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Full Paper

Indazole Synthesis

Functionalizable 1*H*-Indazoles by Palladium Catalyzed Aza-Nenitzescu Reaction: Pharmacophores to Donor-Acceptor Type Multi-Luminescent Fluorophores

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Dedicated to Professor M. V. George on the occasion of his 90th birthday

Abstract: Development of small-molecule-based multi-luminescent fluorophores utilizing simple synthetic methodologies, as well as easily available starting materials, has gained much attention in recent years. Herein, we disclose an efficient protocol for the synthesis of N-protected 1*H*-indazole derivatives with diverse substituents at their 3- and 5-positions via palladium-catalyzed reactions of hydrazones and *p*-benzoquinones. The obtained 1*H*-indazole derivatives can be easily modified into donor-acceptor (D–A)-type chromophores (Indazo-Fluors) with tunable emission properties in both solid and solution state. Owing to the extent of intramolecular charge transfer, Indazo-Fluors exhibit positive solvatochromic emission spanning from blue-green to orange-red. Theoretical studies were undertaken to rationalize the observed trends in the optical properties of Indazo-Fluors. Finally, a triethylene glycol (TEG) appended Indazo-Fluor exhibiting a large Stokes shift and low cytotoxicity allowed us to use it for cell imaging.

Introduction

Luminescent organic molecules with structural flexibility and stability have gained significant attention in various fields of science ranging from material chemistry to biology.[1–3] Over the years, many small-molecules based fluorescent scaffolds have been developed[3] out of which *N*-heterocyclic fluorophores have gained much attention because excellent control over their emission wavelengths are possible either by the extension of conjugation or by introducing donor-acceptor functionalities.[3d–f,4] Among various *N*-heterocyclic azoles studied so far,[4c,5,6] indazole based fluorophores have received less attention in comparison with its counterparts.[6] During the last decade, although the material aspects of indazole at the nascent stage,[7] the chemistry of indazole has gained considerable interest, because of their broad spectrum of biological activities (Figure 1).[8,9] Indazole derivatives as such are found in numerous synthetic pharmaceutical drug molecules. The derivatives of indazole have been successfully used in drug development to afford anti-HIV, anti-tumor, anti-inflammatory etc. drugs.[9] Granisetron, benzydamine, axitinib[9a] are some of the indazole drugs currently available in the market.

As the natural abundance of indazole is rare,[10] chemical synthesis is the major source of this promising molecule, which in fact, stimulated the development of new synthetic routes towards their preparation.[11–13] Intramolecular cyclization of aniline derivatives or hydrazones[12] and transition metal catalyzed C–H activation reactions[13] are the two widely used approaches towards the synthesis of indazoles. However, the major challenges associated with these reported methods are their dependence on a pre-functionalized substrate, usually prepared by multistep synthesis, and harsh reaction conditions. The requisite of a pre-functionalized substrate limits the scope of these approaches to a large extent. Thus, new convergent approaches towards indazoles based on readily available reagents always attract interest.

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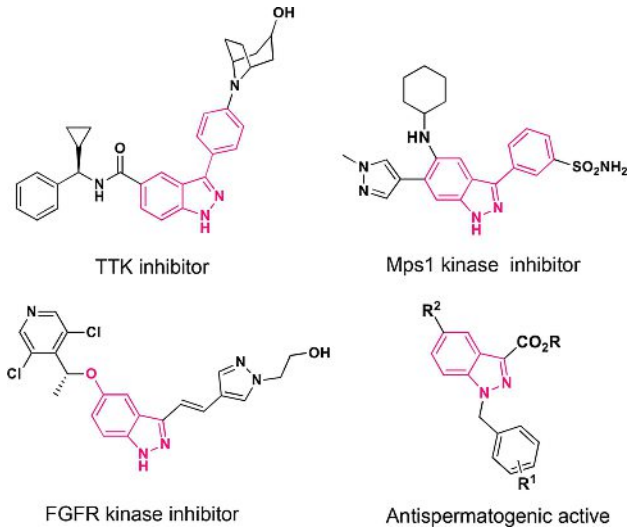


Figure 1. Representative examples of 1*H*-indazole-based bioactive molecules.

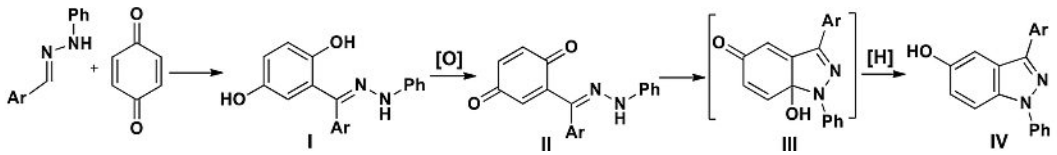
Michael addition of enamines to 1,4-quinones (Nenitzescu reaction) is one of the prominent method towards the synthesis of 5-hydroxy indoles.[14] Indazoles are bioisosteres of indoles[15] and hence the same strategy has been extended towards the synthesis of indazoles where hydrazones (aza-enamines) are used in place of enamines along with 1,4-quinones to afford 5-hydroxy indazoles.[16] Despite it offers an easy access to obtain 5-hydroxy indazoles, the strategy got minimum attention and only a few isolated reports are available in the literature with limited substrate scope and poor conversion.[16] The most widely accepted mechanism for this reaction is depicted in Scheme 1.[16a] The hydroquinone adduct **I** formed by the condensation between benzoquinone and hydrazone, is first oxidized to quinone intermediate **II** followed by intramolecular cyclization to afford a carbinolamine intermediate **III**, which eventually reduced to 5-hydoxy-1*H*-indazole **IV**. The efficiency of the approach is largely dependent on the oxidant that at first converts **I** to **II** and the reduced oxidant will be oxidized back during the reduction of **III** to **IV**. Pd(II) catalyzed oxidation reaction are well known in organic synthesis and *p*-benzoquinone is the most widely used oxidant to maintain Pd^0/Pd^{II} catalytic cycle.[17] Hence, we have speculated the possibility to employ Pd(II) as an oxidant in the aza-Nenitzescu reaction as benzoquinone/hydroquinone intermediates involved in the pathway (Scheme 1) will effectively maintain the catalytic cycle. Detailed investigation has revealed that the reaction is quite effective in presence of catalytic $Pd(OAc)_2$. It is worthy of note that many pharmacologically active 1*H*-indazole derivatives contain substitutions at 3- and 5-positions of the indazole framework with *N*-protecting groups.[18] The present strategy is so unique that it offers a one-pot synthesis of *N*-protected indazole derivatives with diverse substituents at the comparatively more potent 3- and 5-positions, which allows a subsequent functionalization of the indazole framework quite easily.

Although the pharmacological properties of indazoles have been studied extensively, their material chemistry aspects especially photoluminescent properties[6] have gained less attention until Lavis, Ellman and co-workers studied a series of 2*H*-indazole based blue emitting fluorophores synthesized by Rh (III) catalyzed [4 + 1] annulation reaction of azobenzene and aldehydes.[6a] Later, Gao, You and co-workers have reported a library of biheteroaryl fluorophores based on 2*H*-indazoles showing multicolor emission including near-infrared.[6c] Very recently, Tsui, Joo and co-workers have developed a series of blue-emissive 1*H*-indazoles.[6d] These studies have demonstrated the potential of indazoles as fluorophores. However, lack of simple protocols employing low cost starting materials towards the synthesis of indazole-based fluorophores precludes their further exploration. Herein, we describe a simple and efficient synthetic methodology for 5-hydroxy-1*H*-indazoles by the reaction of easily available hydrazones and *p*-benzoquinones. Hence, the developed synthetic route is quite attractive as it affords *N*-aryl protected 1*H*-indazole framework with functionalizable phenyl and hydroxyl groups at C_3 and C_5 positions, respectively. Taking advantage of this aspect, we have synthesized a series of D–A type fluorophores, (Indazo-Fluors) and studied their photophysical properties systematically to unravel a proper structure-luminescent property relationship. In addition, we have also demonstrated the potential of water-compatible version of Indazo-Fluors for cell imaging.

