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Synthesis of Push-Pull Phenazines Possessing pH Responsive Absorption Spectra and Their Application in Selective Detection of Fe³⁺ Ions and Aromatics

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Abstract:

Phenazines bearing electron donor and acceptor groups with high pH sensing behavior were synthesized in moderate to good yields. Theoretical calculations on the frontier molecular orbitals and experimental oxidation potential measurements under variable pHs provide evidence for extensive internal charge transfer at high pH. The well-known strong affinity of the catecholate moiety present in our phenazines is utilized for spectrophotometric detection of Fe³⁺ ion. Fluorescence quenching behavior of the prepared phenazines is utilized for detection and distinction of electron rich and electron poor aromatics.

Key words: Phenazine, push-pull, iron detection, pH sensor, heteroarene

Photoactive organic molecules possessing optical spectra in the visible to NIR regions are relevant for applications in medical and materials sciences.¹ Extensive conjugation through either a polyene system or a polycyclic aromatic structural feature can result in bathochromic shifting of optical spectra from the ultraviolet to visible regions and beyond.² Such molecular arrangement enhances orbital overlap and diminishes gap between the highest occupied and lowest unoccupied molecular orbitals (HOMO→LUMO gap, HLG) leading to the observed-red shifts.³ Attachment of electron donor and acceptor groups in conjugation through a π -system could also impart red-shifted electronic properties in small molecules by reduction in the HLG.⁴ Preparation of such donor-acceptor molecules ideally requires a planar moiety with an extended π -system⁵. Several examples of small molecules bearing such extended conjugation exist, among which phenazine with a pyrazine core and an aromatic π -system, has drawn our attention. This is due to the fact that this class of molecule has established itself as a robust platform for designing of molecular probes for anion and cation detection (**1** and **2**, Figure 1)⁶ as histological fluorescent stains (safranin **3**, Figure 1)⁷, as fluorescent markers in biological assays (2,3-diaminophenazine **4**, Figure 1)⁸, as a strong DNA-intercalators (**5**, Figure 1) and pH sensitive probes (**6** and **7**, Figure 1).⁹

