 3a). A clear isosbestic point was observed at 455 nm, indicating the strong interaction between **4a** and F^- ion. The absorption intensity at 415 nm reaches a plateau after the addition of exactly 20 equivalent of F^- ion and there is no significant enhancement of the absorption intensity on further addition of F^- ion. Moreover, a light orange colour obtained after the addition of F^- ion to the solution of **4a** which was initially colourless. This colour change could be easily observed *via* "naked eye" (Fig. 1). On the contrary, absorption spectrum of **4a** was also studied in the presence of various anions (20 equiv.) such as I^-, Br^-, OAc^-, Cl^-, ClO_4^-, BF_4^-, HSO_4^-, NO_3^- and PF_6^- (tetrabutylammonium salt), however, no considerable change in the colour of the solution as well in the UV–vis spectra was observed.

As shown in Figure 3b, the selectivity of **4a** to F^- ion was also investigated by fluorescence emission spectral titration in acetonitrile. The chemosensor **4a** exhibited an intense emission band at 507 nm (absolute quantum yield, ϕ_f = 0.47), upon the addition of F^- ion (0-20 equiv.) to the solution of **4a** (10 μM) fluorescence intensity at 507 nm decreases gradually and shifted to 534 nm (red shift of 27 nm, absolute quantum yield, ϕ_f = 0.049) (Fig. 3b). Finally the reaction gets saturated with F^- ion and demonstrated a remarkable quenching effect, which is attributed to the formation of receptor-anion (host–guest) hydrogen bonded complex. The result suggested that **4a** can act as a "switched on-off" sensor for F^- ion particularly. As mentioned earlier also, selectivity of chemosensor **4a** towards different anions (I^-, Br^-, OAc^-, Cl^-, ClO_4^-, BF_4^-, HSO_4^-, NO_3^- and PF_6^-) was also investigated which displayed no change in the optical properties. Moreover, no significant fluorescence quenching was observed upon the addition of any other anion. Hence, individual response of **4a** against these anions revealed a remarkable selectivity only for F^- ion binding (Fig. 2b). The selective response of **4a** towards F^- ion was evaluated by adding 5 equiv. of various anions and result showed that there was no interference from other anionic species (Fig. 4a).

The time resolved fluorescence study of chemosensor **4a** was also analysed in the absence and presence of F^- ions, obtained at excitation frequency of 455 nm. As shown in Figure 5b, the fluorescence lifetime decreased from 4.65 ns to 2.47 ns after the addition of F^- ions, indicates strong interaction of receptor **4a** with F^- ions. The binding stoichiometry was obtained from the Job's plot measurement on the basis of fluorescence. The maximum fluorescence intensity appeared at 0.5 mole fraction which clearly expressing 1:1 binding stoichiometry of **4a** with F^- ion (Fig. 4b).[39] The association constant was calculated by using S.V. plot which was found to be 6.2625×10^4 M^{-1} (Fig. 5a) with 0.50 μM detection limit (Fig. 5c).[40–42]

The obvious hypsochromic shift in the absorption band from 485 to 415 nm and remarkable quenching in fluorescence spectra suggests that the π-conjugation was inhibited by the addition of F^- ion to **4a** which may be due to the deprotonation of –OH and –NH protons. To test the deprotonation possibility of –OH and –NH protons as per proposed mechanism, absorption and emission titration of **4a** was also performed with a relatively strong base $[Bu_4N]OH$, which leads to deprotonation. Similar changes in absorption and emission spectra were obtained as those observed with F^- ion (Fig. S29, ESI†). The similar anion sensing properties of **4a** were also observed in the more polar solvent DMSO. It was reported as the protic solvents[43] such as water would compete with the anions for the bonding sites of chemosensor and could therefore disturb their hydrogen bonding interactions. To investigate the applicability of chemosensor **4a** for F^- ion detection in drinking water similar experiments were carried in DMSO-H_2O media and was observed that the receptor was able to demonstrate noticeable changes in both absorption and fluorescence spectra when water content was not more than 10 % (v/v) in DMSO (Fig. S30, ESI†). Thus the chemosensor could be applied for F^- ion detection in environmental water samples and biological samples.