Results and Discussion

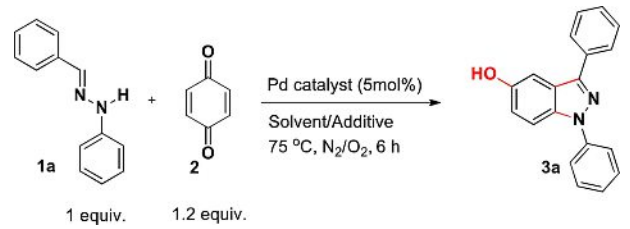
Synthesis

We commenced our investigation by monitoring the reaction between 1-benzylidene-2-phenylhydrazine **1a** (1 equiv.) and *p*-benzoquinone **2** (1.2 equiv.) in the presence of $Pd(OAc)_2$ (5 mol %) in 1,2-dichloroethane (DCE) at 75 °C under O_2 atmosphere and 5-hydroxy-1*H*-indazole **3a** was formed in 41% yield (Table 1, entry 1). Intrigued by the initial result, we carried out the same reaction under N_2 atmosphere and the yield of indazole **3a** was improved to 53% (Table 1, entry 2). In presence of trifluoroacetic acid (TFA) as the additive (3.5 mmol), the yield of **3a** was further increased to 69% (Table 1, entry 4). The other additives like acetic acid, *p*-toluenesulfonic acid (PTSA), pivalic acid,



Scheme 1. The mechanistic pathway of the aza-Nenitzescu reaction for the synthesis of 1*H*-indazoles from hydrazone and *p*-benzoquinone.[16a]

Table 1. Optimization of reaction conditions.[a]



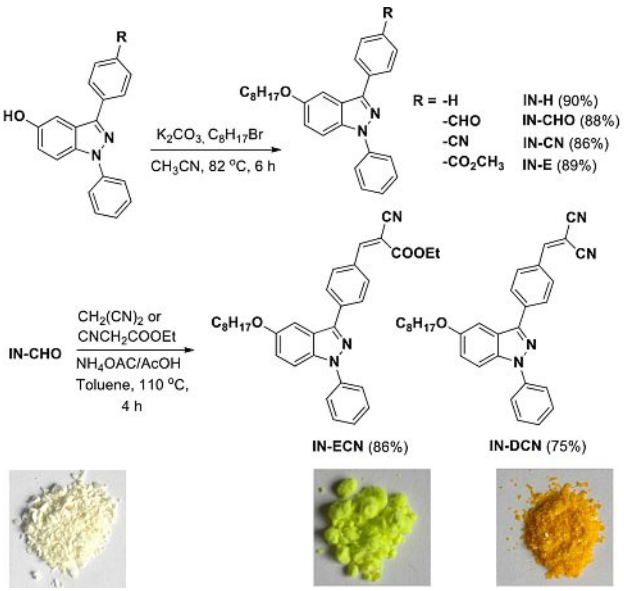
Entry	Catalyst	Additive	Solvent	Yield[b]
1[c]	$Pd(OAc)_2$	–	DCE	41
2	$Pd(OAc)_2$	–	DCE	53
3[d]	–	–	DCE	Nil
4	$Pd(OAc)_2$	TFA	DCE	69
5	$Pd(OAc)_2$	AcOH	DCE	24
6	$Pd(OAc)_2$	PTSA	DCE	trace
7	$Pd(OAc)_2$	pivalic acid	DCE	trace
8	$Pd(OAc)_2$	NaOAc	DCE	29
9	$PdCl_2$	TFA	DCE	46
10	$PdCl_2(PPh_3)_2$	TFA	DCE	44
11	$Pd(TFA)_2$	TFA	DCE	54
12	$Pd(OAc)_2$	TFA	1,2-DME	trace
13	$Pd(OAc)_2$	TFA	CH_3CN	19
14	$Pd(OAc)_2$	TFA	DMSO	trace
15	$Pd(OAc)_2$	TFA	1,4-dioxane	trace
16	$Pd(OAc)_2$	TFA	toluene	44

[a] Reaction condition: **1a** (0.25 mmol), **2** (0.3 mmol), Pd catalyst (5 mol%), with different additives (3.5 mmol) under N_2/O_2 atmosphere at 75 °C for 6 h. [b] Isolated yields. [c] Reaction under O_2 atmosphere. All other trials were performed under N_2 atmosphere. [d] Reaction without palladium catalyst.

NaOAc were found to be ineffective and did not improve the yield of the reaction (Table 1, entries 5–8). Further, we carried out a systematic screening of different palladium catalysts with TFA as the additive (Table 1, entries 9–11), which revealed that $Pd(OAc)_2$ is the best among the other catalysts (Table 1, entry 4). A refining of solvents under identical reaction conditions (Table 1, entries 12–16) showed that 1,2-dichloroethane was the best solvent for the transformation although toluene gave a fair yield of 44% (Table 1, entry 16). Systematic screening of the reaction parameters using various catalysts, additives, solvents and other reaction conditions revealed that the desired 5-hydroxy-1*H*-indazole **3a** was isolated most efficiently when the hydrazone (1 equiv., 0.25 mmol) and *p*-benzoquinone (1.2 equiv., 0.3 mmol) are treated in the presence of $Pd(OAc)_2$ (5 mol%, 0.0125 mmol) and TFA (3.5 mmol) in 1,2-dichloroethane (2 mL) at 75 °C for 6 h.

Under the optimized condition, the substrate scope of the reaction was then tested (Table 2). Both electron rich and deficient hydrazones afforded the product, however, the electron deficient hydrazones led to better conversion. Furthermore, the functional group tolerance of the strategy was noteworthy, as 1*H*-indazoles with functional groups such as aldehyde, cyano, ester, nitro, $-CF_3$, Br, Cl, F, etc. could be isolated in moderate to good yield. In addition to *p*-benzoquinone, 2-methyl-1,4-benzoquinone and 2-chloro-1,4-benzoquinone took part in the reaction with ease and resulted in the formation of a single isomer (see single crystal structure of **16b**, Table 2). However, with 1,4-naphthoquinone, only a trace amount of 1*H*-indazole was formed. In analogy with the mechanism of the previous report based on Fe^{3+}/Fe^{2+} redox couple mediated reaction of hydrazone and *p*-benzoquinone to 5-hydroxy-1*H*-indazoles,[16a] it can be proposed that the Pd(II) in the reaction medium facilitates the oxidation of aza-hydroquinone adduct **I** to benzoquinone adduct **II** and the subsequent reduction of the carbinolamine intermediate **III** to indazole helps to regenerate Pd(II) in the catalytic cycle (Scheme S1 in the Supporting Information). It is worthy of note that the developed synthetic methodology could afford a variety of functionalized 1*H*-indazoles with a better yield in comparison to the earlier reports.[16]

The developed reaction methodology is compatible with a variety of hydrazones and *p*-benzoquinones and offers an opportunity to tune the properties of 5-hydroxy-1*H*-indazoles by selecting appropriate starting materials. As an extended scope of the present methodology and to investigate the photophysical properties, 5-hydroxy-1*H*-indazoles derivatives were structurally modified into a series of luminescent D–A type molecules, Indazo-Fluors **IN-H**, **IN-CHO**, **IN-E**, **IN-CN**, **IN-ECN** and **IN-DCN** (Scheme 2). The design strategy lies with the



