Furo[3,2-c]coumarin-derived Fe$^{3+}$ Selective Fluorescence Sensor: Synthesis, Fluorescence Study and Application to Water Analysis

Norfatirah Muhamad Sarith1,2, Alexander Ciupa3, Stephen Moss1, Peter Myers4, Anna Grace Slater3,4, Zanariah Abdullah2, Hairul Anuar Tajuddin2✉ & Simon Maher1✉

Furocoumarin (furo[3,2-c]coumarin) derivatives have been synthesized from single step, high yielding (82–92%) chemistry involving a 4-hydroxycoumarin 4+1 cycloaddition reaction. They are characterized by FTIR, 1H-NMR, and, for the first time, a comprehensive UV-Vis and fluorescence spectroscopy study has been carried out to determine if these compounds can serve as useful sensors. Based on the fluorescence data, the most promising furocoumarin derivative (2-(cyclohexylamino)-3-phenyl-4H-furo[3,2-c]chromen-4-one, FH), exhibits strong fluorescence (ΦF = 0.48) with long fluorescence lifetime (5.6 ns) and large Stokes' shift, suggesting FH could be used as a novel fluorescent chemosensor. FH exhibits a highly selective, sensitive and instant turn-off fluorescence response to Fe$^{3+}$ over other metal ions which was attributed to a charge transfer mechanism. Selectivity was demonstrated against 13 other competing metal ions (Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Al$^{3+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Co$^{2+}$, Pb$^{2+}$ and Ru$^{3+}$) and aqueous compatibility was demonstrated in 10% MeOH-H$_2$O solution. The FH sensor coordinates Fe$^{3+}$ in a 1:2 stoichiometry with a binding constant, $K_a = 5.25 \times 10^3$ M$^{-1}$. This novel sensor has a limit of detection of 1.93 µM, below that of the US environmental protection agency guidelines (5.37 µM), with a linear dynamic range of ~28 (~2–30 µM) and an R$^2$ value of 0.9975. As an exemplar application we demonstrate the potential of this sensor for the rapid measurement of Fe$^{3+}$ in mineral and tap water samples demonstrating the real-world application of FH as a "turn off" fluorescence sensor.

Coumarin is an aromatic heterocyclic compound made up of two fused six-member aromatic rings, between benzene and pyrone, to form a benzopyrone. The academic literature contains an abundance of information regarding the synthesis and bioactivities of coumarin derivatives1–3. Research involving this ring system has been applied to a wide range of areas including pharmaceuticals4, optical brighteners5, fluorescents6–14 and laser dyes15. Recently, we developed a novel mixture of simple organic fluorescents, including furocoumarin, to generate high purity white light emission when applied as a coating to a commercial UV LED16. Furocoumarins are one of the coumarin derivatives that can be classified into two groups, i. furan fused benzene ring (psoralen and angelicin) and ii. furan fused lactone ring (furo[3,2-c]coumarin, furo[2,3-c]coumarin and furo[3,4-c]coumarins)17. Both psoralen and angelicin compounds are commonly studied because of their abundance in nature compared to the fused furan on the lactone ring17. In this study, furo[3,2-c]coumarin has been chosen as a suitable fluorescent heterocyclic candidate as it gives an excellent yield based on published reports18–20. Furthermore, the synthesis method for furo[3,2-c]coumarin is both efficient and straightforward (one-pot). It is found in natural products, for example, rhizome of Salvia miltiorrhiza Bunge and exhibits potent biological activity (antitumor, antioxidant, anticoagulant, antifungal, anticancer) with several therapeutic applications21. Nair and co-workers reported their preparative procedure which involves a [4 + 1] cycloaddition with in-situ generated heterocyclic coumarin methides and isocyanides19. Since coumarins typically show excellent spectroscopic properties, high stability and...
coagulation) and from corrosion of ferrous materials. In the USA, the environmental protection agency (EPA) liver 52, whilst a lack of iron is related to weakened cognitive growth and decreases the capacity for physical work 53. An excessive amount of iron in the human body can cause toxic damage to various organs including the heart and liver 54, 55. The major source of daily iron intake for humans is from food (e.g., green vegetables contain 20–150 mg/kg 55) with drinking water (assuming an average concentration of 0.3 mg/L) accounting for ~0.6 mg of daily intake. Iron excess are associated with various disorders, such as Alzheimer’s, Parkinson’s disease 49–51 and anemia 52. Among the metal ions, iron is an essential trace element found in living organisms, and both its deficiency and excess are associated with various disorders, such as Alzheimer’s, Parkinson’s disease 49–51 and anemia 52. An excessive amount of iron in the human body can cause toxic damage to various organs including the heart and liver 54, 55, whilst a lack of iron is related to weakened cognitive growth and decreases the capacity for physical work 53. In severe excess it is known to be lethal and death has occurred following human ingestion of ~40 mg/kg 54. In severe excess it is known to be lethal and death has occurred following human ingestion of ~40 mg/kg 54.