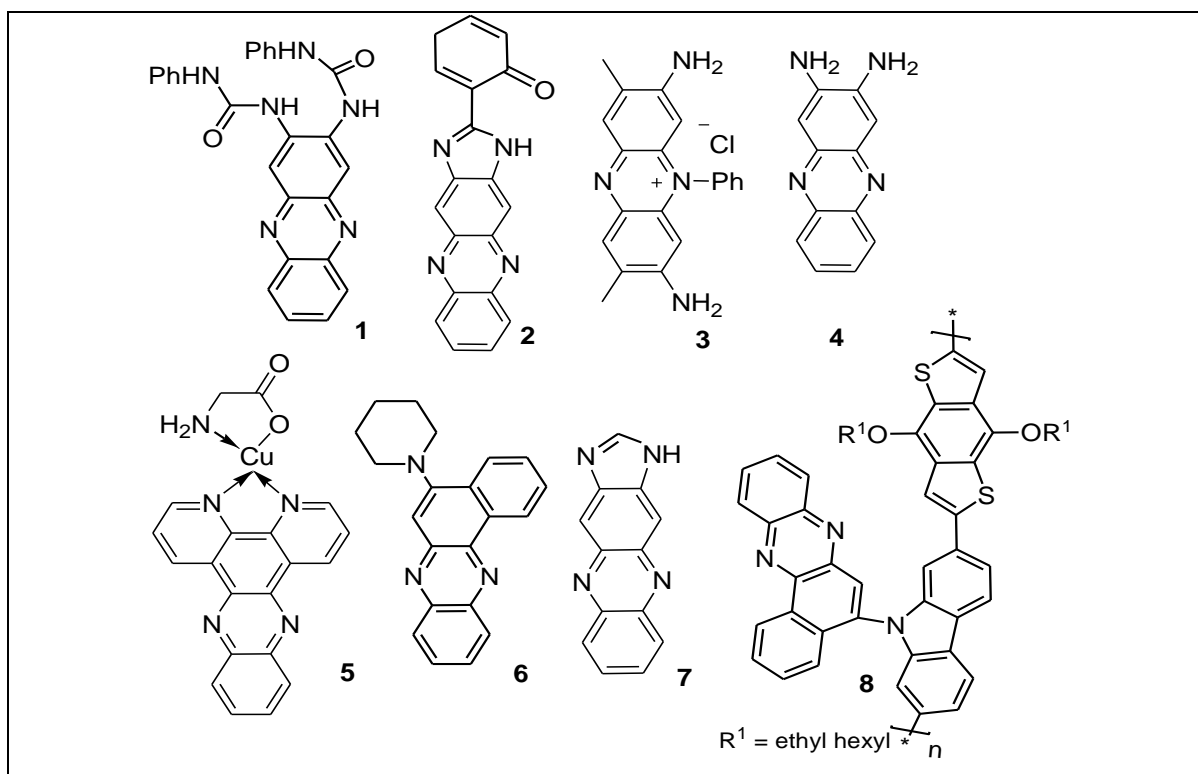


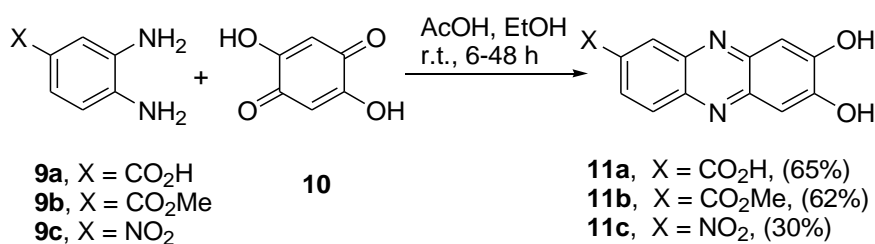
Figure 1. Examples of various photoactive phenazines

Phenazine based polymers have also been used as light sensitive dyes in artificial light harvesting due to its suitable redox properties (**8**, Figure 1).¹⁰ Moreover, a number of natural products with a range of bioactivity exist which contain phenazine as their core structure.¹¹ Most phenazines possess absorption and emission spectra in the visible region above 400 nm and offer a good planar aromatic template for further structural modifications towards construction of molecules with red-shifted optical spectra.

While a number of structural variations on the parent phenazine do exist (Figure 1), with an observed effect on the optical spectra, phenazines containing an electron donating ‘push’ group and an electron withdrawing ‘pull’ group attached directly onto the phenazine moiety, are yet to be constructed. We have recently reviewed the phenazines¹² from the prism of their optical properties leading to their wide-ranging applications. Here we would like to highlight our efforts towards the synthesis of suitably modified phenazines containing for the first time, both electron ‘push’ and ‘pull’ groups in direct conjugation with each other. We believe that such an arrangement could lead to intramolecular charge transfer (ICT)¹³, giving rise to bathochromically shifted optical spectra. Based on the ICT mechanism, herein, we also demonstrate the utility of such molecules in detection of extreme pH, as well as in selective detection of certain metal ions.

Scheme 1 represents the synthesis of our push-pull phenazine systems. The compounds **11a-c** were prepared by the quinoxaline type cross coupling reaction¹⁴ between variously substituted *ortho*-phenylene diamines **9a-c** and 2,5-dihydroxy-1,4-benzoquinone **10**, under acid catalysis. The choice of starting material diamine helped to vary the electron withdrawing component, from carboxylic acid, to ester and nitro group, while the electron donor catechol type moiety originated from the quinone starting material. In a structural sense, compounds **11a-c** represent true ‘push’-‘pull’ phenazines, since at least theoretically, the internal charge transfer from the catecholate moiety to the nitro/ester/acid group would be possible. Whether such a phenomenon could be promoted and observed as shifts in their optical spectra in solution, will be discussed in the subsequent section. The identities of **11a-c** were confirmed on the basis of their proton NMR spectra as well as high resolution mass spectra (HRMS). The proton NMR spectra showed certain characteristic peaks for each compound. Expectedly the most downfield shifted signal originated from the proton *ortho* to the carboxylic acid as well as NO₂ group of **11a** and **11c**. While for **11a** it showed as a singlet at 8.52 ppm, the corresponding signal in **11c** at 8.84 ppm. Interestingly, in basic medium, the singlet at 8.52 ppm of **11a** shifts upfield to 8.24 ppm and the singlet at 7.29 ppm corresponding to the two protons *ortho* to the catechol moiety, also exhibited an upfield shift to 6.59 ppm (Figure S4, SI). One of the problems encountered post synthesis and during characterization was the very poor solubility of our

phenazines in practically any solvent, except trace solubility in DMSO. Hence, some NMR spectra were recorded in d_6 -DMSO with a pinch of solid sodium hydroxide, hoping to deprotonate the catechol moieties of the compounds and obtain a higher concentration in solution.



