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Synthesis and Fluorescent Properties of a New Class of Heterocycles of Isoindole Fused Imidazoles with Phenolic Subunits

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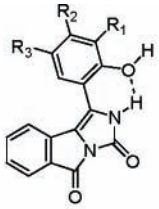
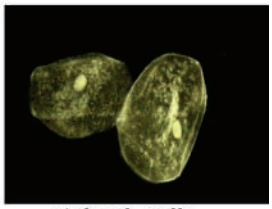
ABSTRACT





A new class of heterocycles of isoindole fused imidazoles with phenolic subunits has been readily synthesized by a two-step one-pot reaction. In aprotic solvent they show high fluorescent properties (Φ_F up to 0.93), but in protic polar solvent fluorescent intensity decreases. They show green fluorescence in weak acidic medium such as acetic acid but lack emission in basic medium. The compounds can also stain human squamous epithelium cells.

Heterocyclic compounds are rich sources of diverse physical, chemical, and biological properties.[1] In medicinal chemistry, they are commonly used as templates to design biologically active agents.[2] Imidazole-based heterocyclic molecules play important roles in various biochemical processes.[3] Therefore, the imidazolyl moiety is being used as a building block in developing new drugs.[3b,4] Moreover the imidazole moiety has wide-range applications in organometallic catalysis,[5] coordination chemistry,[6] and asymmetric catalysis.[7] There are several reports of the synthesis and functionalization of the imidazole moiety.[8] Herein we report an efficient and general procedure for the construction of a new class of heterocycles of isoindole fused imidazoles bearing phenolic subunits. Interestingly this class of molecules is found to exhibit fluorescent properties.

When phenols **1a**−**m** are refluxed in a mixture of ninhydrin and acetic acid, 2-hydroxy-2-(2′-hydroxy-aryl)-1,3-indanediones **2a**−**m** are formed (Scheme 1).[9] The adducts so formed preferentially remain in the cyclic hemiketal form **3a**−**m**.[9a−c] Interestingly refluxing of **3** with urea in the same acetic acid mixture produces 1-aryl-3,5-

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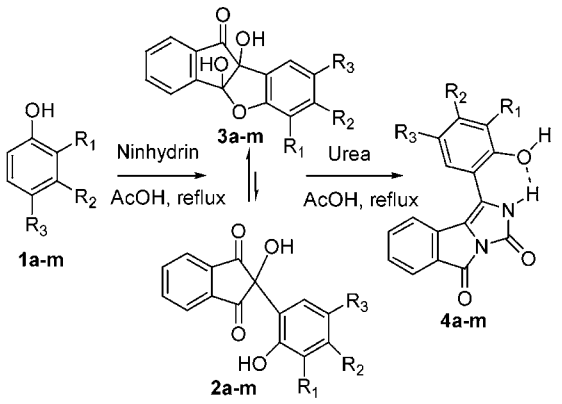
Scheme 1

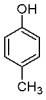
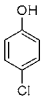
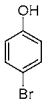
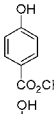
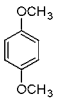


1, 2, 3 and **4**

a: $R_1 = R_2 = R_3 = H$ **b**: $R_1 = CH_3$, $R_2 = R_3 = H$
c: $R_1 = R_2 = H$, $R_3 = CH_3$ **d**: $R_1 = R_3 = H$, $R_2 = CH_3$
e: $R_1 = OCH_3$, $R_2 = R_3 = H$ **f**: $R_1 = R_2 = H$, $R_3 = OCH_3$
g: $R_1 = Cl$, $R_2 = R_3 = H$ **h**: $R_1 = R_2 = H$, $R_3 = Cl$
i: $R_1 = H$, $R_2 = CH_3$, $R_3 = Cl$ **j**: $R_1 = R_2 = H$, $R_3 = Br$
k: $R_1 = OCH_3$, $R_2 = H$, $R_3 = CHO$ **l**: $R_1 = R_2 = H$, $R_3 = CO_2CH_3$
m: $R_1 = R_2 = H$, $R_3 = CO_2C_2H_5$

dioxo-1*H*-imidazo-[3,4-*b*]isoindole **4a**−**m** by condensation reaction (Scheme 1, Table 1).[10] The isolated pure compounds **4a**−**m** are yellow solids. All of the compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy.[11] The X-ray crystal structure of **4f** is shown in Figure 1 where the intramolecular hydrogen bonding is indicated by a broken line.[12] A proposed mechanism for the formation of **4** is depicted in Scheme 2. The nucleophilic attack of urea to either of the carbonyl groups of **2** produces the open chain amide **5**, which undergoes a subsequent intramolecular nucleophilic attack on the other CO to produce the interme-