The study of fluorescent probes for metal ion detection is a vibrant research field, attracting great interest due to both the importance of detecting heavy metals but also because this sensing approach can offer high sensitivity and fast response times with relatively simple instrumentation requirements 23–25. Due to the low concentrations at which metal ions are present, for example in biosystems and in the environment, high-sensitivity probes are essential for practical applications 26–27. In recent years, a large number of fluorescent sensors from coumarin derivatives have been reported for metal ion detection 28, such as Cu 2+ 29–32, Zn 2+ 33–37, Al 3+ 38,39, Mg 2+ 40–42 and Fe 3+ 43–47. Reference 48 gives an overview of some of the sensing materials used for Fe 3+ detection.

The analysis of Fe 3+ is of great importance for various application areas including biomedical 58, environmental 59 and aquatic 60. In previous work successful attempts have been reported for the detection of Fe 3+ 43–47. However, in each case, selectivity is not demonstrated for some heavy metals (that exhibit properties similar to those of Fe 3+ ) which could interfere with detection 41. For example, we note that Ru 3+, which amongst the variety of transition metal ions, theoretically, has the greatest similarity to Fe 3+, is not tested for potential interference. Ruthenium is mainly used in the electronics 42–44 and chemical industries 55, 66, but it also used for biomedical purposes such as anti-cancer drugs 67, 68. Therefore, for any Fe 3+ fluorescent probe, it is important to extensively demonstrate selectivity, testing with other heavy metals including ruthenium, as it can be present in the environment 69, biological systems 70 and water 71 samples.

Herein, for the first time, we perform a fluorescent study of furo[3,2-c]coumarin derivatives. In particular, we show that the derivative, 2-(cyclohexylamino)-3-phenyl-4H-furo[3,2-c]chromen-4-one (FH), is as an effective fluorescent sensor which exhibits high selectivity for Fe 3+, tested against 13 other competing metal ions, including Ru 3+ and Fe 2+. Finally, we demonstrate the potential of this novel chemosensor for the rapid measurement of Fe 3+ in real water samples.

Results and discussions

The structures of the furocoumarin derivatives (FH, FCI, and FNO 2 ) were characterized by 1H NMR and FTIR. These results are in good agreement with the chemical structures for furocoumarin from the literature 18,19. Table 1 summarizes the UV-Vis and fluorescence spectroscopy data of FH, FCI and FNO 2 . Fig. S1, shows the UV-Vis spectra of FH, FCI and FNO 2 in ethanol. In Fig. 1, the fluorescence spectra of FH and FCI show higher intensity than FNO 2 . The main contributing factor responsible for the high fluorescence intensity of furocoumarin is related to its planar and rigid structure 24. Fluorescence of FNO 2 was severely quenched, contrary to the responses for FH and FCI. Chloro- in FCI is a weaker electron withdrawing group (EWG) than -NO 2 in FNO 2 , however, the chloro-substituent can also donate through the aromatic ring, which has a high electron density, as the atom is enriched with non-bonding electrons. Therefore, it can be through a π-electron delocalization promoter rather than a nitro group, which acts as a relatively strong EWG as illustrated in Fig. 2. In this case, chlorophenyl would be a donor group to the furocoumarin moiety (an acceptor group). It has been reported that the EWG decreases electron density of the aromatic ring with the exception of the halogen substituent group 72. The EWG of the nitro group in the benzene ring (nitroaromatic) has empty π*-orbitals of low energy, which are good acceptors of electrons. Therefore, electron-rich fluorescent molecules can potentially undergo strong quenching via a photoinduced electron transfer (PET) 74, fluorescence resonance energy transfer (FRET) or electron exchange energy transfer with nitroaromatics 76,77. Hence, we attribute the higher fluorescence intensity to the chloro- over the nitro- substituent.