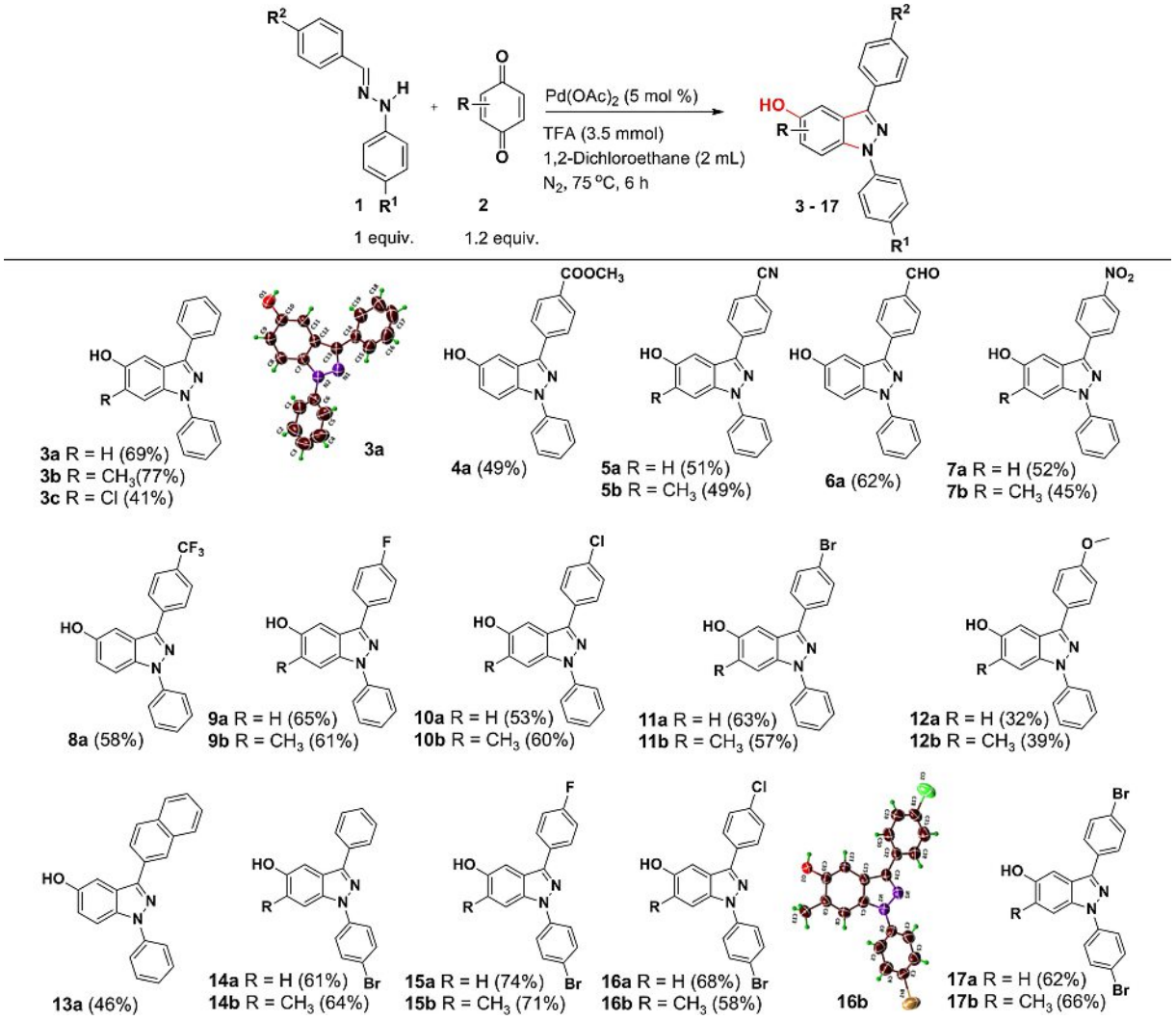
Scheme 2. Synthesis of Indazo-Fluors by modification of 5-hydroxy-1*H*-indazoles.

tuning of optical properties of 1*H*-indazole derivatives by modulating the ground state polarizability by changing electron withdrawing ability of the appended acceptor moieties.

Photophysical Studies

The introduction of octyloxy group (D) and appropriate acceptor (A) groups were very fruitful, as the non-luminescent hydroxy 1*H*-indazole turned to be multi luminescent fluorophores. Upon illumination with 365 nm UV light, Indazo-Fluors **IN-E**, **IN-CN**,

Table 2. Substrate scope of Pd catalyzed aza-Nenitzescu synthesis of 5-hydroxy-1*H*-indazoles.[a]



[a] Standard reaction conditions: **1** (0.25 mmol), **2** (0.3 mmol), $Pd(OAC)_2$ (5 mol%), TFA (3.5 mmol), DCE (2 mL), under N_2 atmosphere at 75 °C for 6 h.

IN-ECN and **IN-DCN** showed very good solid-state photoluminescence that can be tuned from blue, green to yellow-orange (Figure 2). To gain insight about the photophysical properties of Indazo-Fluors, absorption, emission, quantum yield (Φ_F) and lifetime (τ) studies were conducted both in solid- and solution-states (Figure S1 and Table 3). In the solid-state, **IN-E** and **IN-CN** exhibited the emission maxima at 417 ($\Delta\nu_{St}$ = 2686 cm^{-1}) and 412 nm ($\Delta\nu_{St}$ = 2609 cm^{-1}) with Φ_F values of 0.21 and 0.16, respectively. However, molecules **IN-ECN** and **IN-DCN** with strong acceptor groups and π-spacer displayed red-shifted emission maxima at 498 ($\Delta\nu_{St}$ = 3616 cm^{-1}) and 570 nm

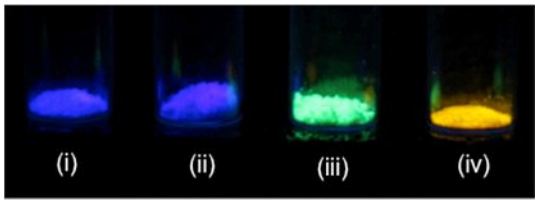


Figure 2. Solid-state photoluminescence of Indazo-Fluors under 365 nm UV light illumination, (i) **IN-E**, (ii) **IN-CN**, (iii) **IN-ECN** and (iv) **IN-DCN**.

Table 3. Solid-state photophysical properties of Indazo-Fluors.

Compounds	λ_{abs} (nm)	λ_{em} (nm)	$\Delta\nu_{St}$ (cm^{-1})[a]	Φ_F[b]	τ_{av} (ns)[c]
IN-E	375	417	2686	0.21	1.36
IN-CN	372	412	2609	0.16	1.16
IN-ECN	422	498	3616	0.20	2.60
IN-DCN	441	570	5132	0.18	11.6

[a] $\Delta\nu_{St} = \nu_{abs} - \nu_{em}$, Stokes shift. [b] Solid-state quantum yields were measured using an integrated sphere set up.[20] [c] Average lifetime.

($\Delta\nu_{St} = 5132\ cm^{-1}$) with Φ_F values of 0.20 and 0.18, respectively. The longer lifetime value (11.6 ns) of **IN-DCN** indicates that this molecule is densely packed in the solid-state due to strong intermolecular dipole-dipole interaction.[19]

To understand the effect of electron withdrawing substituent on the photophysical properties of Indazo-Fluors, the absorption and emission spectra were recorded in THF (Figure 3) and data have summarized in Table 4. The 1*H*-indazole derivative **IN-H**, devoid of any acceptor moiety exhibited absorption maximum (λ_{abs}) at 337 nm ($\log\varepsilon = 4.31$), which was bathochromically shifted to 347 ($\log\varepsilon = 4.43$) and 348 nm ($\log\varepsilon = 4.36$) for **IN-E** and **IN-CN** having electron accepting moieties -COOMe and –CN, respectively. With the introduction of –CHO group to 1*H*-indazole framework, the push-pull D–A system with octyloxy donor, **IN-CHO** exhibited a bathochromic shift of 1583 cm^{-1} in the absorption maximum ($\lambda_{abs} = 356$ nm, $\log\varepsilon = 4.41$). Further, introduction of π-extended acceptor such as –CH=CH–(COOEt)(CN) resulted in shifting the absorption maxima to 394 nm ($\log\varepsilon = 4.52$) with a significant bathochromic shift of 4293 cm^{-1} for **IN-ECN**. While, the bathochromic shift was found to be maximum of 5402 cm^{-1} with –CH=CH-$(CN)_2$ acceptor moiety, the molecule **IN-DCN** exhibited the absorption maximum at 412 nm ($\log\varepsilon = 4.54$).

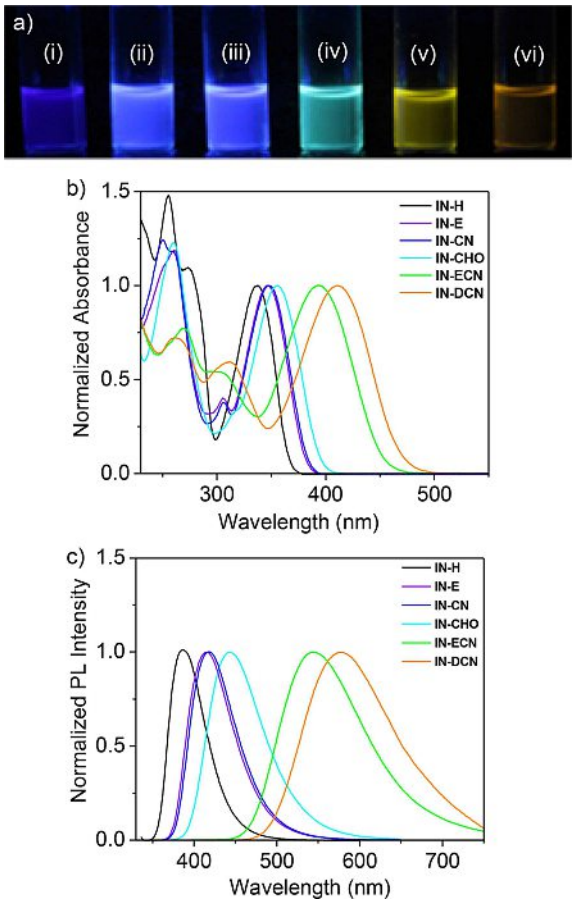


Figure 3. a) Fluorescence image of THF solution of Indazo-Fluors under 365 nm UV light illumination, (i) **IN-H**, (ii) **IN-E**, (iii) **IN-CN**, (iv) **IN-CHO**, (v) **IN-ECN**, and (vi) **IN-DCN**. The b) absorption and c) photoluminescence spectra of Indazo-Fluors in THF. All the studies were conducted at a concentration of 1×10^{-5} M.

