Pd-Catalyzed Four-Component Sequential Reaction Delivers a Modular Fluorophore Platform for Cell Imaging

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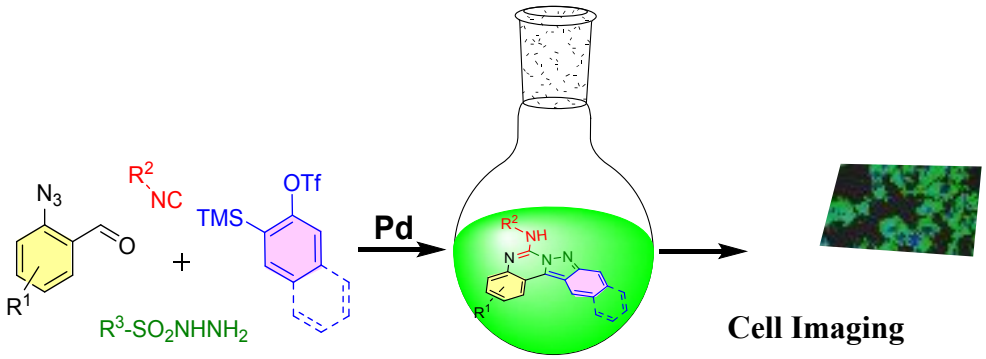


Abstract

A Pd-catalyzed cascade reaction of four versatile privileged synthons is described. The sequential reaction involves the formation of five new chemical bonds by concatenating three distinct chemical steps. One of the derivatives exhibited absorption in the visible region, fluorescence with high quantum yield and excellent photostability. Its application is explored in live cell imaging, which exhibited cytoplasmic and mitochondrial specific staining with no toxicity.

Introduction

The development of organic fluorophores is essential for the progress in the area of chemosensors,[1] medical diagnostic,[2] photoactive materials,[3] biological labels, and probes.[4] A chemical probe that can enter the cell with minimal perturbation with living systems is an excellent tool for studying morphological and physiological processes of the live cell. The dyes available commercially such as rhodamine, fluorescein, Nile red, cyanines, BODIPY, etc. typically exhibit small Stokes shift, leading to background interferences in investigating biological processes due to reabsorption of emitted photons.[5] Moreover, the poor photostability of these dyes also limits their application in bioimaging applications, where prolonged illumination is required.[6] In addition, poor cell permeability and cytotoxicity could prove to be the Achilles heel for any popular fluorescent dye. Therefore, *de novo* design and development of safe, rapid and efficient organic fluorophore continues to be an active area of research.

Traditionally, organic fluorophores are synthesized by standard synthetic approaches that typically involve multistep routes.[7] over the decade, transition metal catalyzed cascade reactions emerged as an important strategy for generating sophisticated fluorophores, which cannot be achieved through classical reactions.[8] The cascade reactions carry out multiple transformations in a single-pot without isolating any intermediates, enabling construction of unique fluorescent chemotypes that were inaccessible through conventional approaches shown in Fig.1[9] For the first time in 2011, Müller combined Pd-catalyzed Sonogashira coupling with a Michael addition in a one-pot fashion for the synthesis of merocyanines [Eq(1)].[10, 11] In 2013, Perumal employed Pd-catalyzed carbopalladation/C-H activation protocol for the synthesis of tetrasubstituted olefin xanthines that exhibited yellow fluorescence [Eq(2)].[12] Tietze reported Pd-catalyzed cascade reaction that involved concatenation of Sonogashira coupling, double, carbometallation of triple bonds and C-H arylation steps in 2014 [Eq(3)].[13] In 2015, Glorius prepared a fluorescent polycyclic framework for the detection of metal ions by Rh-catalyzed coupling of arylpyridines and carbenoid generated from pyridotriazole [Eq(4)].[14] Recently, alkyne annulations were elegantly utilized by Hua[15] Cheng,[16] Wang,[17] and Patil[18] for the construction of polycyclic fluorophores [Eq(5)]. Of note, one of the major limitations of these strategies is the prerequisite of a completely fabricated precursor generated by a multi-step route. Thus, any strategy that generates sophisticated fluorophore from simple and easily available starting materials in one-pot are highly desirable for the development of organic fluorophores.