| Compounds | [M] | Abs | Molar Abs | λ ex (nm) | λ em (nm) | Φ F | Stokes shift (nm) | τ (ns) |
|-----------|-----|-----|-----------|-----------|-----------|------|------------------|------|
| FH        | 1.00 × 10⁻⁴ | 0.37 | 2.00 × 10⁴ | 375       | 492       | 0.48 | 127              | 5.61 |
| FCI       | 1.00 × 10⁻⁴ | 0.20 | 3.70 × 10⁴ | 375       | 491       | 1.00 | 126              | 4.17 |
| FNO 2     | 1.00 × 10⁻³ | 0.22 | 5.70 × 10⁴ | 380       | 440       | nd   | 60               | nd   |

Table 1. Concentration [M], Absorbance (Abs), fluorescence lifetimes (τ) and quantum yield (Φ F) for fluorescence properties of furocoumarin derivatives in ethanol solution. nd = not determined.
Fluorescence and UV–Vis titration studies of FH with other metal ions.

The photophysical complexation studies of FH with an extensive series of metal salts including: Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Mn\(^{2+}\), Fe\(^{2+}\), Fe\(^{3+}\), Al\(^{3+}\), Ni\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Co\(^{2+}\), Pb\(^{2+}\) and Ru\(^{3+}\) in methanol, was performed using fluorescence spectroscopy. As shown in Fig. 3, the mixture of FH with Fe\(^{3+}\) was the only test sample that exhibited no fluorescence emission (i.e., turn-off) in the wavelength range from 430 to 700 nm. Remarkably, in the presence of 50 μM of various metal ions, fluorescence spectra of FH exhibited an appreciable fluorescence emission except in the case of Fe\(^{3+}\), which resulted in a noticeable turn-off fluorescence response. This fluorescence spectral change was also observed visually when examined with a UV transilluminator (380 nm) as illustrated in Fig. S2. The interaction of FH with...
Fe$^{3+}$ leads to an immediate fluorescence turn-off, while for the other metal ions, a slight fluorescence quenching is observed by the naked eye. As mentioned, the planar and rigid structure of the FH molecule makes it a highly fluorescent compound. However, when chelation occurs, there is a transfer of charges within the fluorescent ligand-metal system which then causes fluorescence quenching$^{8,9}$. Therefore, it can be inferred that the fluorescence quenching of FH in the presence of Fe$^{3+}$ is due to a ligand-metal charge transfer (LMCT) mechanism. This suggestion is supported by considering the paramagnetic nature of Fe$^{3+}$ with an unpaired d shell, this would take part in the energy and/or electron transfer processes leading to quenching of the fluorescence$^{80,81}$. We suspect, when Fe$^{3+}$ binds with FH, the fluorescent opens a non-radiative deactivation channel induced by the unpaired d shell, resulting in fluorescence quenching due to electron transfer$^{82}$. Thus, the mechanism of LMCT could happen promptly due to the strong paramagnetic quenching property of Fe$^{3+}$, leading to a severe fluorescence quenching effect (i.e., turn-off) to coordinate between FH and Fe$^{3+}$.

To gain a quantitative evaluation of the relation between the change in emission intensity of FH and the amount of Fe$^{3+}$ interaction, a fluorescence titration experiment was carried out with varying concentrations of Fe$^{3+}$ (Fig. 4). The emission intensity of the peak at 511 nm was systematically quenched by increasing the concentration of Fe$^{3+}$ from 5 to 50 μM. Moreover, the emission intensity at 511 nm was linearly proportional (correlation coefficient, R$^2$ > 0.99) to the concentration of Fe$^{3+}$ over the range of 0–30 μM, with a limit of detection of 1.93 μM (Fig. S3). These observations revealed that FH is suitable for use as a sensor for the quantitative measurement of Fe$^{3+}$

Figure 4. Fluorescence emission spectra of FH (0.5 μM) titrated with Fe$^{3+}$ (0–100 equiv.) in methanol.