The emission spectra of 1*H*-indazole-based fluorophores in THF covered a full range of wavelengths from 388 (**IN-H**) to 578 nm (**IN-DCN**) (Figure 3a and c). The model derivative **IN-H** showed a Stokes shift of 3900 cm^{-1} with Φ_F of 0.31. The molecules **IN-E** and **IN-CN** displayed the emission maxima around 420 nm with a substantial increase in Stokes shift and Φ_F values (Table 4). In comparison to **IN-H**, the emission maximum of **IN-CHO** exhibited a red shift of 3200 cm^{-1} ($\lambda_{em} =$ 443 nm) along with a Stokes shift value of 5516 cm^{-1}. **IN-ECN** and **IN-DCN** showed the largest red shift of 7391 cm^{-1} ($\lambda_{em} =$ 544 nm, $\Delta\nu_{St} = 6998\ cm^{-1}$) and 8472 cm^{-1} ($\lambda_{em} = 578$ nm, $\Delta\nu_{St} = 6970\ cm^{-1}$), respectively. In THF, acceptor functionalized Indazo-Fluors showed almost similar lifetime decay values (~ 2.5 ns), while the model derivative **IN-H** showed a relatively shortened lifetime decay value of 1.57 ns (Table 4).

In general, the D – A type fluorophores are prone to exhibit solvent dependent absorption and fluorescence characteristics resulting from the induction of dipole moment due to the transfer of electron density from donor to acceptor and its interaction with dipole of the solvent.[22] For solvatochromism experiments, we chose a variety of solvents having difference in polarity viz methyl cyclohexane (MCH), toluene, dichloromethane, chloroform, THF and acetone. The UV/Vis and photoluminescence studies revealed that for compounds **IN-H**, **IN-E**, **IN-CN**, and **IN-CHO**, the absorption and emission maxima are only moderately shifted from nonpolar (MCH) to polar (acetone) solvent indicating that ground state is less polar with little difference between ground and excited state structures (Tables S1–S4, Figures S2–S5). However, molecules **IN-ECN** and **IN-DCN** exhibited tunable photoluminesce from blue to red

Table 4. Effect of substituents on the photophysical properties of Indazo-Fluors in THF.

Compounds	λ_{abs} (nm)	logε	λ_{em} (nm)	$\Delta\nu_{St}$ (cm^{-1})[a]	Φ_F	τ (ns)
IN-H	337	4.31	388	3900	0.31[b]	1.57
IN-E	347	4.43	420	5009	0.67[b]	2.44
IN-CN	348	4.36	421	4983	0.69[b]	2.69
IN-CHO	356	4.41	443	5516	0.24[b]	1.48
IN-ECN	394	4.52	544	6998	0.43[c]	2.67
IN-DCN	412	4.54	578	6970	0.43[c]	2.62

[a] $\Delta\boldsymbol{\nu} = \boldsymbol{\nu}_{abs} - \boldsymbol{\nu}_{em}$, Stokes shift. Fluorescence quantum yields (±5% error) were determined by using [b] quinine sulfate as the standard ($\Phi_F = 0.54$ in 0.1 M H_2SO_4), [c] rhodamine 6G ($\Phi_F = 0.95$ in ethanol) as the standard.[21]

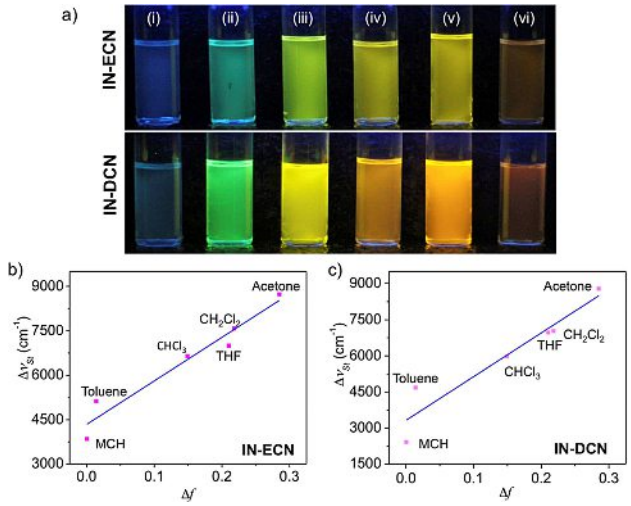


Figure 4. a) Fluorescence images of Indazo-Fluors **IN-ECN**, and **IN-DCN** in solvents of different polarity under 365 nm UV light illumination (1×10^{-5} M, i = MCH, ii = toluene, iii = $CHCl_3$, iv = THF, v = CH_2Cl_2, vi = acetone); b) The plot Stokes shift ($\Delta\nu_{St}$) versus solvent orientational polarizability (Δf) for **IN-ECN** and **IN-DCN**.

while moving from nonpolar to polar solvents (Figures 4a, S6–S8). The obtained photophysical parameters have been summarized in Table 5 and S5.

The positive solvatochromism exhibited by Indazo-Fluors with increase in solvent polarity can be correlated to dipole moment upon photoexcitation. The change in ground to excited state dipole moment of the Indazo-Fluors upon photoexcitation can be determined from Lippert-Mataga Equation (1).[23]

$$\Delta\nu_{St} = \frac{2\Delta f}{4\pi\varepsilon_0 hca^3}\left(\mu_e - \mu_g\right)^2 + \text{constant} \qquad (1)$$

Where $\Delta\nu_{st}$ is the Stokes shift, μ_e and μ_g are permanent dipole moment in the excited and ground state, respectively, ε_0 is the relative permittivity of vacuum, h is the Planck's constant, a is Onsager radius. The Onsager radii[24] of Indazo-Fluors were calculated from optimized ground state structures which are obtained from quantum chemical calculations by DFT method at M06-2X/6-311 + G(d,p) level. The plot of $\Delta\nu_{st}$ versus orientational polarizability of solvents Δf (Lippert plot) showed a good linear correlation between $\Delta\nu_{st}$ and Δf except for model derivative **IN-H**. The orientational polarizability of solvents were calculated using the Equation 2 (Table S6).[23]

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \qquad (2)$$

Where n is the refractive index and ε is the static dielectric constant of the solvents used. Having the value of Onsager radius and the slope of the Lippert plot (Figures 4b,c S9 and Table S7), the changes in dipole moment (μ_e–μ_g) of Indazo-Fluors after optical excitation were estimated to be 19.4, 16.7, 10.7, 6.7, 6.19 and 2.81 D for **IN-DCN** (a = 5.932 Å), **IN-ECN** (a = 5.769 Å), **IN-CHO** (a = 4.926 Å), **IN-CN** (a = 4.886 Å), **IN-E** (a = 4.466 Å) and **IN-H** (a = 3.774 Å), respectively. It should be noted that with an increase in the electron withdrawing ability of the acceptor substituents, a linear increase in $\mu_e - \mu_g$ values was obtained for Indazo-Fluors, which can be correlated to the significant charge polarizability because of the charge separation upon photoexcitation. As a result of large dipole moment in the excited state, the excited state are stabilized by the reorientation of the solvent dipoles. The extent of stabilization of polarized excited state depends on the solvent polarities. This in fact accounts for the color tunability in the emission exhibited by **IN-ECN** and **IN-DCN** while moving from nonpolar to polar solvents.

Theoretical Investigations

The electronic ground state geometry, frontier molecular orbital (FMO) arrangement and the energy gap between FMOs were then optimized with DFT method using Gaussian 09 program at M06-2X level in conjunction with 6-311 + G(d,p) basis set.[25] From the computed FMOs (Figure 5) it is clear that in HOMO for all the molecules, the distribution of MO coefficients are predominantly localized on the octyloxy substituted indazole ring. In LUMO, for **IN-E**, **IN-CN** and **IN-CHO**, the MO coefficients are concentrated over the acceptor appended C_3 phenyl ring of the indazole skeleton, whereas for **IN-ECN** and **IN-DCN** the MO coefficients are localized mainly over the acceptor groups. Since HOMO and LUMO are distinctly localized over donor and acceptor groups, it imparts a charge transfer character to the Indazole Fluors and accounts for the observed photophysical properties. In case of **IN-H**, HOMO and LUMO are found to be

Table 5. Effect of solvents on the photophysical properties of **IN-DCN**.