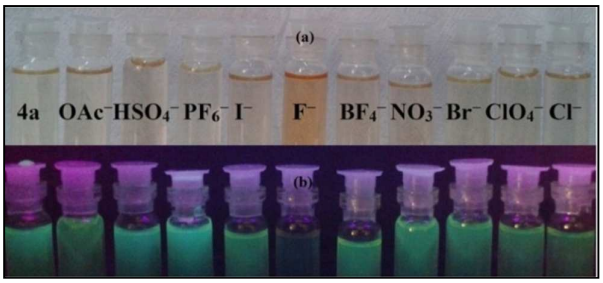

Fig. 1. (a) Colourimetric and (b) fluorimetric naked-eye detection of **4a** in the absence and presence of different anions (20 equiv.) in CH_3CN.

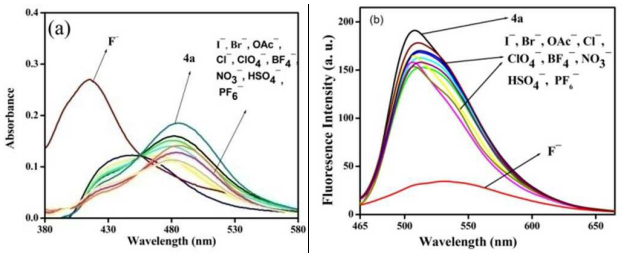

Fig. 2. (a) Absorption and (b) fluorescence emission spectra of **4a** (1.0×10^{-5} M) in the absence and presence of 20 equiv. of different anions in CH_3CN. λ_{ex} = 455 nm.

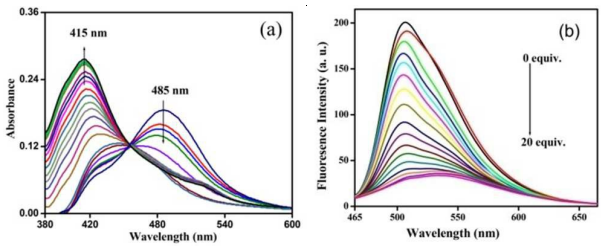

Fig.3. (a) Absorption and (b) fluorescence emission spectral responses of **4a** (1.0×10^{-5} M) towards varying F^- ion concentrations (0 to 20×10^{-5} M) in CH_3CN. λ_{ex} = 455 nm.

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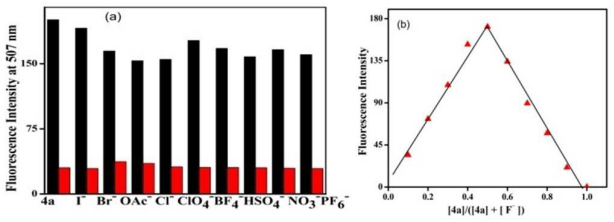
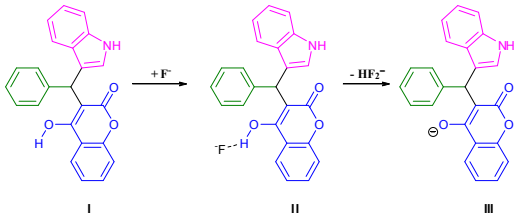


Fig.4. (a) Interference studies of F^- with **4a** (1:5), black bars represent the emission intensity of [**4a**-other anions] system and red bars show [**4a**-F^--other anions] system (b) Jobs plot shows 1:1 stoicheometry in CH_3CN.



Scheme 2: Proposed sensing mechanism of **4a** through deprotonation in the presence of F^- ion.