**Competition experiment using fluorescence spectroscopy.** To further investigate the practical applicability of FH as a selective sensor for Fe$^{3+}$, a competition experiment was carried out for FH in the presence of Fe$^{3+}$ mixed with other metal ions (Na$^{+}$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Mn$^{2+}$, Al$^{3+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Co$^{2+}$, Pb$^{2+}$, Ru$^{3+}$). Interestingly, the fluorescence emission intensity was quenched in every case after mixing Fe$^{3+}$ with each of the candidate metal ions (Fig. 5). Thus, FH shows great promise as a highly selective and sensitive fluorescence turn-off sensor for the detection of Fe$^{3+}$ even in the presence of other analogous ions (in particular, Fe$^{2+}$ and Ru$^{3+}$). Furthermore, based on the general trend in Fig. 5, it is apparent that 3+ cations tend to exhibit stronger binding that effects fluorescence quenching of FH. This may be due to stabilization of the binding with an anion (NO$^3$ $^-$); 2 bonds at FH and one bond with anion. Consider, for example Al$^{3+}$, where the cation can bind in a similar way. This tridentate binding is certainly more stable than the other 2+ cations with bidentate binding. It is also apparent that Fe$^{3+}$ shows better binding with FH than Fe$^{2+}$, which can be attributed to the cationic radii, since Fe$^{3+}$ is much smaller than Fe$^{2+}$ about half the size of the Fe$^{3+}$ radius$^{83}$. When considering 1+ cations it is interesting that Na$^{+}$ also quenches FH but with K$^+$ to a lesser extent. This is probably related to the single bond with FH that is not very stable. Moreover, Na$^{+}$ has better electronegativity compared to K$^+$, which one expects promotes better binding with FH.

**Proposed sensing mechanism.** To study the reasonable binding mode of FH and Fe$^{3+}$, mass spectrometry analysis has been carried out and supports the formation of a 1:2 FH-Fe$^{3+}$ complex. As illustrated in Fig. S6, FH exhibits an intense protonated peak at m/z 360.21, while in the presence of Fe$^{3+}$, a peak at m/z 595.55 is observed, which is attributed to the formation of a protonated FH:(Fe$^{3+}$NO$_3$)$^-$ complex. For the mentioned results above, as well as the Job’s plot (Fig. S4), we suspect that the sensing mechanism for the 1:2 binding modes of the FH-Fe$^{3+}$ complex is as suggested in Fig. 6. IR spectroscopy was used to elucidate the coordination mode
between FH and Fe³⁺ (Fig. S7), shows the FTIR spectra of FH before and after the addition of Fe³⁺. A shift in the characteristic absorption band in the FTIR spectra confirmed the coordination behavior for FH-Fe³⁺. Upon the introduction of Fe³⁺, an extremely broad peak appeared between 3665 and 3125 cm⁻¹, which is attributed to the involvement of nitrogen from the primary amine (NH) and oxygen from furan in the binding of Fe³⁺. Furthermore, the stretching vibration frequency of the pyrone carbonyl (C=O) at 1720 cm⁻¹ is shifted to 1605 cm⁻¹.

Fluorescence and UV–vis titration studies of FH with other metal ions (in water/methanol (9:1, v/v)). Fluorescence quenching in protic solvents is a common problem with previously reported fluorescence sensors. In order to confirm FH is not susceptible to this issue and to demonstrate a real-world sample application, the photophysical properties of sensor FH were examined in a predominantly aqueous environment, water/methanol (9:1, v/v) at 5 µM. This composition of 9:1 v/v water/methanol was at the maximum solubility of FH in water. Changes to the fluorescence properties of FH caused by various metal ions are shown in Fig. 7. The result shows Fe³⁺ also produces significant quenching in the fluorescent emission of FH. The other tested metals only show relatively insignificant changes, except Co²⁺, Na⁺ and K⁺. So, it can be concluded that FH also has high selectivity for recognition of Fe³⁺ in a predominantly aqueous solution. The fluorescence spectra of FH (5 µM) in water/methanol (9:1, v/v), in the presence of various concentrations of Fe³⁺ ion (0.2–8 equiv.), are shown in Fig. 8, which shows quenching in the fluorescent emission of FH when the concentration of Fe³⁺ is increased. A Job’s plot of FH with Fe³⁺ also indicates the formation of a 1:2 complex (Fig. S8). A competitive assay (Fig. 9) confirms that FH can still detect Fe³⁺ even in the presence of other heavy metals. Thus, in a predominantly aqueous solution, FH exhibits high selectivity for Fe³⁺ over the other tested metal ions except Co²⁺, Na⁺ and K⁺.
Figure 7. Fluorescence spectra of FH (5 μM) in the presence of different metal ions (10 equiv.) in water/methanol (9:1, v/v).