Table 1. Preparation of **4** from Ninhydrin, Phenols/Methoxy Aromatics (**1a**−**o**), and Urea

entry	phenols/ aromatics	**1**	time[a] (h)	yield % of **4**[b]	
1		**1a**	2.5	**4a**	80
2		**1b**	3.5	**4b**	70
3		**1c**	2.5	**4c**	76
4		**1d**	3.0	**4d**	72
5		**1e**	2.5	**4e**	60
6		**1f**	2.5	**4f**	68
7		**1g**	4.0	**4g**	76
8		**1h**	3.5	**4h**	75
9		**1i**	4.0	**4i**	80
10		**1j**	3.5	**4j**	82
11		**1k**	4.0	**4k**	61
12		**1l**	4.0	**4l**	63
13		**1m**	4.0	**4m**	60
14		**1n**	8.0	**4n**	62
15		**1o**	8.0	**4o**	65

[a] Reaction time after addition of urea. [b] Yields are for isolated products.

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(10) **Typical Procedure for Preparation of 4.** A mixture of ninhydrin (0.25 g, 1.4 mmol) and phenol **1a** (4.2 mmol) was refluxed in AcOH (6.0 mL) until the formation of adduct **2a** was complete (monitored by TLC).[9] Then urea (1.0 g, 16.6 mmol) was added to the above reaction mixture, which was refluxed further for 2.5 h. The reaction mixture turned into red color. The cold reaction mixture was poured into ice-cold water. The yellow solid product was filtered and purified by column chromatography over silica gel (petroleum ether/ethyl acetate 70/30 v/v) to give 0.31g of **4a** (80% yield). The compound was crystallized from acetone.

(11) **4a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.64 (bs, -NH), 7.80 (d, J = 7.8 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.41−7.32 (m, 3H), 7.05 (d, J = 8.2 Hz, 1H), 6.96 (t, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.4, 152.3, 144.5, 131.3, 129.1, 128.3, 127.8, 126.6, 124.2, 122.4, 119.0, 117.6, 116.4, 113.8, 113.4, 111.8.

(12) X-ray crystal structure analysis for **4f**: formula $C_{17}H_{12}N_2O_4 \cdot H_2O$, M = 326.30, yellow crystal 0.35 × 0.25 × 0.20 mm^3, a = 7.103(1), b = 13.709(1), c = 14.869(1) Å, V = 1447.9(2) Å^3, ρ_{calc} = 1.497 g cm^{-3}, μ = 0.940 mm^{-1}, empirical absorption correction (0.734 ≤ T ≤ 0.834), Z = 4, orthorhombic, space group $P2_12_12_1$ (No. 19), λ = 1.54178 Å, T = 223 K, ω and φ scans, 12370 reflections collected (±h, ±k, ±l), [(sinθ)/λ] = 0.60 Å^{-1}, 2564 independent (R_{int} = 0.033) and 2552 observed reflections [$I \geq 2\ \sigma(I)$], 231 refined parameters, R = 0.027, wR^2 = 0.073, max. residual electron density 0.12 (−0.13) e Å^{-3}, Flack parameter −0.07(16), hydrogen atoms at water from difference Fourier calculation, other calculated and refined as riding atoms. CCDC 610504 contains the supplementary crystallographic data for this paper.