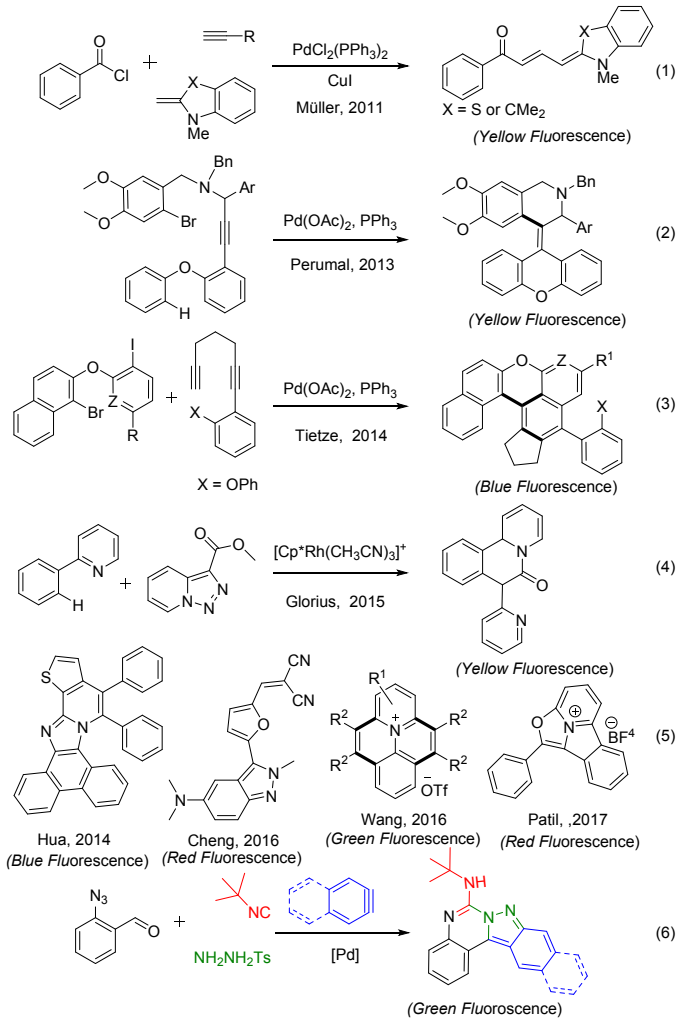
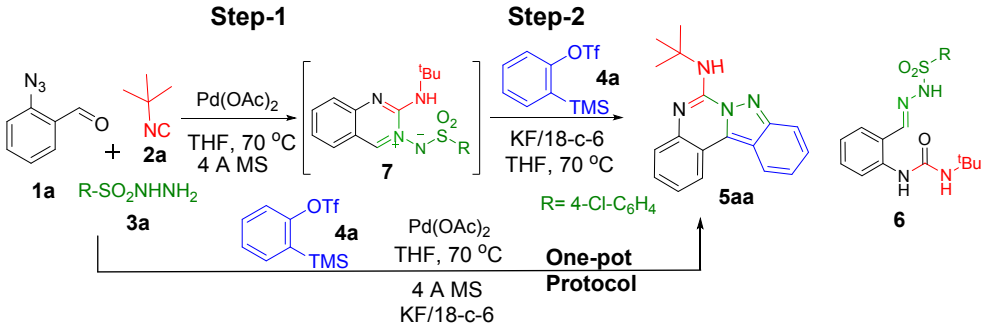


Figure 1. Transition metal catalysed cascade reactions for the synthesis of sophisticated fluorophores.