Figure 8. Fluorescence emission spectra of FH (5 μM) titrated with Fe$^{3+}$ (0.2–8 equiv.) in water/methanol (9:1, v/v).

Figure 9. Competitive experiments in the FH + Fe$^{3+}$ system with interfering metal ions. FH (5 μM), Fe$^{3+}$ (50 μM) and other metals (50 μM) in water/methanol (9:1, v/v). Excited at 374 nm and emission measured at 511 nm.
reported and the characterization data agree with previous studies18,19. This was obtained by recrystallization from diethyl ether to produce up to 85% yield. These compounds have been synthesized by mixing 4-hydrocoumarin, benzaldehyde derivatives, and cyclohexyl isocyanide under reflux conditions within 24 h using singlestep high yielding chemistry (82–92% yield). All compounds are purified from recrystallization preventing the need for time consuming column chromatography and showing that this chemistry is amenable to automated high throughput synthesis and screening technologies. Both FH and FCI produce strong fluorescence intensity whilst FNO2 does not, as a result of strong electron withdrawing from –NO2 causing fluorescence quenching of furocoumarin. Furthermore, the fluorescence study has led us towards a successful demonstration of a novel coumarin-based fluorescent (FH) ratiometric chemosensor, with an LMCT mechanism attributed to the recognition of Fe3+ in methanol and also in water/methanol (9:1, v/v). FH formed 1:2 complexes with Fe3+ and exhibited a fluorescence turn-off response to Fe3+ ions. Extensive competitive selectivity experiments in methanol have been performed for Na+, K+, Mg2+, Ca2+, Mn2+, Fe2+, Al3+, Ni2+, Cu++, Zn++, Co++, Pb2+ and Ru3+ demonstrating that FH has higher selectivity towards Fe3+ (fluorescence turn-off) than other analogous ions and other previously reported Fe3+ sensors (to the best of our knowledge). In an aqueous environment the probe selectivity reduces but the “turn off” effect is still operational confirming water does not fully quench fluorescence. The potential of this sensor has been further highlighted by testing with untreated mineral and tap water samples. This result sets the foundation for a second generation of sensors with improved sensing properties and water solubilizing groups with the real potential of developing a fully aqueous furocoumarin based sensor, which is the subject of future work.

**Materials** and Methods

**Materials.** All reagents were purchased from commercial suppliers and used without further purification. The salts used in stock solutions of metal ions were Al(NO3)3·9H2O, CaCl2, CoCl2·6H2O, Cu(NO3)2·4H2O, FeCl3·4H2O, Fe(NO3)3·9H2O, KOH, MgCl2·6H2O, NaOH, NiCl2·6H2O, Pb(NO3)2, RuCl3·H2O, Zn(NO3)2·6H2O.

**Instrumentation.** 1H NMR (400 MHz) spectra were acquired on a Bruker AVANCE 400 MHz NMR Spectrometer using TMS (tetramethylsilane) as internal standard. All stock solutions of the samples for both UV-Vis and Fluorescence studies were prepared at 0.1mM in different solvents (ethanol, chloroform and ethyl acetate) and diluted in 10 mL with appropriate concentrations. UV-vis absorption and fluorescence spectra of the furocoumarin derivatives (in solution) were recorded on a Cary 60 UV-Vis spectrophotometer and CARY Eclipse Fluorescence Spectrometer, respectively. Excitation and emission monochromator band pass were kept at 5 nm using a quartz cell cuvette (1×1 cm). The absolute quantum yields were calculated using quinine sulfate in 0.1 M H2SO4 as a standard. Fluorescence lifetime measurements were performed with the use of an FLS 1000 Spectrometer (Edinburgh Instruments, Livingston, UK) at room temperature. In these experiments the fluorescence lifetimes of the furocoumarin derivatives in methanol were measured using the photon counting technique (requiring at least 10,000 photons per second to be counted because the signal-to-noise ratio becomes unsatisfactory at lower count rates25) with an excitation wavelength set to 374 nm in all the cases. UV-vis absorption and fluorescence spectra of FH and all metal ions were performed with the use of a Cary 5000 UV-Vis-NIR Spectrophotometer (Agilent Technologies) and FLS 1000 Spectrometer (Edinburgh Instruments, respectively). Paper spray ionization mass spectrometry (PSI-MS)18–20 was performed on a Waters Xevo TQ-MS (Waters, Wilmslow, UK).