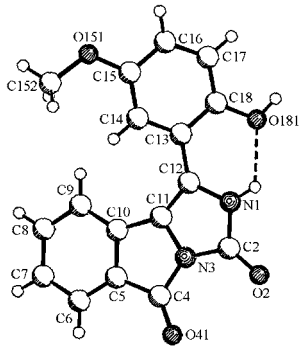
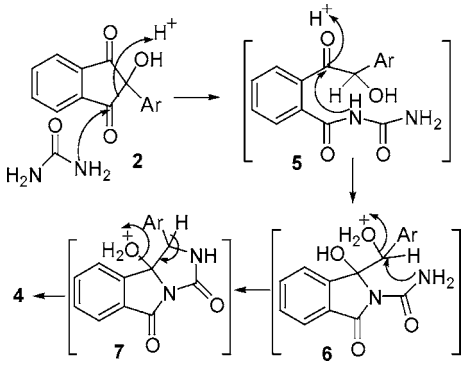


Figure 1. X-ray crystal structure of **4f**. The broken line indicates intramolecular hydrogen bonding.

diate **6**. The isoindole fused imidazole ring **7** is formed by another intramolecular nucleophilic attack of the free -NH_2 group. Finally dehydration produces **4**.

Scheme 2



The purified compounds **4a**−**m** were dissolved in several solvents to study their spectroscopic properties (Table 2). For all compounds, the emission maxima shift to the red wavelength region with increasing solvent polarity. The most bathochromic emission is found in methanol as solvent. Concurrently, the fluorescence quantum yields (Φ_F) decrease with increasing solvent polarity. The highest Φ_F values are measured in dichloromethane, and the lowest are measured in protic polar methanol. Probably the protic polar solvent disrupts the six-membered intramolecular hydrogen bond in **4** and solvates the molecules with intermolecular hydrogen bonding. This may influence the emission properties. The significant fluorescence quenching of compounds **4e**, **4f**, and **4k** in methanol is attributed to extensive solvation for the presence of methoxy phenolic units (Scheme 1, Table 2).

As a result of the insolubility of **4** in aqueous media, the influence of pH on the photophysical properties could not be recorded accurately. Instead, similar studies were carried out in organic solution. In spectrophotometric study when a solution of **4c** in methanol is basified gradually with methanolic triethylamine, the absorbance of the band maxima

Table 2. Spectroscopic Data of **4** in Three Solvents

4	solvent	λ_{abs} (nm)	λ_{em} (nm)	Φ_F [a]
4a	CH_2Cl_2	394	477	0.59
	CH_3CN	393	480	0.48
	CH_3OH	396	498	0.38
4b	CH_2Cl_2	392	473	0.60
	CH_3CN	391	480	0.52
	CH_3OH	395	496	0.15
4c	CH_2Cl_2	393	475	0.62
	CH_3CN	394	480	0.51
	CH_3OH	399	500	0.25
4d	CH_2Cl_2	393	474	0.63
	CH_3CN	393	481	0.51
	CH_3OH	397	498	0.14
4e	CH_2Cl_2	396	471	0.75
	CH_3CN	393	478	0.51
	CH_3OH	395	496	0.02
4f	CH_2Cl_2	399	481	0.66
	CH_3CN	395	480	0.46
	CH_3OH	401	500	0.01
4g	CH_2Cl_2	391	470	0.65
	CH_3CN	388	476	0.46
	CH_3OH	393	492	0.13
4h	CH_2Cl_2	395	475	0.78
	CH_3CN	390	475	0.61
	CH_3OH	393	493	0.28
4i	CH_2Cl_2	399	478	0.61
	CH_3CN	393	479	0.56
	CH_3OH	395	496	0.30
4j	CH_2Cl_2	396	474	0.61
	CH_3CN	390	475	0.49
	CH_3OH	400	492	0.32
4k	CH_2Cl_2	390	467	0.93
	CH_3CN	390	473	0.33
	CH_3OH	398	490	0.04
4l	CH_2Cl_2	395	474	0.86
	CH_3CN	390	477	0.46
	CH_3OH	394	491	0.35
4m	CH_2Cl_2	399	474	0.59
	CH_3CN	391	476	0.49
	CH_3OH	395	490	0.40
4n	CH_2Cl_2	406	488	0.57
	CH_3CN	401	492	0.48
	CH_3OH	401	507	0.39
4o	CH_2Cl_2	399	462	0.58
	CH_3CN	396	466	0.51
	CH_3OH	397	484	0.24