3.3 Nature of interaction between chemosensor 4a and F^- ions:

To gain an insight about the mechanism of interaction between **4a** and F^- ion, ^{1}H-NMR titration experiment was performed in DMSO-d_6. The ^{1}H-NMR spectra of **4a** show NMR peaks as singlet at δ 11.65, 10.91 and 6.09 ppm which are attributable to the –OH, –NH and –CH proton, respectively and all aromatic protons appeared within the region δ 6.89–8.03 ppm. Upon the addition of 0.5 equiv. of $[Bu_4N]F$ to the solution of **4a** in DMSO-d_6, the most noticeable change was observed in –OH proton. The singlet at δ 11.65 ppm corresponding to –OH proton completely disappeared due to deprotonation. This was confirmed by the appearance of broad signal corresponding to HF_2^- at around 16.0 ppm in presence of excess F^- ions.[44] Alternatively a slight upfield shift of -NH proton was noticed (from δ 10.91 to 10.84 ppm), reflecting the increase in electron density (Fig. 6). Thus, the ^{1}H-NMR experiment clearly supported the hypothesis that the F^- ions preferably interacts with the receptor –OH rather than –NH. We also carried out computational DFT and TD-DFT studies in gas phase, to further appreciate the binding procedures of F^- ion with chemosensor **4a**.[45] The DFT and TD-DFT calculations were performed for the optimized structure of chemosensor **4a** and **4a**+F^- to the B3LYP/6-31G(d) level using Gaussian 03 program. DFT calculation results showed that the –OH bond length was changed by 0.975 Å to 1.446 Å after addition of F^- ions. So, one conclude from these data deprotonation of the -OH protons of chemosensor **4a**. TD-DFT calculations were performed, to interpret further the absorption properties of **4a** and **4a**+F^- complexes. As shown in Table S1, Fig. S34 and Fig. S35 ESI†, the lowest energy transitions of **4a** arise from the HOMO→LUMO, HOMO-2→LUMO, HOMO-3→LUMO and HOMO-4→LUMO whereas in the **4a**+F^- originated from HOMO→LUMO and HOMO-1→LUMO. We also calculated the UV-Vis absorption spectra of chemosensor **4a** and **4a**+F^- in gaseous state by TD-DFT. The absorption spectra indicates a systematic blue shift from λ_{max} = 478 nm to λ_{max} = 321 nm, in chemosensor **4a** when binding with F^- ion. The blue shift in absorption maxima of chemosensor **4a** after binding with F^- ion could be understand in terms of increases energy gap between HOMO and LUMO of **4a**+F^- (Fig. 7). For chemosensor **4a**, the HOMO was distributed on the indole scaffold while the LUMO was distributed on the coumarin moiety with energy deference ΔE = 3.432 eV. Whereas, for **4a**+F^-, the HOMO was distributed on the whole indole and coumarin system while LUMO was distributed on coumarin scaffold with energy deference ΔE = 4.220eV. This clearly indicates the sensing of the fluoride ion. Therefore, the interaction of F^- ion with -OH of chemosensor **4a** was result of deprotonation due to charge transfer, which also support the blue shift in the UV-Vis. absorption spectra of **4a** with F^- ion and naked eye colour change.

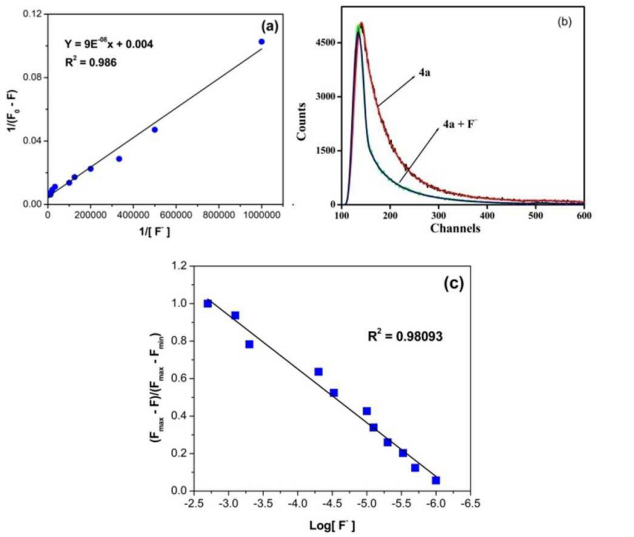


Fig.5. (a) Stern-Volmer plot (b) Life time decay plot (c) Limit of detection for binding of F^- ion with chemosensor **4a**.

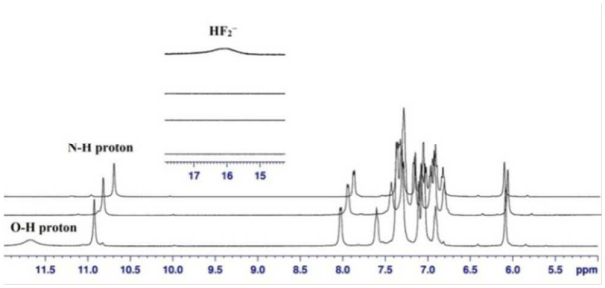


Fig. 6. ^{1}H-NMR titrations of **4a** with F^- ion in DMSO-d_6.