**Synthesis of furo[3,2-c] coumarin derivatives.** Equimolar amounts of 4-hydroxycoumarin and benzaldehyde derivatives were dissolved in benzene (0.2 M) and heated under reflux (Fig. 10). After 30 minutes, cyclohexyl isocyanide (1 eq.) was added to the reaction mixture and further refluxed for 24 h. The pure compound was obtained by recrystallization from diethyl ether to produce up to 85% yield. These compounds have been reported and the characterization data agree with previous studies18,19.

| Water samples | Added (µM) | Found (µM) | Recovery (%) | RSD (%) |
|---------------|-----------|------------|--------------|---------|
| Mineral water | 2.0       | 2.1        | 105          | 0.71    |
|               | 10.0      | 10.0       | 100          | 5.71    |
|               | 20.0      | 19.3       | 96.5         | 9.60    |
| Tap water     | 2.0       | 2.5        | 125          | 0.5     |
|               | 10.0      | 9.8        | 98           | 6.9     |
|               | 20.0      | 18.3       | 91.5         | 4.2     |

Table 2. Analytical results of FH-Fe3+ in water samples.

**Determination of Fe3+ in real water samples.** To investigate the applicability of the FH sensor in real-istic environmental samples, recovery studies were carried out in mineral drinking water and tap water samples doped with Fe3+, using fluorescence emission spectroscopy. Testing on these water samples was performed without any sample pre-treatment except for the addition of FH, Fe3+ and allowing 1 minute for mixing. From Table 2, we can see that the recoveries of Fe3+ were from 91.5% to 125%. These data indicate that FH as a sensor has significant potential for the practical detection of Fe3+ in various aqueous samples where other potentially competing species are present.

**Conclusion**

In summary, we have successfully synthesized and for the first time, characterized, the fluorescence properties of furocoumarin derivatives (FH, FCI and FNO2). These were synthesized by mixing 4-hydrocoumarin, benzaldehyde derivatives, and cyclohexyl isocyanide under reflux conditions within 24 h using singlestep high yielding chemistry (82–92% yield). All compounds are purified from recrystallization preventing the need for time consuming column chromatography and showing that this chemistry is amenable to automated high throughput synthesis and screening technologies. Both FH and FCI produce strong fluorescence intensity whilst FNO2 does not, as a result of strong electron withdrawing from –NO2 causing fluorescence quenching of furocoumarin. Furthermore, the fluorescence study has led us towards a successful demonstration of a novel coumarin-based fluorescent (FH) ratiometric chemosensor, with an LMCT mechanism attributed to the recognition of Fe3+ in methanol and also in water/methanol (9:1, v/v). FH formed 1:2 complexes with Fe3+ and exhibited a fluorescence turn-off response to Fe3+ ions. Extensive competitive selectivity experiments in methanol have been performed for Na+, K+, Mg2+, Ca2+, Mn2+, Fe2+, Al3+, Ni2+, Cu++, Zn++, Co++, Pb2+ and Ru3+ demonstrating that FH has higher selectivity towards Fe3+ (fluorescence turn-off) than other analogous ions and other previously reported Fe3+ sensors (to the best of our knowledge). In an aqueous environment the probe selectivity reduces but the “turn off” effect is still operational confirming water does not fully quench fluorescence. The potential of this sensor has been further highlighted by testing with untreated mineral and tap water samples. This result sets the foundation for a second generation of sensors with improved sensing properties and water solubilizing groups with the real potential of developing a fully aqueous furocoumarin based sensor, which is the subject of future work.
2. (Cyclohexylamino)-3-phenyl-4H-furo[3,2-c]chromen-4-one. FH, 92% yield, light yellow powder, m.p. = 120–122 °C, FTIR = 3250 (NH), 2925–2850 (cyclohexane), 1720 (C=O of pyrone), 1570 (C=C of pyrone), 1H NMR = 1.18–2.08 (m, 10H), 3.55–3.58 (m, 1H), 4.29 (d, J = 8.32Hz 1H), 7.27–7.31 (m, 2H), 7.39 (d, J = 4 Hz, 1H), 7.43 (t, J = 8H, 3H), 7.52 (d, J = 8 Hz, 2H), 7.77 (d, J = 8 Hz, 1H), 1H NMR spectrum of FH as shown in Fig. S9. UV-Vis = 375 nm (in ethanol).