We report herein development of four-component reaction (4-CR) of 2-azidobenzaldehyde, isocyanide, sulfonyl hydrazides and aryne for the synthesis of fluorescent indazolo[2,3-*c*]quinazoline in a one-pot [Eq(6)]. The 4-CR involves concatenation of three sequential transformations: (i) formation of azomethine imine catalyzed by palladium,[19] (ii) cyclocondensation with hydrazides,[19] and (iii) carboamination of aryne.[20] Overall, there is a formation of five new chemical bonds. To the best of our knowledge, this is the first report of one-pot synthesis of indazolo[2,3-*c*]quinazoline[20] from four simple and easily available precursors. This work led to a discovery of an interesting and new organic fluorophore, benzo[5,6]indazolo[2,3-*c*]quinazoline **5bf** with high quantum yield and excellent photostability, rapid penetration through biological membranes, and low cytotoxicity at a concentration of investigation. Its application in cell imaging was conferred by confocal, flow cytometric and 3D cell culture analysis.

Result and discussion

Our work commenced with the screening of various parameters for the synthesis of azomethine imine (step-1, scheme 1) from a mixture of 2-azidobenzaldehyde **1a**, tert-butyl isocyanide **2a** and 4-chlorophenylsulfonyl hydrazide **3a** in toluene at room temperature. Various Pd-catalyst including $Pd(PPh_3)_4$, $Pd(PPh_3)_2Cl_2$, $PdCl_2$, and $Pd_2(dba_3)$, solvents including DCE, THF, dioxane were screened (Refer ESI for detail). In general, $Pd(OAc)_2$ (7.5 mol%) in THF at 70 °C found to be an optimal reaction condition. The use of 4 Å molecular sieves was essential to avoid a side product, urea **6**. Then, we shifted our focus on carboamination of arynes step-2 Scheme 1). Benzyne generated from 2-(trimethylsilyl)-phenyltriflate **4a** (1.1 equiv) reacted with azomethine imine **7** (1 equiv) in the presence of KF/18-c-6 (3 equiv) in THF under an argon atmosphere at 70 °C to furnish **5aa** in 82 % isolated yield (Refer SI for detail). Both optimization studies revealed that THF can be used as a common platform to combine the formation of azomethine imine and carboamination of aryne steps. Pleasingly, combining these two reactions was very much feasible upon sequential addition of Pd-catalyst and fluoride source. This encouraged us to develop a one-pot four-component sequential reaction as depicted in scheme 1.