Scheme 1. Synthesis of push-pull phenazines

Figure 2 represents a colorimetric ‘naked-eye’ analysis of compounds **11a-c**, on treatment with a small amount of solid sodium hydroxide. While the DMSO solutions of phenazines **11a** and **11b** did not exhibit any color change upon NaOH addition, the solution of dihydroxy nitrophenazine **11c** turned purple in presence of base. This color change was reversible and the original orange-yellow color of the DMSO solution was restored upon addition of a few drops of conc. HCl. Thus, based on the upfield shifting of the proton NMR spectra of the phenazines and this reversible colorimetric change observed for **11c** under strongly basic conditions, we can propose that our phenazines undergo deprotonation of the acidic hydrogen atoms. The anionic species generated could therefore be capable of intramolecular charge transfer (ICT) to the pyrazine core. This would be further elaborated when the solutions of phenazines were treated separately under conditions of varying pHs and their absorption spectral changes were monitored.

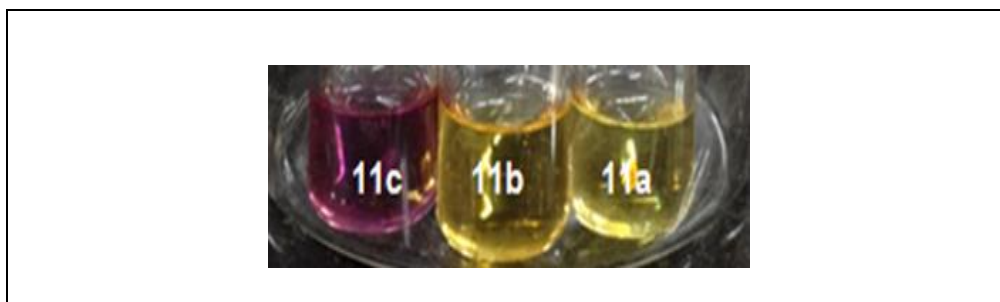


Figure 2 Observed color changes upon addition of a small pinch of solid NaOH to DMSO solutions of phenazines 11a-c.

Figure 3 represents the pH dependent absorption spectral changes in compounds **11a-c**. We chose three different pHs representing the neutral, strongly acidic and strongly basic conditions (pHs 7, 1 and 11 respectively). A dilute solution of the phenazines in DMSO was used as the stock solution from which an aliquot of 0.1 mL was taken and diluted with 2 mL of the solutions possessing the

different pHs. Figure 3A represents the UV-visible spectrum of phenazine carboxylic acid **11a**. At pH 7 the compound has a broad band at 427 nm (λ_{max}) and a stronger band at 300 nm and a shoulder at 270 nm, which is the strongest absorption peak. When the spectrum of **11a** recorded at a pH of 11, we observed a modest red shift of 16 nm for the λ_{max} out to 443 nm. The other two bands mostly remained unchanged with marginal alterations in intensity. Under acidic conditions represented by pH 1, the λ_{max} for **11a** was observed to be blue shifted to 417 nm. Compound **11b** did not exhibit any noticeable spectral shifts on proceeding from pH 7 to pH 11, but at pH 1 the λ_{max} under neutral medium of 446 nm blue shifted to 416 nm. The most important spectral shifting occurred in nitrophenazine **11c**. It exhibited two broad bands of nearly equal intensity at 340 nm and at 443 nm in neutral solution. Upon recording the spectrum in solution of pH 11, the 340 nm band red shifted to 370 nm and the λ_{max} at 440 nm red shifted to 475 nm. There was an increase in intensity by ~50% for both the bands, once again maintaining the nearly equal intensity for both the bands. Under acidic pH 1, we observed blue shifting of the 340 nm band to 318 nm and 440 nm band to 420 nm, without any loss of intensity. Interestingly, spectral shifts observed for all compounds, under extreme acid or basic conditions were reversible.