Solvents	λ_{abs} (nm)	logε	λ_{em} (nm)	$\Delta\nu_{St}$ (cm^{-1})[a]	Φ_F	τ (ns)
MCH	431	4.21	455, 482	2412	0.009[b]	0.25
Toluene	415	4.58	515	4679	0.31[c]	0.88
$CHCl_3$	423	4.48	566	5973	0.42[d]	2.06
THF	412	4.54	578	6970	0.43[d]	2.62
CH_2Cl_2	419	4.62	594	7031	0.36[d]	2.21
Acetone	406	4.56	631	8783	0.03[d]	0.33

[a] $\Delta\nu_{St} = \nu_{abs} - \nu_{em}$, Stokes shift. Fluorescence quantum yields (±5% error) were determined by using [b] quinine sulfate as the standard (Φ_F = 0.54 in 0.1 M H_2SO_4), [c] fluorescein as the standard (Φ_F = 0.93 in 0.1 M NaOH), [d] rhodamine 6G (Φ_F = 0.95 in ethanol) as the standard.

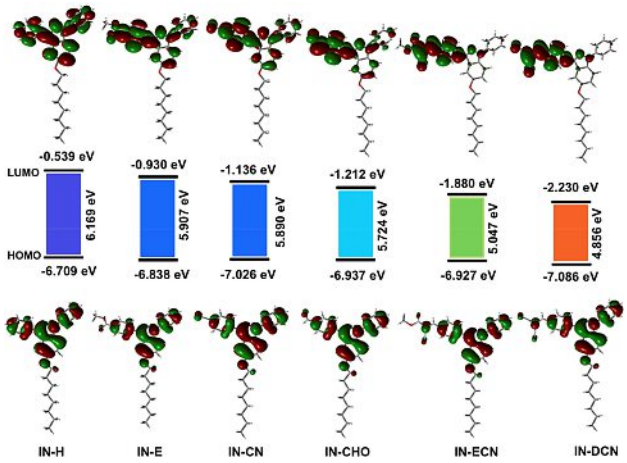


Figure 5. Calculated molecular orbitals (HOMO and LUMO) and energy levels of Indazo-Fluors.

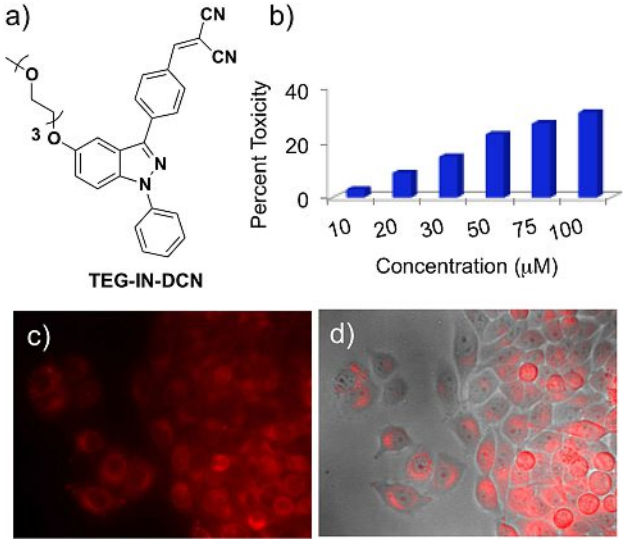


Figure 6. a) Molecular structure of water compatible Indazo-Fluor **TEG-IN-DCN**. b) Cytotoxicity of **TEG-IN-DCN** was assessed based on MTT assay in HeLa cell lines. Different concentrations of **TEG-IN-DCN** were evaluated and values plotted are the mean of three different experiments. c) Fluorescence images of HeLa cells incubated with **TEG-IN-DCN** in 1% DMSO/phosphate buffer (10.0 μM, pH 7.4, λ_{ex} = 440 nm, λ_{em} = 570 nm). d) merged image of fluorescent and bright-field images.

located over the indazole core and the lack of charge transfer character can be attributed to its solvent polarity insensitive optical properties. With increase in electron withdrawing ability of appended acceptor moieties of Indazo-Fluors, a considerable stabilization of LUMO energy levels are observed in comparison to the HOMO levels. This in turn results in a reduced HOMO-LUMO energy gap on moving from **IN-H** (6.169 eV) to **IN-DCN** (4.856 eV). Having the optimized structures, the UV/Vis absorption spectra of Indazo-Fluors including vertical excitations were estimated in solution state by TD-DFT method employing polarizable continuum model applying self-consistent reaction field in nonpolar MCH and polar acetone solvents using PBE1PBE functional for **IN-H**, **IN-E**, **IN-CN** and **IN-CHO** and M06-2X functional for **IN-ECN** and **IN-DCN**. TD-DFT computation studies revealed that the transition from ground state (S_0) to first excited state (S_1) having the major contributions from HOMO→LUMO transition and is responsible for the lowest energy absorption band in the absorption spectrum (Figure S10 and Tables S8, S9). While the FMOs are well located over the donor and acceptor moieties of Indazo-Fluors, the lowest energy absorption of these molecules possess a profound charge transfer character.

Modification of Indazo-Fluors for Bio-Imaging

The excellent photophysical properties of Indazo-Fluors, especially their large Stokes shift values prompted us to explore these fluorophores for cell imaging experiments. For this purpose, we modified **IN-DCN** by introducing water compatible TEG (Figure 6a) with anticipation that it can provide better solubility in buffer medium used for the cell imaging purpose. The detailed photophysical studies of **TEG-IN-DCN** conducted in solvents of different polarity indicate that the introduction of oligoether chain did not substantially alter its optical properties (Figure S11 and Table S10). We then evaluated the photophysical properties of **TEG-IN-DCN** in 1% DMSO/phosphate buffer (10 μM, pH 7.4, Figure S12). As inferred from the absorption and photoluminesce spectra, **TEG-IN-DCN** possess a large Stokes shift value of 8509 cm^{-1}, an ideal requirement for bio-imaging. From the bio-imaging point of view, the photostability of dye is a crucial criterion for the reason that the high-energy laser beam often used for imaging may lead to the photobleaching and limits its applicability as fluorescent probe. Thus, the photostability of **TEG-IN-DCN** was investigated and it was understood that the dye is stable with respect to 418 nm light irradiation over a period of 20 min (Figure S13). Further, cell toxicity studies were carried out in HeLa cell lines. For this purpose, cells were treated with different concentration of **TEG-IN-DCN** ranging from 10 to 100 μM in 10% DMSO/phosphate buffer (pH 7.4) and after a 15 min of treatment, it was found that both the molecule was less than 20% toxic up to 30 μM concentration (Figure 6b). Therefore, a concentration less than 30 μM was used for further cell imaging studies. The incubation of **TEG-IN-DCN** (10 μM) for 10 min in HeLa cells exhibited marked internalization of the dyes as represented in Figure 6c and d.

Conclusions

In summary, we have established a Palladium catalyzed protocol for the synthesis of 1*H*-Indazoles by reactions of hydrazones and *p*-benzoquinones. The reported reaction shows good functional group tolerance with moderate to good yields. The developed protocol affords *N*-protected 1*H*-indazoles with phenyl group at 3rd position and hydroxy group at 5th position, which allow late stage modifications as a fluorophore. The developed Indazo-Fluors exhibit tunable photoluminescence

properties both in solid as well as in solution states with good quantum yield and large Stokes shift values. The π-extended fluorophores **IN-ECN** and **IN-DCN** showed solvatochromic photoluminescence, while TEG appended water compatible analogue, **TEG-IN-DCN** has been successfully used for demonstrating HeLa cell imaging. Overall this study opens up a door to develop 1*H*-indazole based fluorophores for applications such as target specific imaging probe useful for theranostic (visible, near infrared and multi-photon) and stimuli responsive security as well as sensing materials.

Experimental Section

Synthesis-general techniques: Unless otherwise stated, all reagents and catalysts were purchased from commercial suppliers and used without further purification. The solvents were purified and dried by standard methods prior to use. All reactions under standard condition were carried out in a Schlenk tube under nitrogen atmosphere and monitored by thin layer chromatography (TLC) on silica gel plates (Merck $60F_{254}$; 0.2 mm). The spots were visualized with UV light (254 and 365 nm) or by dipping the plate in an iodine chamber. All synthesized compounds were purified by silica gel column chromatography (60–120 mesh size) with hexane-ethyl acetate solvent mixture as eluent.