2. (Cyclohexylamino)-3-(4-chlorophenyl)-4H-furo[3,2-c]chromen-4-one. FCI, 90% yield, bright crystalline yellow, m.p. = 110–112 °C, FTIR = 3289 (NH), 2930–2857 (cyclohexane), 1707 (C=O of pyrone), 1593 (C=C of pyrone), 1H NMR = 1.16–2.07 (m, 10H), 3.57 (br, 1H), 4.21 (s, 1H), 7.33–7.28 (m, 1H), 7.41–7.39 (m, 4H), 7.47 (d, J = 6.4Hz 2H), 7.77 (d, J = 7.6Hz 1H), 1H NMR spectrum of FCI as shown in Fig. S10. UV-Vis = 375 nm (in ethanol).

2. (Cyclohexylamino)-3-(4-nitrophenyl)-4H-furo[3,2-c]chromen-4-one. FNO2, 85% yield, reddish orange powder, m.p. = 145–147 °C, 3389 (NH), 2929–2851 (cyclohexane), 1736 (C=O of pyrone), 1574 (C=C of pyrone), 1H NMR = 1.19–2.11 (m, 10H), 3.67 (m, 1H), 4.60 (d, J = 7.96Hz 1H), 7.34 (t, J = 6.80Hz 1H), 7.45–7.40 (m, 2H) 1H NMR spectrum of FNO2 as shown in Fig. S11. UV-Vis = 380 nm (in ethanol).

Fluorescence spectral responses of FH to metal ions. The analysis was conducted for two different solvent systems: pure methanol and a water/methanol mixture (9:1; v/v). All stock solutions of the furocoumarin (FC) and various metal ions (Mg2+, Ca2+, Mn2+, Fe2+, Fe3+, Al3+, Ni2+, Cu2+, Zn2+, Co2+, Pb2+ and Ru3+) were analyzed at a concentration of 0.001 M, except Na+ and K+ at 0.2 M in methanol. Then, each of the metal ions were diluted to 50 μM, while FH was diluted to 0.5 μM in methanol. For the water/methanol solvent system, FHM was diluted to 5 μM.

For testing, FH was mixed with each of the metal ions for up to 1 minute (by stirring until no layers could be visually observed) after which UV-Vis and fluorescence analysis were carried out. The fluorescence emission spectra were recorded from 430 to 700 nm with an excitation wavelength at 374 nm. Both excitation and emission slits were set at 1 nm. For the competing analysis, the fluorescence changes of FH in methanol were measured by the treatment of 50 μM Fe3+ ion in the presence of 50 μM other interfering metal ions. All of the background metal ions tested showed no interference with the detection of Fe3+ by competitive experiment.

Received: 9 October 2019; Accepted: 19 March 2020;
Published online: 04 May 2020

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Acknowledgements
N.M.S. acknowledges Dual PhD Program (University of Malaya-University of Liverpool) sponsored by University of Malaya, MyBrain15 (MyPhd), FRGS grant (FP070-2018A), Faculty Program - Research University Grant (GPF048B-2018), and UMRG grant (RG540-15AFR), postgraduate grant (PG356-2016A) and the Materials Innovation Factory for access to FLS1000 and Cary 5000 instruments. A.G.S acknowledges a Royal Society-EPSRC Dorothy Hodgkin Research Fellowship.
Author contributions
S.M. and H.A.T. designed the project. Experiments were performed by N.M.S. The manuscript and figures were prepared by N.M.S. and S.M. Various aspects of the research ideas described were initiated and developed by A.C., S. Moss., P.M., A.G.S. and Z.A. All authors reviewed the manuscript and supplementary information.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-63262-7.

Correspondence and requests for materials should be addressed to H.A.T. or S.M.

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