We hypothesize based on our proton NMR evidence (upfield shifting of relevant resonances) that under basic conditions all acidic hydrogen atoms on the phenazines are deprotonated and the anionic species could give rise to greater charge transfer into the phenazine core. While charge transfer from the carboxylate anion into the electron deficient phenazine takes place to a moderate extent as seen in the small red-shift in compound **11a**, the presence of the electron withdrawing nitro group renders the phenazine core of **11c** significantly more electron deficient. Hence, internal charge transfer from the catecholate anion in **11c** (after deprotonation) would be strongly promoted, as reflected by the strong (35-40 nm) red shift of the both the main bands. We also believe that under strongly acidic conditions the electron donor ability of the catechol moiety in all of **11a-c** is reduced, hence leading to a reduction in degree of charge transfer and the observed blue shifting on bands. Similar observations of blue shifting of optical spectra under acidic pH and red shifting of optical spectra under basic pH were reported in literature for structurally diverse phenazines of the type **6** and **7** (Figure 1).^{9b, c}

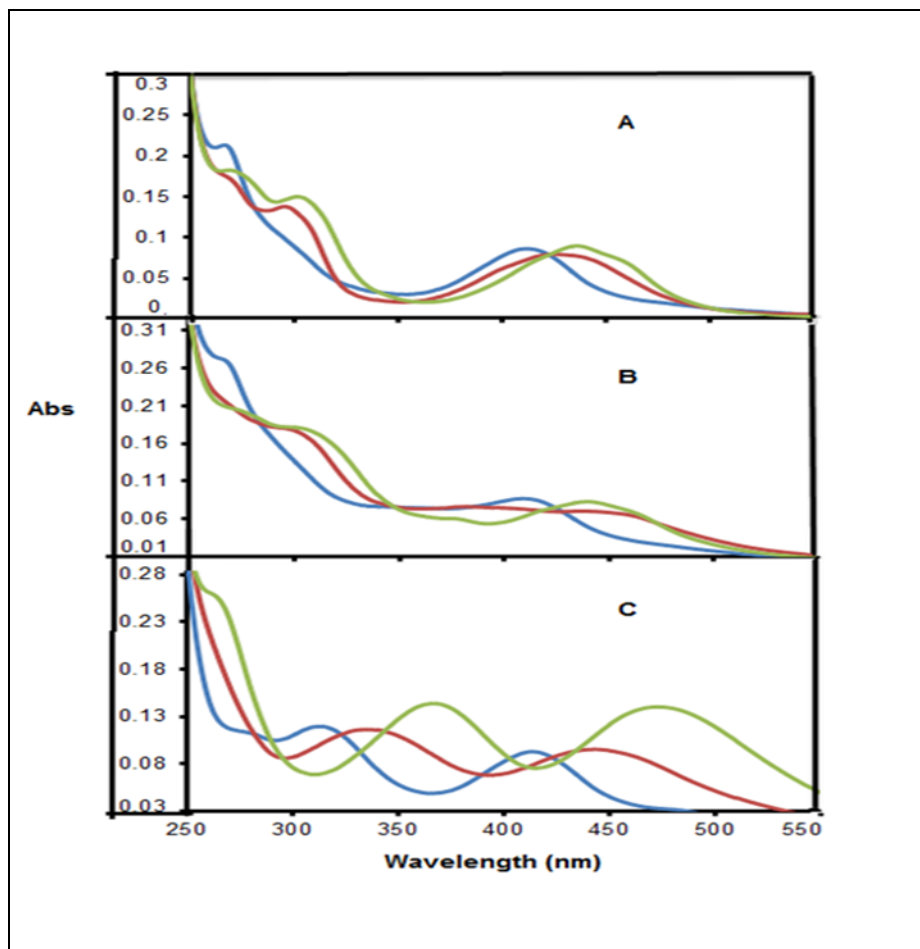


Figure 3. UV-visible spectra of phenazines **11a-c** recorded in aq. media of different pHs. From a stock solution of the phenazines in DMSO was taken 0.1 mL and diluted further by 2 mL of the aq. solution of a specified pH. A: **11a**, B: **11b**, C: **11c**. Green trace: pH 11, red trace: pH 7, blue trace: pH 1.