Synthesis-characterization techniques: Melting points were determined with JSGW melting point apparatus and were uncorrected. ^{1}H NMR (400 and 500 MHz) and ^{13}C NMR (100 and 125 MHz) spectra were recorded in $CDCl_3$, DMSO-d_6 and Acetone-d_6 on Bruker Avance III 400 MHz, Bruker Avance DPX 500 MHz spectrometer with tetramethyl silane (TMS; $\delta_H = 0$ ppm) as an internal standard and chemical shifts were reported in ppm relative to TMS. The resonance multiplicity is described as s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets), td (triplet of doublets). Infrared spectra were recorded on JASCO FTIR-4100 using KBr pellet and only intense peaks were reported. Electrospray ionization (ESI) high-resolution mass spectra were recorded on using Thermo Scientific Exactive mass spectrometer. Single crystal X-ray diffraction were collected by means of Bruker SMART APEX diffractometer with graphite-monochromated Mo-K_α ($\lambda = 0.71073$ Å) radiation source.

General procedure for the synthesis of 5-hydroxy-1*H*-indazole derivatives: To an oven dried Schlenk tube equipped with magnetic stirrer, *N*-phenylhydrazone (0.25 mmol, 1 equiv.), *p*-benzoquinone (0.3 mmol, 1.2 equiv.), $Pd(OAc)_2$ (5 mol%) were added followed dry 1,2-dichloroethane (2 mL) and trifluoroacetic acid (3.5 mmol). It was then heated at 75 °C for 6 h under N_2 atmosphere. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate and filtered through celite pad. The filtrate was then neutralized with $NaHCO_3$ solution and organic layer extracted with ethyl acetate. The ethyl acetate layer was finally washed with brine solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was then purified by silica gel column chromatography using hexane-ethyl acetate as the eluent.

1,3-diphenyl-1*H*-indazol-5-ol (3a): Following the general procedure, reaction of 1-benzylidene-2-phenylhydrazine **1a** (0.25 mmol, 49 mg) with *p*-benzoquinone **2** (0.3 mmol, 32.4 mg) afforded the desired product **3a** as a white solid in 69% yield (49.4 mg); m.p. 176–178 °C; FT-IR (KBr): $\nu_{max} = 3435$, 1594, 1497, 1359, 1197 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.90$ (dd, $J = 8.2$ Hz, 1 Hz, 2H), 7.70 (dd, $J = 8.6$ Hz, 1 Hz, 2H), 7.59 (d, $J = 9.2$ Hz, 1H), 7.48-7.42 (m, 4H), 7.36-7.32, (m, 2H), 7.30-7.26 (m, 1H), 6.98 (dd, $J = 9.2$, 2.4 Hz, 1H), 4.90 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 151.0$, 145.1, 140.1, 136.2, 133.2, 129.4 (2C), 128.8 (2C), 128.1, 127.5 (2C), 126.5, 123.7, 122.7 (2C), 118.0, 111.7, 104.6 ppm; HRMS (ESI) calcd. for $C_{19}H_{15}N_2O[M+H]^+$ 287.1179 found 287.1187.

Methyl 4-(5-hydroxy-1-phenyl-1*H*-indazol-3-yl)benzoate (4a): Following the general procedure, reaction of methyl 4-((2-phenylhydrazono)methyl)benzoate **1b** (0.25 mmol, 63.5 mg) with *p*-benzoquinone **2** (0.3 mmol, 32.4 mg) afforded the title product **4a** as white solid in 49% yield (42.1 mg); m.p. 214–216 °C; FT-IR (KBr): $\nu_{max} = 3335$, 1696, 1597, 1496, 1288, 1109 cm^{-1}; ^{1}H NMR (500 MHz, DMSO- d_6): $\delta = 9.63$ (s, 1H), 8.17-8.13 (m, 4H), 7.83 (dd, $J = 8.5$, 1 Hz, 2H); 7.78 (d, $J = 9$ Hz, 1H), 7.62 (t, $J = 8$ Hz, 2H), 7.45-7.42 (m, 2H), 7.12 (dd, $J = 9.2$, 2.2 Hz, 1H), 3.91 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 165.9$, 153.5, 142.3, 139.4, 137.5, 134.8, 129.8 (2C), 129.6 (2C), 128.7, 126.8 (2C), 126.7, 123.3, 122.1 (2C), 119.0, 112.0, 103.1, 52.1 ppm; HRMS (ESI) calcd. for $C_{21}H_{17}N_2O_3$ $[M+H]^+$ 345.1234 found 345.1243.

4-(5-hydroxy-1-phenyl-1*H*-indazol-3-yl)benzonitrile (5a): Following the general procedure, reaction of 4 ((2-phenylhydrazono) methyl)benzonitrile **1c** (0.25 mmol, 55.3 mg) with *p*-benzoquinone **2** (0.3 mmol, 32.4 mg) afforded the desired product **5a** in 51% yield (39.6 mg) as an ivory colored solid; m.p. 200–202 °C; FT-IR (KBr): $\nu_{max} = 3341$, 2933, 2232, 1596, 1502, 1258, 1112 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 9.64$ (s, 1H), 8.18 (d, $J = 8.5$ Hz, 2H), 8.02 (d, $J = 8.5$ Hz, 2H), 7.83 (d, $J = 7.5$ Hz, 2H), 7.78 (d, $J = 9$ Hz, 1H), 7.62 (t, $J = 7.5$ Hz, 2H), 7.46-7.43 (m, 2H), 7.13 (dd, $J = 9$, 2 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 153.6$, 141.7, 139.3, 137.5, 134.9, 132.9, 129.6 (2C), 127.3 (2C), 126.9, 123.2., 122.2 (2C), 119.1, 118.8, 112.1, 110.1, 103.0, 99.5 ppm; HRMS (ESI) calcd. for $C_{20}H_{14}N_3O$ $[M+H]^+$ 312.1131 found 312.1140.

4-(5-hydroxy-1-phenyl-1*H*-indazol-3-yl)benzaldehyde (6a): Following the general procedure, reaction of 4 ((2-phenylhydrazono) methyl)benzaldehyde **1d** (0.25 mmol, 56 mg) with *p*-benzoquinone **2** (0.3 mmol, 32.4 mg) afforded the desired product **6a** as yellow solid in 62% yield (48.7 mg); m.p. 206–208 °C; FT-IR (KBr): $\nu_{max} = 3299$, 2920, 2848, 2728, 1686, 1588, 1489, 1204, 1136 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 10.14$ (s, 1H), 9.72 (s, 1H), 8.29 (d, $J = 8.4$ Hz, 2H), 8.15 (dd, $J = 6.6$, 1.8 Hz, 2H), 7.89 (dd, $J = 8.6$, 1 Hz, 2H), 7.84 (d, $J = 9.2$ Hz, 1H), 7.70–7.66 (m, 2H), 7.53-7.48 (m, 2H), 7.19 (dd, $J = 9.2$, 2.4 Hz, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 192.6$, 153.6, 142.2, 139.4, 138.6, 135.3, 134.9, 130.2 (2C), 129.7 (2C), 127.1 (2C), 126.8, 123.3, 122.2 (2C), 119.1, 112.0, 103.1 ppm; HRMS (ESI) calcd. for $C_{20}H_{15}N_2O_2$ $[M+H]^+$ 315.1128 found 315.1136.

General procedure for the synthesis of derivatives IN-H, IN-E, IN-CN and IN-CHO: To a stirring solution of 1,3-diphenyl-1*H*-indazol-5-ol (0.5 mmol, 1 equiv.) and K_2CO_3 (1 mmol, 2 equiv.) in acetonitrile (3 mL) in a reaction tube, 1-bromooctane (0.6 mmol, 1.2 equiv.) was added and the reaction mixture was kept stirring at 82 °C for 6 h. After the completion of reaction, the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The ethyl acetate layer was then washed with water and organic layer extracted with ethyl acetate. The combined ethyl acetate layer was washed with brine, dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The residue was purified by column chromatography using hexane and ethyl acetate solvent mixture as eluent. Yields of the reaction were 86–90%.

5-(octyloxy)-1,3-diphenyl-1*H*-indazole (IN-H): Following the general reaction procedure, reaction of 1,3-diphenyl-1*H*-indazol-5-ol **3a** (0.5 mmol, 143 mg) with 1-bromooctane (0.6 mmol, 115.8 mg) afforded the desired product **IN-H** as white solid in 90% yield (179.4 mg); m.p. 56–58 °C; FT-IR (KBr): $\nu_{max} = 3055$, 2926, 2858, 1603, 1494, 1256, 1121 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.00$–7.98 (m,

2H), 7.78 (dd, J=8.5, 1 Hz, 2H), 7.68 (d, J=9.0 Hz, 1H), 7.55–7.51 (m, 4H), 7.42, (t, J=7.2 Hz, 1H), 7.39 (d, J=2 Hz, 1H), 7.35 (t, J=7.2 Hz, 1H), 7.13 (dd, J =9.2, 2.2 Hz, 1H), 4.04 (t, J=6.5 Hz, 2H), 1.86–1.80 (m, 2H), 1.53–1.47 (m, 2H), 1.40–1.25 (m, 8H), 0.89, (t, J=7 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=155.0, 145.4, 140.2, 136.0, 133.5, 129.4 (2C), 128.8 (2C), 128.0, 127.6 (2C), 126.4, 123.6, 122.6 (2C), 119.2, 111.6, 102.0, 68.8, 31.8, 29.4, 29.3, 29.2, 26.1, 22.6, 14.1 ppm; HRMS (ESI) calcd. for $C_{27}H_{31}N_2O$ $[M+H]^+$ 399.2436 found 399.2443.