[a] Determined with reference to quinine sulfate in 0.1 M H_2SO_4 (Φ_F = 0.54).

at 399 nm steadily decreases (Figure 2). At the same time a new band maxima develops around 480 nm with increase of base concentration. In fluorimetric study a steady decrease of fluorescence intensity (at λ_{em} 498 nm) with addition of base is observed, but without development of any band maxima during the titration. The result indicates that the phenolate ions of **4** are nonfluorescent, causing a lack of fluorescence property in basic medium. When compounds **4** are dissolved in acetic acid, a green fluorescence is observed, but fluorescence quenching is observed when methanolic solutions of **4** are gradually acidified with a strong

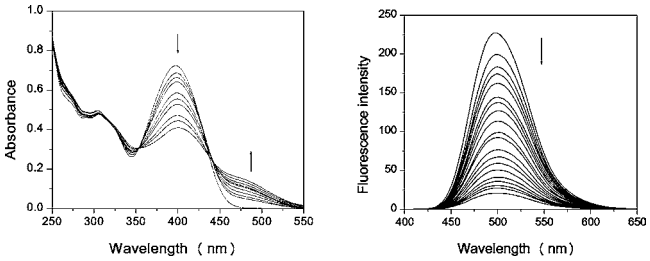


Figure 2. Spectrophotometric (left) and fluorimetric (right) titration of **4c** ($\sim 10^{-5}$ M in MeOH) with methanolic triethylamine.

acid such as trifluoroacetic acid. The study in acid medium shows that intramolecular H-bonding is not the only factor responsible for fluorescent property. The compounds **4n** and **4o**, where the phenolic -OH groups are replaced by -OMe (Figure 3), also show a decrease of fluorescence quantum

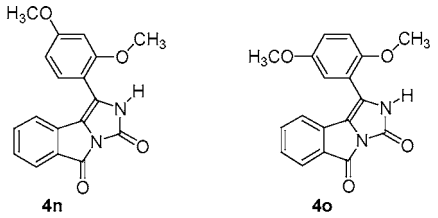


Figure 3. Structure of compounds **4n** and **4o**.

yields on going from dichloromethane to methanol (Table 2). Fluorescence quenching is observed when a methanolic solution of **4o** is titrated with methanolic triethylamine. Interestingly crystals of **4** also show fluorescent property when observed under a fluorescence microscope (Figure 4).

Fluorescent molecular probes are widely used in chemistry, physics, biology, and medicinal sciences.[13] One of the most important applications is the characterization of (bio)chemical

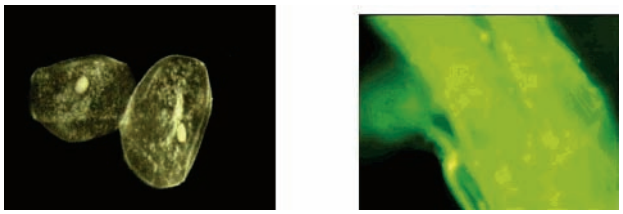


Figure 4. Images of cells stained by a solution of **4a** in methanol/water (1:5 v/v) (left) and crystal of **4a** (right), both under fluorescence microscope.

processes, both in vitro and in vivo. Preliminary studies show that **4** can readily stain human squamous epithelium cells (Figure 4). Interestingly the nucleus of the cell has been found to stain prominently. The development of cell-permeable DNA fluorescence sensor with high selectivity and sensitivity has been of great interest.[13a,14] Further studies on the staining properties of these molecules may produce useful results.

In summary, we have developed an efficient procedure for synthesis of isoindole fused imidazoles with phenolic subunits from easily available starting materials. The compounds show green fluorescence in neutral and weakly acidic medium but lack fluorescent emission in basic medium. The compounds are also found to stain human squamous epithelium cells particularly the nuclei.

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Supporting Information Available: Experimental procedures, spectroscopic data, ^{1}H and ^{13}C NMR spectra, fluorescence emission and excitation spectra of **4**, X-ray crystalographic data of **4f** in CIF format, and enlarged image of stained cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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