In order to account for the observed pH dependent spectral shifts, the energies and electron distribution of the frontier molecular orbitals under various conditions were theoretically computed. We selected phenazine **11c** for our calculations since the spectral shifts in this species were the most prominent. Compound **11c** has been studied at the Density Functional Theory (DFT) level of calculation. The ground state geometries of the actual compound and different possible compounds in alkaline and acidic conditions were optimized using the B3LYP/6-311G* methodology (Fig. S1) while the time dependent density function theory (TDDFT) has been utilized for low-lying excitations at the ground state geometries. The latter has been also attempted at the ZINDO/CI level of theory. Considering the fact that in the neutral condition compound **11c** exists, the absorption peak arising from the HOMO → LUMO excitation ($\Delta E_{\text{HOMO-LUMO}} = 3.42 \text{ eV}$) can be expected near 420 nm (Table S1) which is followed by other stronger peaks towards lower wavelength side starting

from 360 nm. In the alkaline medium, there exists two possibilities; either both the hydroxyl groups (**11c-Alk1**) or one of them (**11c-Alk2**) becomes deprotonated. In both the cases the HOMO-LUMO gap becomes almost half of the neutral species indicating a significant shift in the absorption peak position. In case of **11c-Alk1**, a strong peak is observed near 523 nm arising from the HOMO-1. LUMO excitation followed by peaks with higher oscillator strength values; on the other hand, in **11c-Alk2** a slightly weaker peak near 487 nm arising from the HOMO to LUMO+1 excitation can be seen while a strong peak can be observed near 390 nm arising from the HOMO-2 → LUMO excitation at the TDDFT level of calculation. The ZINDO/CI predicted values of the strongest peak positions arise near 453 nm and 420 nm for **11c-Alk1** and **11c-Alk2**, respectively. These results indicate the better possibility of the existence of the **11c-Alk1** form in comparison to **11c-Alk2**. Under acidic condition, the catechol hydroxyl groups are expected to be protonated. The intramolecular H-bonding between the 2 hydroxyl groups on the adjacent carbon atoms can be visualized from Figure 4. Interestingly, calculations rule out any biprotonated species, since it seems to have no peak in the range of our interest, while the monoprotinated species (**11c-Acid**) shows a slight blue shift of the HOMO → LUMO dominated peak in comparison to that of the neutral species (**11c**) at the TDDFT level. The corresponding energy gap of the two frontier orbitals is roughly 3.47 eV. The semi-empirical level CI calculation has predicted a strong peak near 314 nm for this species arising from the HOMO to LUMO+1 excitation.

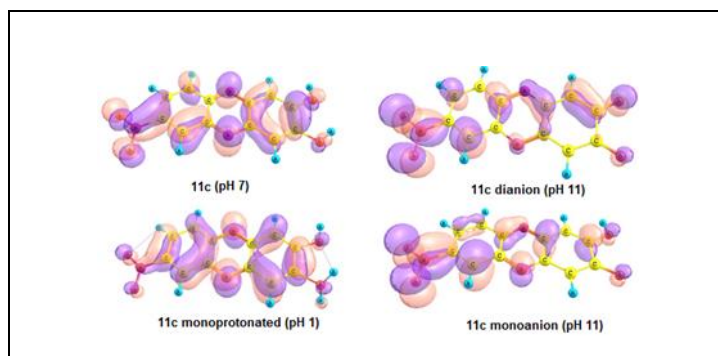


Figure 4 LUMO picture of phenazine 11c under different pHs.

The significant red-shift of the λ_{\max} value in alkaline medium and the blue-shift in acid medium becomes apparent if we see the LUMO pictures (Figure 4) of all the above-mentioned species. The two possible species in alkaline medium (**11c-Alk1** and **11c-Alk2**) are very good push-pull types of systems. The electronic charge cloud on LUMO in these systems (Figure 4) shifts away significantly from the catechol side towards the adjacent nitro group in comparison to their HOMOs. This significant amount of charge transfer stabilizes the excited state in the polar solvent and thereby creates the red-shift in the spectrum. On the contrary, in all other species (**11c**, **11c-Acid**) the shift in

charge cloud is not that significant. There is a marginal difference in the HOMO-LUMO gap (~ 0.05 eV) between **11c** and **11c-Acid** which reflects in the slight blue-shift in the peak position of the latter.