Methyl 4-(5-(octyloxy)-1-phenyl-1*H*-indazol-3-yl)benzoate (IN-E): Following the general reaction procedure, reaction of methyl 4-(5-hydroxy-1-phenyl-1*H*-indazol-3-yl)benzoate **4a** (0.5 mmol, 172 mg) with 1-bromooctane (0.6 mmol, 115.8 mg) afforded the desired product **IN-E** as a white solid in 89% yield (203.2 mg); m.p. 100–102 °C; FT-IR (KBr): ν_{max}=3051, 2926, 2858, 1712, 1608, 1494, 1271, 1115 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.12 (dd, J=6.5, 1.5 Hz, 2H), 8.02 (dd, J=6.7, 1.7 Hz, 2H), 7.71-7.69 (m, 2H), 7.61 (d, J=9 Hz, 1H), 7.48 (t, J=8 Hz, 2H), 7.32-7.29 (m, 2H), 7.07 (dd, J=9.2, 2.2 Hz, 1H), 3.98 (t, J=6.5 Hz, 2H), 3.89 (s, 3H), 1.80-1.74 (m, 2H), 1.46-1.40 (m, 2H), 1.33-1.17 (m, 8H), 0.81 (t, J=6.7 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=165.9, 154.3, 143.0, 138.9, 137.0, 135.1, 129.1 (2C), 128.4 (2C), 128.3 (2C), 126.2, 125.8, 122.5 (2C), 121.7, 118.3, 110.8, 100.7, 67.7, 51.1, 30.8, 28.3, 28.3, 28.2, 25.1, 21.6, 13.0 ppm; HRMS (ESI) calcd. for $C_{29}H_{33}N_2O_3$ $[M+H]^+$ 457.2486 found 457.2512.

4-(5-(octyloxy)-1-phenyl-1*H*-indazol-3-yl)benzonitrile (IN-CN): Following the general reaction procedure, reaction of 4-(5-hydroxy-1-phenyl-1*H*-indazol-3-yl)benzonitrile **5a** (0.5 mmol, 155.6 mg) with 1-bromooctane (0.6 mmol, 115.8 mg) afforded the desired product **IN-CN** as a white solid in 86% yield (182 mg); m.p.=82–84 °C; FT-IR (KBr): ν_{max}=3055, 2926, 2853, 2210, 1603, 1499, 1261, 1115 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.06 (d, J=8.5 Hz, 2H), 7.73 (d, J=8.5 Hz, 2H), 7.69 (dd, J=8.2, 1.2 Hz, 2H), 7.62 (d, J=9 Hz, 1H), 7.49 (t, J=8 Hz, 2H), 7.32 (t, J=7.5 Hz, 1H), 7.27 (d, J=2 Hz, 1H), 7.09 (dd, J=9, 2 Hz, 1H), 3.98 (t, J=6.5, 2H), 1.80-1.75 (m, 2H), 1.47–1.41 (m, 2H), 1.34–1.18 (m, 8H), 0.82 (t, J=6.7 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=155.6, 143.0, 139.8, 138.1, 136.2, 132.6 (2C), 129.5 (2C), 127.7 (2C), 127.1, 123.3, 122.8 (2C), 119.5, 119.0, 112.0, 111.1, 101.4, 68.8, 31.8, 29.7, 29.4, 29.2, 26.1, 22.6, 14.1 ppm; HRMS (ESI) calcd. for $C_{28}H_{30}N_3O$ $[M+H]^+$ 424.2383 found 424.2395.

4-(5-(octyloxy)-1-phenyl-1*H*-indazol-3-yl)benzaldehyde (IN-CHO): Following the general reaction procedure, reaction of 4-(5-hydroxy-1-phenyl-1*H*-indazol-3-yl)benzaldehyde **6a** (0.5 mmol, 157 mg) with 1-bromooctane (0.6 mmol, 115.8 mg) afforded the desired product **IN-CHO** as a white solid in 88% yield (188 mg); m.p. 72–74 °C; FT-IR (KBr): ν_{max}=3071, 2920, 2848, 1697, 1614, 1499, 1266, 1121 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=10.02 (s, 1H), 8.13 (d, J=8.5 Hz, 2H), 7.97(dd, J=6.5, 1.5 Hz, 2H), 7.71 (dd, J=8.5, 1 Hz, 2H), 7.63 (d, J=9.5 Hz, 1H), 7.49 (t, J=8 Hz,, 2H), 7.34–7.31 (m, 2H), 7.09 (dd, J=9.2, 2.2 Hz, 1H), 3.99 (t, J=6.5 Hz, 2H), 1.81–1.75 (m, 2H), 1.50–1.41 (m, 2H), 1.32-1.18 (m, 8H), 0.82 (t, J=6.7 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.9, 155.5, 143.7, 139.9, 139.6, 136.2, 135.5, 130.3 (2C), 129.5 (2C), 127.7 (2C), 127.0, 123.5, 122.8 (2C), 119.4, 111.9, 101.6, 68.3, 31.8, 29.4, 29.3, 29.2, 26.1, 22.6, 14.1 ppm; HRMS (ESI) calcd. $C_{28}H_{31}N_2O_2$ for $[M+H]^+$ 427.2380 found 427.2390.

General procedure for the synthesis of derivatives IN-ECN and IN-DCN: To an oven dried reaction tube charged with a magnetic stirrer, 4-(5-(octyloxy)-1-phenyl-1*H*-indazol-3-yl)benzaldehyde **IN-CHO** (1 equiv.), active methylene compound (1.1 equiv.) and toluene (2 mL) were added followed by ammonium acetate (20 mol%) and acetic acid (80 mol%). The reaction mixture was then heated at 102 °C for 1 h. After the completion of reaction, the reaction mixture was cooled to room temperature, toluene was evaporated off, and residue was washed with distilled water and extracted with ethyl acetate. The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was then purified by silica gel column chromatography with hexane and ethyl acetate as eluent.

Ethyl 2-cyano-3-(4-(5-(octyloxy)-1-phenyl-1*H*-indazol-3-yl)phenyl) acrylate (IN-ECN): Following the general procedure, reaction of 4-(5-(octyloxy)-1-phenyl-1*H*-indazol-3-yl)benzaldehyde **IN-CHO** (0.5 mmol, 213.3 mg) with ethyl cyano acetate (0.55 mmol, 62.3 mg) afforded the desired product **IN-ECN** as a bright green solid in 86% yield (224 mg); m.p. 124–126 °C; FT-IR (KBr): ν_{max}=2926, 2848, 2215, 1723, 1598, 1494, 1264, 1112 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.31 (s, 1H), 8.20-8.16 (m, 4H), 7.78 (dd, J=8.2, 2.2 Hz, 2H), 7.70–7.68 (m, 1H), 7.56 (t, J=7.7 Hz, 2H), 7.41–7.38 (m, 2H), 7.16 (dd, J =9.2, 2.2 Hz, 1H), 4.41 (q, J=7 Hz, 2H), 4.08 (t, J=6.5 Hz, 2H), 1.89–1.83, (m, 2H), 1.54–1.44 (m, 2H), 1.41 (t, J=7.7 Hz, 3H), 1.38–1.29 (m, 8H), 0.89 (t, J=6.7 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=162.7, 155.6, 154.4, 143.4, 139.9, 138.6, 136.2, 131.8 (2C), 130.6, 129.5 (2C), 127.7 (2C), 127.0, 123.5, 122.8 (2C), 119.5, 115.8, 111.9, 102.2, 101.4, 68.8, 62.7, 31.8, 29.4, 29.3, 29.2, 26.1, 22.6, 14.2, 14.1 ppm; HRMS (ESI) calcd. for $C_{33}H_{36}N_3O_3$ $[M+H]^+$ 522.2751 found 522.2759.