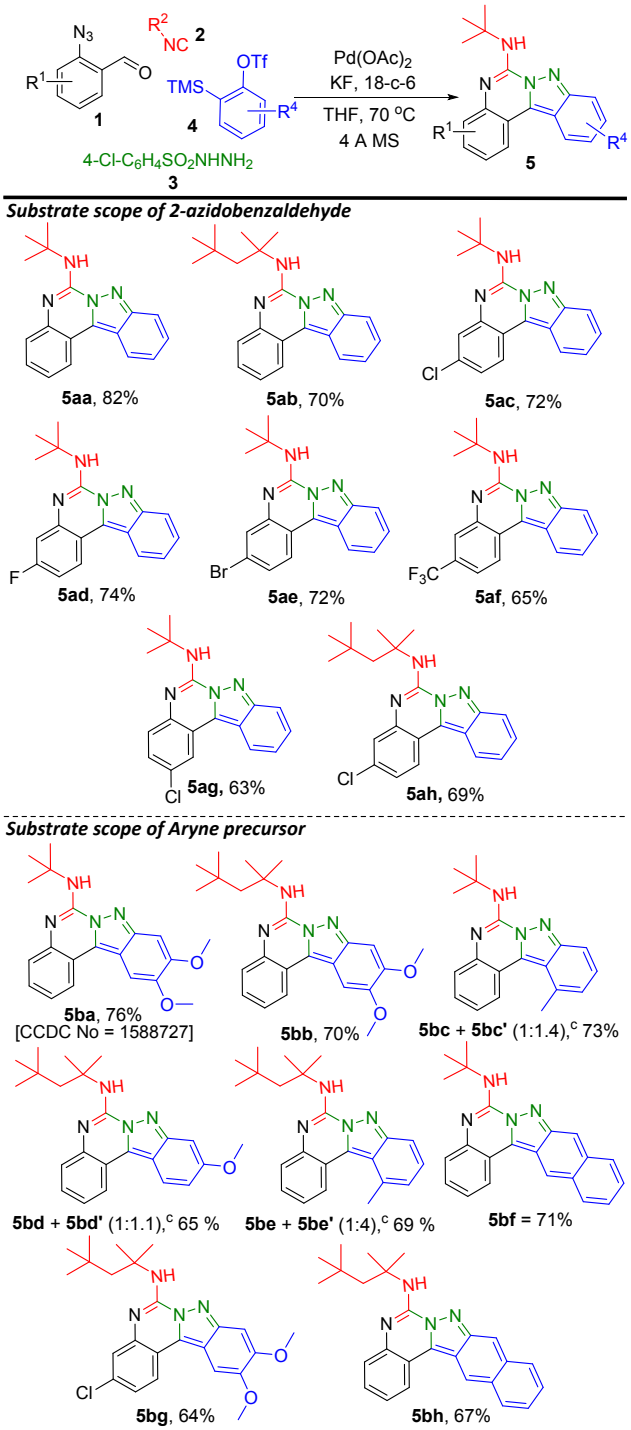


Scheme 1: Development of four-component reaction.

With these optimal reaction conditions in hand, we examined the scope and limitation of the four-component reaction. We embarked our studies with a range of 2-azidobenzaldehyde derivatives (Table 1). Various substitutions on 2-azidobenzaldehydes such as F, Cl, Br, and CF_3 were well tolerated (table 1, **5ac-5ag**). The chemical integrity of reactive to functional groups such as Cl and Br remained unperturbed in the optimized reaction condition providing a chemical handle for further transformations. Fluorinated substitutions in **5ad**, would contribute to enhancing their pharmacodynamics and pharmacokinetic properties. Alkyl isocyanides participated well in the 4-CR to produce the title compound. In contrast, reactions involving aryl isocyanides failed to react with 2-azidobenzaldehyde to furnish the carbodiimide

intermediate. Next, we studied the diversity of aryne precursors. Pleasingly, various symmetric arynes participated the 4-CR under a standard condition to produce **5ba** and **5bb** in moderate to good yield. Symmetric arynes generated from the corresponding precursors **4b** and **4e** participated the 4-CR under a standard condition to produce title compounds in moderate to good yield. Also, the unsymmetric arynes generated from precursors **4b** and **4c** afforded a mixture of regioisomers, **5bc** in a 1:1.4 ratio, **5bd** in a 1:1.1 ratio, and **5be** in an equimolar ratio.

Table 1: Substrate scope of 4-component reaction.[a]



[a]Representative procedure: 2-Azidobenzaldehyde (0.10 mmol), isocyanide (0.12 mmol), 4-chlorophenylsulfonyl hydrazide (0.11 mmol), Pd catalyst (0.075 mmol), 4 Å MS (300mg), aryne precursor (0.12 mmol), KF (0.3 mmol), 18-Crown-6 (3.0 equiv) and THF (2 ml) as solvent under Argon balloon at 70 °C stirred for 12 h, [b]Isolated yield, [c]inseparable mixture, but we were unable to tell which one is the major isomer.