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4. Application

4.1 Visual colour changes on test papers:

To investigate the potential application of **4a** as an anion sensor in solid state, we prepared a test kit using Whatman-40 filter paper coated with acetonitrile solution of **4a** (10 µM) followed by drying in air. The colour of the test strip changed to bright orange only with F^- ions supporting the practical applicability of the receptor **4a** (Fig. 8). This experiment shore up that compound **4a** has the potential to detect F^- ions in solid state.

4.2 Ocular Colorimetric fluoride detection from mouth rinser and toothpaste:

Inspired by the results of test kit, we were moved to test its capacity in detecting F^- ions from commercially available toothpaste (131 mg in 1.0 g) and mouth rinser (0.01 % w/v). The chemosensor **4a** (1.0×10^{-5} M) can effectively sense fluoride from mouth rinser and toothpaste by appearance of light orange colour to the colourless solution (Fig. 9). We have directly used commercially available mouth rinser and in case of toothpaste a 10 mL 10^{-6} (M) water solution was prepared to conduct F^- ions detection.

4.3 Biological Evaluation:

Synthesized compounds were evaluated for their *in vitro* antibacterial activity against Gram-positive bacteria (*B. subtilis* and *S. aureus*), Gram-negative bacteria (*E. coli* and *S. Flexneri*) and antifungal potential against *Candida albicans* fungal strain. The results are summarized in Table 3.

4.3.1 Antibacterial activity:

The compounds **4d**, **4e**, **4f**, **4l**, and **4n** (MIC: 3.12, 0.39, 1.56, 3.12 and 1.56 mg/ml respectively) show excellent antibacterial activity against *B. subtilis* compared to the standard drugs. Compound **4e** (MIC: 0.39 mg/ml) and, compounds **4d** and **4l** (MIC: 3.12 mg/ml each) exhibit equal antibacterial activity than positive controls against *B. subtilis*. In case of *S. aureus*, the compounds show equal or lower activity than Ampicillin and Cefadroxil drugs. Compound **4d** (MIC: 0.78 mg/ml) and, compounds **4l** and **4m** (MIC: 1.56 mg/ml) were found equally potent as the standard drugs against *S. aureus*. However, compounds **4i** and **4j** (MIC: 0.39 mg/ml) show better activity than positive controls against *E. coli* while compound **4l** (MIC: 0.78 mg/ml) and, compounds **4d** and **4m** (MIC: 1.56 mg/ml) show equal activity to the reference drugs. Compounds **4d**, **4g**, **4i** and **4l** show higher antibacterial activity (MIC: 3.12 mg/ml) compared to Ampicillin and Cefadroxil against *Shigella flexneri*. Thus, the electron releasing group (CH_3, OCH_3 and 2-methyl indole moiety) containing compounds such as **4e**, **4f** and **4n** has shown excellent (MIC: 0.39 mg/ml) to good (MIC: 1.56 mg/ml) activity against *B. subtilis*. Against *S. aureus* also, the electron donating group (2-methyl indole moiety and 4-substituted phenyl) carrying compounds (**4l** and **4m**) exhibit equal (MIC: 1.56 mg/ml) antibacterial activity compared to Cefadroxil. Against *E. coli*, compounds **4d**, **4g**, **4i**, **4j**, **4k**, **4l** and **4m** show excellent activity compared to Ampicillin and Cifadroxil regardless of presence of electron releasing or donating group. Against *Shigellaflexneri*, all compounds exhibit lower activity compared to the standard drugs without depending on the presence of any functional group.

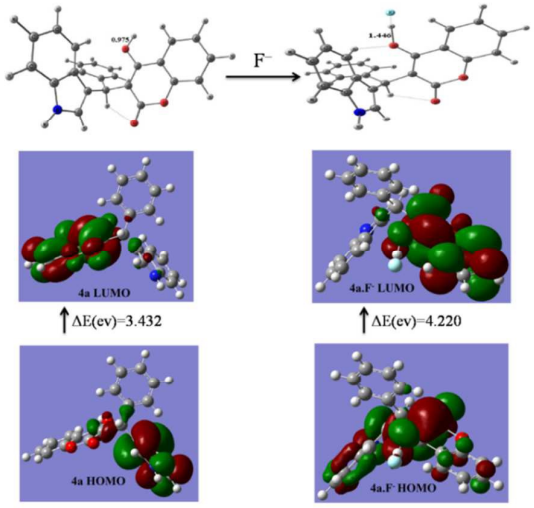

Fig. 7. DFT optimised structure and HOMO-LUMO band gap of chemosensor **4a** and **4a**+F^-.