In order to understand the extent of delocalization that the molecule possesses under different pHs correlating to the variations in degree of push-pull charge transfer, the differential pulse voltammetric study was carried out for the compound **11c**. The experiment was performed in dimethylsulfoxide using tetrabutylammonium hexafluorophosphate as the supporting electrolyte, under two different pHs, 7 and 11. The pH of the solution was adjusted to 11 using aqueous NaOH. The first oxidation wave under pH 7 is at 1209 mV and that under pH 11 is 814 mV (Figure 5). The decreased potential requirement for oxidation in the case of pH 11 indicates ease of oxidation owing to greater degree of electron richness of the molecule. This finding supports the proposed internal charge delocalization in **11c** from the anionic part to the $-\text{NO}_2$ group as revealed through the theoretical calculations and exhibited in the LUMO picture(s) in Figure 4 under pH 11.

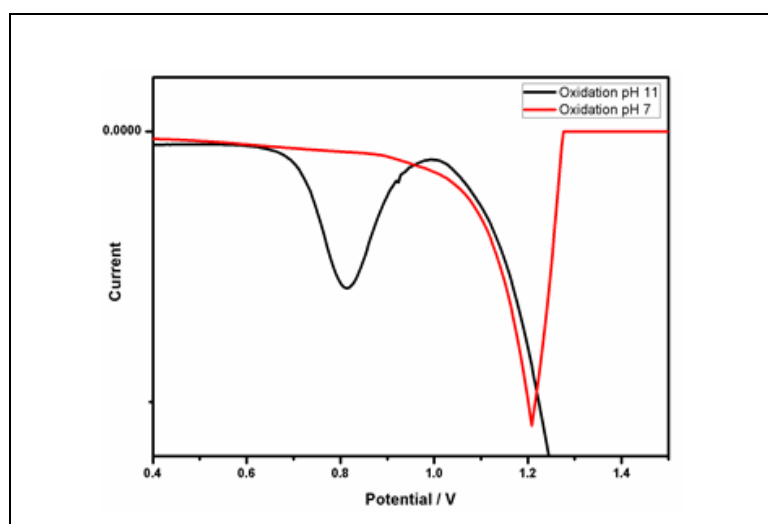


Figure 5. Differential pulse voltammetric plots of compound **11c** at pHs 7, 11.

Our nitrophenazine **11c** bearing a catechol moiety proved useful in selective detection of Fe^{3+} metal ion from a selection of other metal ions such as Cu^{2+} , Fe^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Ag^+ , Sn^{2+} , Zn^{2+} . In a typical experiment, to a dilute solution of the phenazine **11c** in DMSO-water were added aliquots of aq. solutions of the mentioned metal ions. Except Fe^{3+} in all other cases no significant changes were observed in the UV-visible spectrum of **11c**. In the case of addition of Fe^{3+} ion solution, the two main absorption bands of **11c** around 340 nm and 440 nm disappear and a new broad band around 300 nm appears with ten-fold increase in intensity (Figure 6). Clearly, complexation of the Fe^{3+} with the catechol unit of **11c** stops the ICT from the phenolic oxygens into the electron deficient phenazine core and the nitro group; thereby blue shifting the absorption spectrum.

Numerous examples of Fe(III)-tris catecholates complexes are known in literature and they are very stable on account of their very high formation constants, compared to similar complexes with other metal ions, a fact that has been postulated on the basis of the hard-soft acid base (HSAB) principle.¹⁵ Hence, our observed selectivity of nitrophenazine catechol **11c** towards Fe³⁺ ion is well in line with the existing knowledge on formation of high stability Fe(III)-tris catecholate complexes. Fe³⁺ being a hard cation is expected to form a stable complex with the hard anionic catecholate moiety. Another similar example regarding phenazine based metal ion recognition, is the reported strong blue-shifting of 2,3-diamino phenazine (**4**) absorption spectrum and disappearance of the longer wavelength bands, upon Cu²⁺ metal ion complex formation.¹⁶