To elucidate the mechanistic detail of sequential 4-CR, we focused our attention on the formation of azomethine imine **7**. We monitored the progress of 3-CR of **1a**, **2a** and **3a** (step-1, scheme 1) using ^{1}H NMR spectroscopy (Fig. 2A). The results revealed that **8** was formed as a predominant intermediate, which was

readily converted into 3-CR adduct **7**. This observation strongly supports Path A (Fig 2B) for the formation of **7**. However, an alternate path B (Fig 2B) cannot be ruled out. Thus, to further gain meaningful insights of 3-CR, we carried out the chemical kinetics of individual steps of 2-component reaction depicted in Fig. 2B. Examining the first step of these pathways revealed that formation of **8** was four times faster than the formation of **9** under 2-CR. Similarly, the second step of path A, 2-CR between **8** and **3a** was nine times faster than the second step of path B. The latter produced **7** in meager 35% isolated yield, and most of the starting material was remained unchanged even after prolonging the reaction for a long time. Thus, the chemical kinetics studies of individual 2-CR support the result obtained in 3-CR. Both these studies ruled out path B for the formation of **7** and unequivocally established that the reaction is traversed via path A (refer Section S1.7 of SI for details).

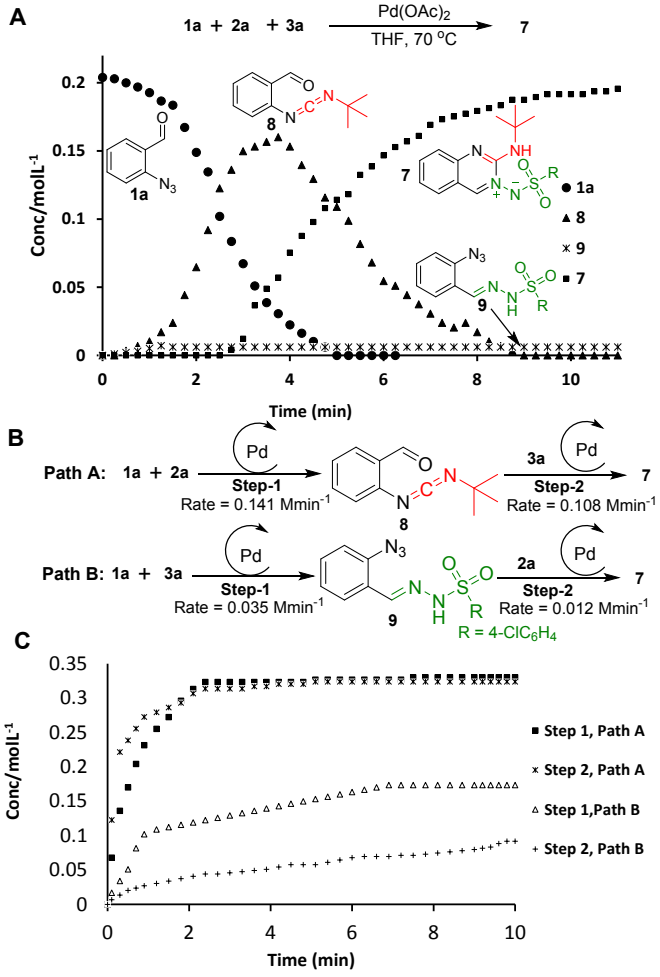
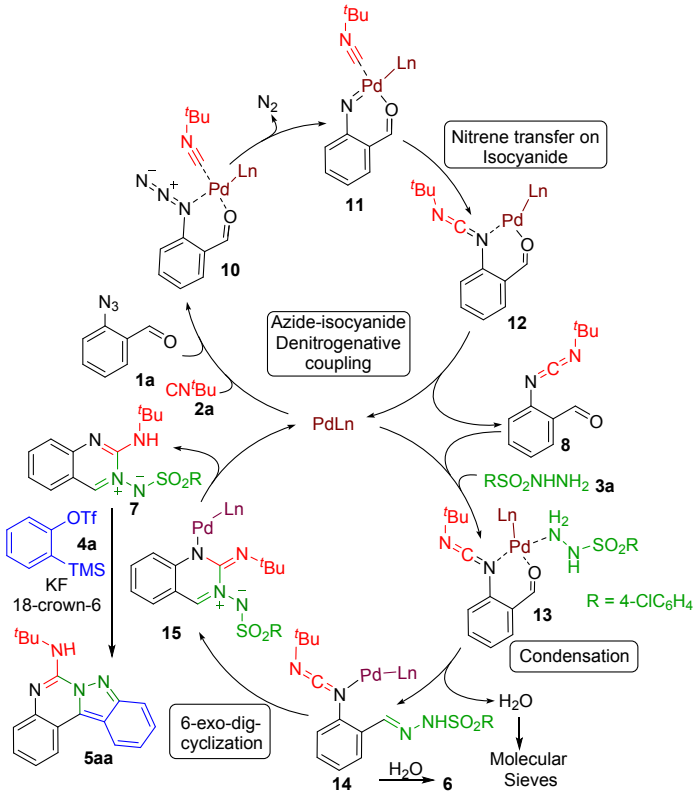