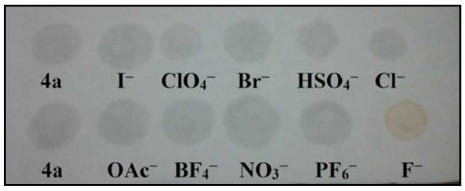

Fig. 8. Colour change of test paper containing **4a** (10 µM) with different anion.

Table 3. Minimum inhibitory concentration (MIC, mg/ml^{-1}) of synthesized compounds **4a-n** against bacterial and fungal strains.

	Gram-positive		Gram-negative		Fungus
	B.subtilis	*S.aureus*	*E.coli*	*S.flexneri*	*C.albicans*
4a	>100	>100	>100	50	25
4b	25	25	12.5	50	**3.12**
4c	50	25	>100	12.5	50
4d	**3.12**	**0.78**	**1.56**	**3.12**	6.25

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4e	**0.39**	12.5	50	12.5	12.5
4f	**1.56**	25	25	12.5	12.5
4g	>100	12.5	**0.78**	**3.12**	25
4h	6.25	25	12.5	6.25	25
4i	50	**3.12**	**0.39**	**3.12**	25
4j	6.25	6.25	**0.39**	6.25	**0.39**
4k	12.5	**3.12**	**1.56**	>100	**3.12**
4l	**3.12**	**1.56**	**0.78**	**3.12**	12.5
4m	6.25	**1.56**	**1.56**	12.5	25
4n	**1.56**	>100	>100	50	**3.12**
Ampicillin	0.39	0.78	1.56	0.78	-
Cefadroxil	3.12	1.56	0.78	1.56	-
Fluconazole	-	-	-	-	3.12

4.3.2 Antifungal activity:

Compounds were subjected to MIC (minimum inhibitory concentration) determination by microdilution method[46] and the results are summarized in Table 3. The standard reference drugs Fluconazole was selected as positive control in antifungal activity assay. Compound **4j** exhibits excellent antifungal activity (MIC: 0.39 mg/ml) against *C. albicans*.

Moreover, compounds **4b**, **4k** and **4n** show similar antifungal activity (MIC: 3.12 mg/ml) as Fluconazole against *C. albicans* while others show lower activity. Thus, compound **4j** (carrying electron releasing group -OCH_3 on the indole moiety) shows excellent antifungal activity against *C. albicans* compared to standard drug.

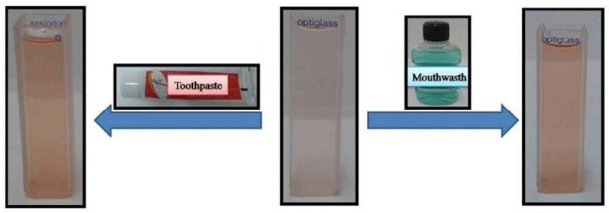

Fig. 9. F^- ion detection; **4a** before and after exposure to commercially available toothpaste and mouth rinser.

5. Conclusions

In conclusion, an economical, eco-friendly, simple and efficient method was developed for the synthesis of indole-coumarin heterocycle adducts. The products were obtained in high purity with easy work up procedure, short reaction time and excellent yields under solvent free and grinding conditions.

The series of chemosensor **4a-4n** displayed a highly selectivity and sensitivity towards F^- ion. Significant blue shift in absorption spectra and remarkable fluorescence quenching was observed in the presence of F^- ion, while other anions show no significant changes in optical properties. Chemosensor **4a** also exhibited very low detection limit of 0.50 µM for F^- ions. The experimental results are proved by ^{1}H-NMR titration and computational DFT and TD-TFT calculations. Naked-eyes detection on the basis of colour change for fluoride ion was realized successfully in aqueous solution.

Furthermore, compounds **4a-4n** exhibited good biological activity**,** particularly compounds **4e**, **4f** and **4i** exposed excellent biological activity against Gram-positive bacteria, while compounds **4j** and **4k** exhibited highest activity towards Gram-negative bacteria, even as compound **4k** exhibited the highest activity against pathogenic fungi *C. albicans* in the MIC method.

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Graphical Abstract

'Naked-Eye' Colorimetric/Fluorimetric Detection of F^- Ion by Biologically Active 3-((1H-Indol-3-yl)methyl)-4-hydroxy-2H-chromen-2-one Derivatives

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