2-(4-(5-(octyloxy)-1-phenyl-1*H*-indazol-3yl)benzylidene)malononitrile (IN-DCN): Following the general procedure, reaction of 4-(5-(octyloxy)-1-phenyl-1*H*-indazol-3 yl)benzaldehyde **IN-CHO** (0.5 mmol, 213.3 mg) with malononitrile (0.55 mmol, 37 mg) afforded the desired product **IN-DCN** as an orange-yellow solid in 75% yield (178 mg); m.p. 134–136 °C; FT-IR (KBr): ν_{max}=2926, 2858, 2220, 1572, 1494, 1256, 1115 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.22 (d, J=8.5 Hz, 2H), 8.07 (d, J=8.5 Hz, 2H), 7.80 (s, 1H), 7.77 (dd, J=8.5, 1 Hz, 2H), 7.70 (d, J=9 Hz, 1H), 7.57 (t, J=7.7 Hz, 2H), 7.43-7.39(m, 2H), 7.17(dd, J=9.2, 2.2 Hz, 1H), 4.07 (t, J=6.5 Hz, 2H), 1.89-1.83 (m, 2H), 1.55-1.49 (m, 2H), 1.42-1.29 (m, 8H), 0.89 (t, J=6.7 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.1, 155.8, 142.8, 140.0, 139.7, 136.3, 131.4 (2C), 130.0, 129.6 (2C), 127.9 (2C), 127.2, 123.5, 122.9 (2C), 119.6, 114.0, 112.9, 112.1, 101.4, 81.6, 68.8, 31.8, 29.4, 29.3, 29.2, 26.1, 22.6, 14.1 ppm; HRMS (ESI) calcd. for $C_{31}H_{31}N_4O$ $[M+H]^+$ 475.2492 found 475.2501.

Synthesis of 4-(5-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1-phenyl-1*H*-indazol-3-yl)benzaldehyde (TEG-IN-CHO): Triethylene glycol monomethyl ether tosylate was prepared according to literature procedure.[26] To a stirring solution of 4-(5-hydroxy-1-phenyl-1*H*-indazol-3-yl)benzaldehyde **6a** (1 equiv., 1 mmol, 314 mg) and K_2CO_3 (2 equiv., 2 mmol, 276 mg) in acetonitrile (5 mL) under argon, triethylene glycol monomethyl ether tosylate (1.5 equiv., 1.5 mmol, 478 mg) was added and the reaction mixture was kept stirring at 82 °C for 16 h. After the completion of reaction, the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure and the residue was dissolved in chloroform. The chloroform layer was washed with brine solution, dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The residue was purified by column chromatography using chloroform and methanol as eluent. The carbaldehyde **TEG-IN-CHO** was obtained as a pale green viscous solid in 78% yield (360 mg); FT-IR (KBr): ν_{max}=3062, 2877, 1698, 1565, 1502, 1262, 1211, 1113 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=10.06 (s, 1H), 8.18 (d, J=8.5 Hz, 2H), 8.01 (d, J=8 Hz, 2H), 7.75 (d, J=8.5 Hz, 2H), 7.67 (d, J=9.5 Hz, 1H), 7.54 (t, J=8 Hz, 2H), 7.43 (d, J=2 Hz, 1H), 7.37 (t, J=7.5 Hz, 1H), 7.18 (dd, J=9.2, 2.2 Hz, 1H), 4.23 (t, J=4.7 Hz, 2H), 3.92 (t, J=4.7 Hz, 2H), 3.77 (t, J=4.7 Hz, 2H), 3.70 (t, J=4.5 Hz, 2H), 3.65 (t, J=4.5 Hz, 2H), 3.54 (t, J=4.5 Hz, 2H), 3.36 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=191.9, 155.1, 143.6, 139.8, 139.4, 136.3, 135.5, 130.3 (2C), 129.5 (2C), 127.7 (2C), 127.0, 123.4, 122.8 (2C), 119.4, 111.9, 102.0, 71.9, 70.8, 70.6, 70.5, 69.7, 68.1, 59.0 ppm; HRMS (ESI) calcd. for $C_{27}H_{29}N_2O_5$ $[M+H]^+$ 461.2076 found 461.2083.

Synthesis of 2-(4-(5-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1-phenyl-1*H*-indazol-3-yl)benzylidene)malononitrile (TEG-IN-DCN): To an oven dried reaction tube charged with a magnetic stirrer, 4-(5-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1-phenyl-1*H*-indazol-3-yl) benzaldehyde **TEG-IN-CHO** (1 equiv., 0.5 mmol, 230.3 mg), malononitrile (1.1 equiv., 0.55 mmol, 37 mg) and toluene (2 mL) were added followed by ammonium acetate (20 mol%), acetic acid (80 mol%). The reaction mixture was then heated at 102 °C for 4 h. After the completion of reaction, toluene was evaporated; reaction mixture was washed with distilled water and extracted with chloroform. The chloroform layer was washed with brine solution, dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. The residue was then purified by silica column chromatography with chloroform and methanol as eluent. The desired product **TEG-IN-DCN** was formed as bright orange solid in 70% yield (178 mg): m.p. 82–84 °C; FT-IR (KBr): ν_{max} = 2923, 2222, 1626, 1579, 1495, 1266, 1113 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.20 (d, J = 8.5 Hz, 2H), 8.05 (d, J = 8.5 Hz, 2H), 7.80 (s, 1H), 7.76 (d, J = 8 Hz, 2H), 7.70 (d, J = 9 Hz, 1H), 7.56 (t, J = 7.7 Hz, 2H), 7.43-7.39 (m, 2H), 7.20 (dd, J = 9.2, 2.2 Hz, 1H), 4.26 (t, J = 4.7 Hz, 2H), 3.94 (t, J = 4.7 Hz, 2H), 3.79 (t, J = 4.7 Hz, 2H) 3.71 (t, J = 4.7 Hz, 2H), 3.66 (t, J = 4.5 Hz, 2H), 3.55 (t, J = 4.5 Hz, 2H), 3.37 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 159.1, 155.4, 142.9, 139.9, 139.7, 136.4, 131.5 (2C), 130.0, 129.6 (2C), 127.8 (2C), 127.2, 123.4, 122.9 (2C), 119.6, 114.0, 112.9, 112.1, 101.8, 81.6, 71.9, 70.8, 70.6, 70.5, 69.7, 68.2, 59.0 ppm; HRMS (ESI) calcd. for $C_{30}H_{29}N_4O_4$ $[M+H]^+$ 509.2189 found 509.2201.

Optical measurements: The electronic absorption spectra were recorded on a Shimadzu spectrophotometer (UV-2600 and 2401PC). The fluorescence spectra were recorded on a SPEX-Fluorolog-3 FL3–221 spectrofluorimeter. Optical studies in solution state were carried out in a 1 cm quartz cuvette. Emission spectra in the solid state were recorded using the front face geometry. Relative quantum yield in the solution state and absolute quantum yield in the solid state were determined as per the standard procedure.[20–22] Fluorescence lifetime measurements were carried out using IBH (model 5000 DPS) time-correlated single photon counting system. The lifetime values were obtained using DAS6 decay analysis software. The quality of the fit has been judged by the fitting parameters such as χ^2 (< 1.1) as well as the visual inspection of the residuals.

Cell culture and treatment: HeLa cells were obtained from the National Centre for Cell Sciences, Pune, India. Cells were maintained in DMEM supplemented with 10% FBS, 1% antibiotic–antimycotic mix at 37 °C under 5% CO_2 atmosphere.

Cytotoxicity assay: MTT assay was performed to check the cytotoxicity of **TEG-IN-DCN**. Different concentrations of **TEG-IN-DCN** (10, 20, 30, 50 and 100 μM in DMSO) were used to carry out the assay. Briefly, cells after incubation with the compound for 15 min, were washed and MTT (0.5 g L^{-1}, dissolved in DMEM, was added to each well for the estimation of mitochondrial dehydrogenase activity as described previously by Mosmann.[27] After an additional 90 min of incubation at 37 °C in a CO_2 incubator, 10% SDS in DMSO was added to each well and the absorbance at 570 nm of solubilized MTT formazan products were measured after 45 min, using a micro-plate reader (BIOTEK-USA). Results were expressed as percentage of cytotoxicity (Equation 3):

$$\text{Percentage of toxicity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbacne of control}} \times 100 \quad (3)$$

Cell internalization studies using TEG-IN-DCN: Cells were seeded in 96 well black clear bottom plates (BD Biosciences, Franklin Lakes, BJ) and after attaining 40% confluency the cells were taken for carrying out the experiments. The cells were then incubated with **TEG-IN-DCN** (10 μM, 1% DMSO/phosphate buffer, pH 7.4) for 10 min. This was followed by phosphate-buffer saline (PBS) wash of cells (2 times) to remove unbound dye. After resuspending the cells in PBS, cells were visualized under a fluorescent microscope (Pathway 855, BD Bioscience, USA) equipped with filters 440 nm (excitation channel) and 570 nm (emission channel).

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Conflict of Interest

The authors declare no conflict of interest.

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