Figure 2. Chemical Kinetics, reaction conditions: $Pd(OAc)_2$, THF, 70 °C. (B) A Plot of concentration *vs*. time for 3-CR. (C) A Plot of concentration *vs*. time for 2-CR. Monitoring temporal progress of the two-component reaction using ^1H NMR spectroscopy.

Based on these experimental findings and literature precedence,[21] a plausible reaction mechanism was sketched as shown in Scheme 2. Initially, the coordination of 2-azidobenzaldehyde **1a** and isocyanide **2a** with $Pd(OAc)_2$ affords a complex **10**, which undergoes extrusion of dinitrogen to afford nitrene intermediate **11**. An intra-molecular isocyanide transfer over nitrene generates carbodiimide **8** in a concerted fashion.

Then this reactive carbodiimide enters into the second catalytic cycle of Pd(II) and coordinates with palladium metal, where its aldehydic functional group condenses with 4-chlorophenylsulfonyl hydrazide **3a** to furnish the hydrazone **14**, and further 6-*exo-dig* cyclization produces azomethine imine **7**. Aryne generated *in situ* from 2-trimethylsilyl aryl triflate **1a** using KF and 18-c-6, then reacts with **7** to furnish the title compound indazolo[2,3-*c*]quinazoline **5aa.**



Scheme 2: Plausible Mechanism of 4-CR

To our delight, indazolo[2,3-*c*]quinazolines exhibit excellent, photoluminescent properties and, of these benzo[5,6]indazolo[2,3-*c*]quinazoline **5bf** emits light in the green region due to extended conjugation, while the rest of compounds (**5aa** to **5be**) emit light in the blue region. CIE chromaticity coordinates of the **5bf** (Fig. 3A) clearly indicated fluorescence emission of **5bf** is falling in the green region. **5bf** exhibits a high quantum yield of 68% and a larger Stokes shift in the chloroform solution with compared to some of the known dyes (Refer SI for detail). Next, the stability of **5bf** was examined in DMSO solution at 454 nm for 30 min. The change in fluorescence intensity is negligible with time (Fig 3B), which implies that **5bf** is a highly photostable compound.

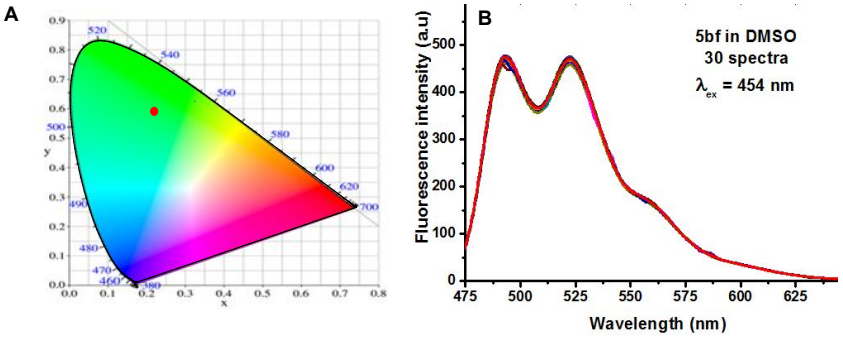


Figure 3: (A) CIE chromaticity diagram of **5bf** in solution (B) Fluorescence emission spectra of **5bf** in DMSO were continuously recorded by exciting at 454 nm for 30 spectra (~ 30 min), a plot of fluorescence intensity (a.u) *Vs* wavelength (nm).