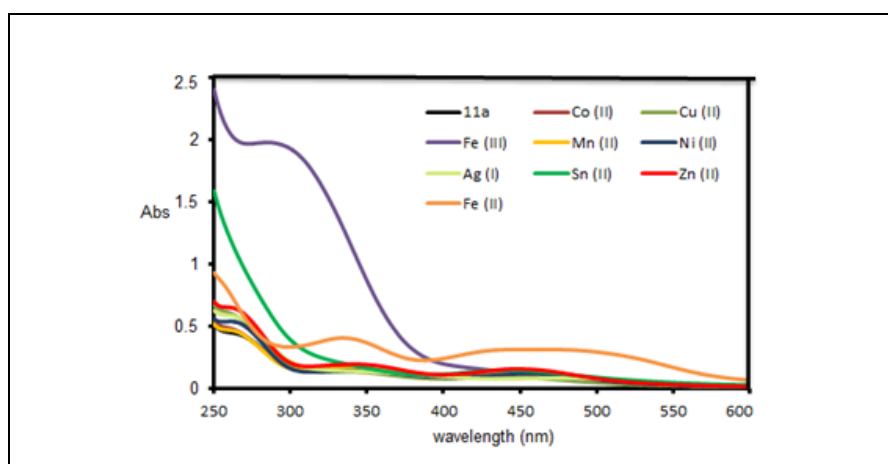


Figure 6. Titration of **11c** (2 μM) in 5% DMSO/water with aq. solutions of various metal ions (20 μM).

Compound **11a** was weakly fluorescent and its fluorescence spectrum was sensitive to presence of other aromatic analytes in the medium. Figure 6 depicts the effects of presence of aromatic compounds such as catechols and dinitroveratrole among others. There is no major alteration of the peak position or intensity of the fluorescence spectrum of **11a** upon addition of electro rich compounds. However, yet another type of electron rich species, the *ortho*-phenylene diamine, did not alter the fluorescence intensity of **11a**, but mildly blue shifted the spectrum. Finally, in presence of **11a** the electron poor dinitrobenzene, reduced its fluorescence intensity by 30%, a fact that is relevant in developing new analytical techniques for the selective detection of nitroaromatics.¹⁷

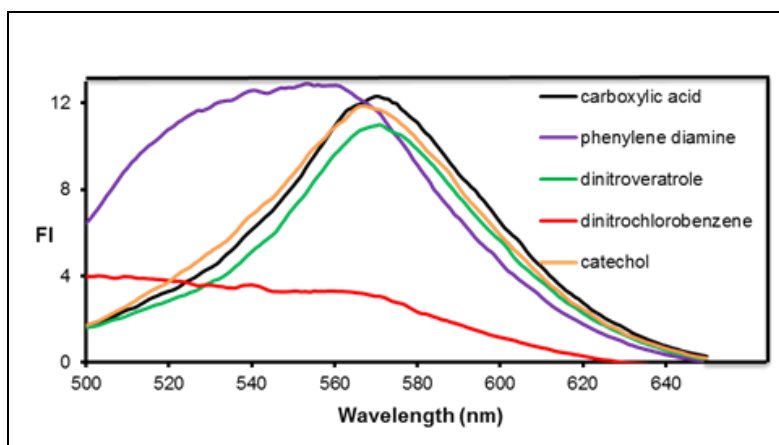


Figure 7. Fluorescence spectra of 11a (0.5 μ M, black trace) upon addition of various quenchers (5 μ M) in 10mL DMSO. (Amount was 1.2 mL of 11a + 0.2 mL of quencher).

In conclusion, we report the first examples of push-pull phenazines, which also exhibit pH responsive colorimetric and UV-visible spectral alterations. Theoretical calculations backed up by experimental studies on their oxidation potentials prove that under strongly basic pH, the phenazines undergo extensive charge delocalization from the electron rich to the electron poor portions. Since the UV-vis absorption spectra lie in the visible region above 400 nm, the presence of high pH can thus be easily detected spectrometrically as well as by the naked eye. This fact is significant in development of high pH responsive molecular probes based on our phenazines. Additionally, the Fe^{3+} ion selective behavior of our phenazine is relevant in the context chelation therapy applied for combatting physiological distresses arising from excess iron levels in human body.¹⁸ The findings about selective detection of nitroaromatics through fluorescence quenching, is relevant from the prism of trace explosives detection, most of which are indeed nitroaromatic molecules. We here propose to utilize our findings in developing of newer more easily soluble varieties of our push-pull phenazines and hope to mimic their various solution properties in thin film forms, towards more practical devices.

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