Encouraged by a high quantum yield and photostability of **5bf**, we next explored the feasibility of **5bf** for cell imaging studies on MDA-MB 231 cancer cells shown in Fig. 4. Briefly, the variable concentration of **5bf** was used to treat cells for indicating time points followed by incubation in normal DMEM media for 24 h. The confocal imaging of the cells indicates short (10 min) and long (6 h) exposure to **5bf** exhibit similar staining, indicating that very short exposure to the compound is sufficient for absorption by the cells. (Fig 4a and 4b). Furthermore flow cytometry analysis of the cells as well as isolated organelles exhibited cytoplasmic and mitochondrial specific staining; however, no staining of the nucleus of MDA-MB 231 cell was observed (Fig 4c, and Fig S17 of SI). Dye retention analyses by flow cytometry using MDA-MB 231 cells after 10 min and 6 h staining, and with media for indicated time points showed that cells were retaining the dye even after prolonged incubation for 96 h. (Fig 4d). Further, to validate whether the fluorogenic property of **5bf** remains intact, we explored hPBMCs, owing to their peculiar morphology and low division rates when unexposed to mitogenic compounds, the results disclosed that **5bf** entered cells rapidly (within 10 min) and exhibited brighter staining owing to its excellent ϕf value. These results implicated that metabolic rates of a cell may not be affecting the entry of **5bf** inside cells. (Fig 4e). Moreover, the staining time of 10, 30 or 60 min did not show the significant difference in stain intensity (Fig 4e). Pleasingly, **5bf** showed negligible toxicity in MTT assay on both MDA-MB 231 and human peripheral blood mononuclear cells (hPBMCs) pretreated at 5 μM concentration for 48 h (Fig 4f). In addition, **5bf** exhibited fluorescence in DMSO but owing to poor solubility, it failed to fluoresce in PBS solution. On prolonged exposure of DMSO solution of **5bf** to both UV and was studied on MDA-MB 231 cancer cells. A briefly variable concentration of **5bf** was used to treat cells for indicating time points followed by incubation in normal media for 24 h. The visible light, its photoluminescence remained unperturbed and, no quenching was observed. For further analysis, **5bf** was incubated with the 3-D culture of MDA-MB 231 cells for 2 days, which showed that cells inside the 3D culture mass were showing fluorescence (Fig 4g) indicating it's potential in the cell as well as tissue culture experiments. These observations indicate that has an excellent scope as a fluorescent dye in the live cell imaging of mitochondria and cytoplasm, where any potential genotoxicity by the dye has to be avoided. Furthermore higher retention time also makes it an ideal candidate to study different cell types in co-culture experiments.

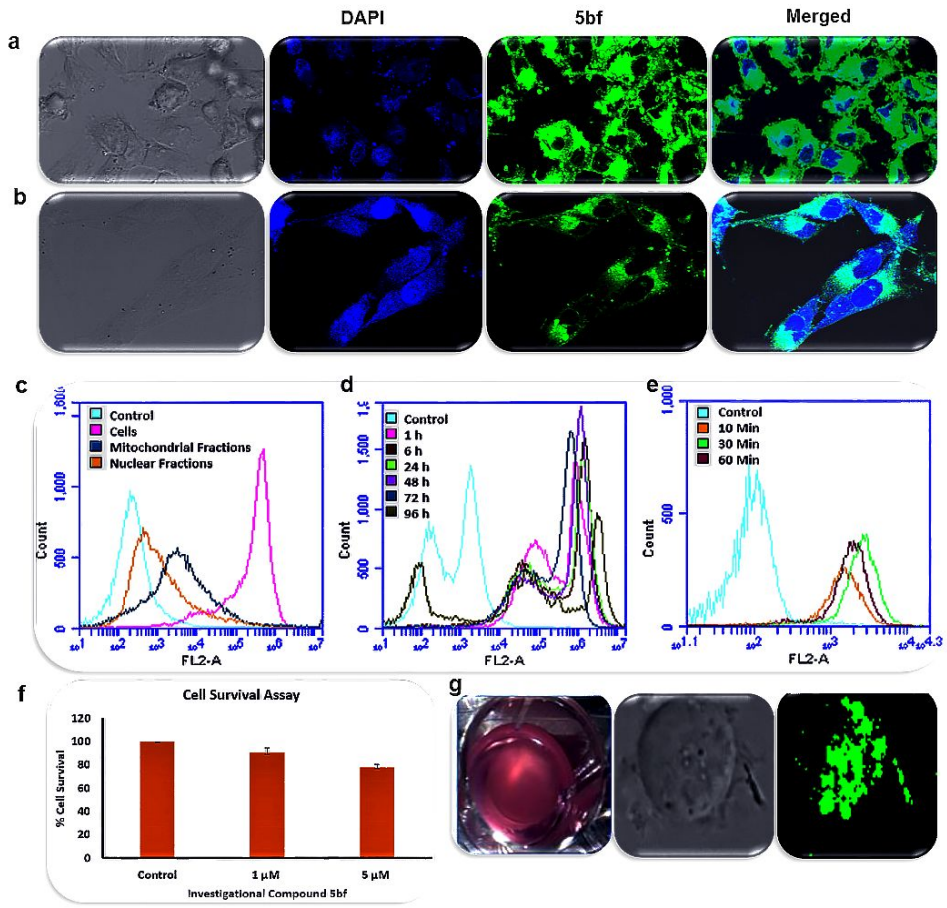


Figure 4: Confocal scanning images of MDA-MB 231 cells after 15 min (a) and 6 hours (b) staining. The picture represents the phase contrast image, nuclear staining (blue), compound **5bf** staining (green) and merge. c) Flow cytometric analyses of nuclear and mitochondrial fractions. d) Flow cytometer analyses of cells stained with **5bf** for 30 minutes followed by incubation of cells at different time points. e) Comparison of the intensity of staining by **5bf** at different time points using flow cytometer in hPBMCs. g) 3D culture image showing culture dish, phase contrast, and **5bf** green fluorescence

Conclusion

In conclusion, we developed a new four-component reaction for accessing fluorescent indazolo[2,3-*c*]quinazolines in one-pot from simple and easily available precursors. The 4-CR involves the. formation of five new chemical bonds and concatenation of three individual reaction sequences, which is confirmed by the chemical kinetic experiment. Of these, benzo[5,6]indazolo[2,3-*c*]quinazoline **5bf** exhibited green fluorescence under visible light with high quantum yield and excellent photostability. The application of this dye for cell imaging has been described. These results formed a basis for the development of new fluorescent probes for the selective detection of various proteins and mitochondria in live cells.

Experimental Section:

General Considerations

All the reactions were carried out under argon in a dried reaction vessel with Teflon screw caps. THF was freshly dried and distilled over Na-benzophenone and kept under an inert atmosphere. All other solvent used in synthesis were purchased from Spectrochem. Aliphatic isocyanides were purchased from Sigma-

Aldrich and Alfa Acer. Aryne precursor were purchased from TCI and used without any further purification. All starting reagents were synthesized by the reported literature. Other reagents were purchased from Aldrich or Spectrochem used as such without purification. Analytical TLC was performed using 2 x 4 cm plate coated with a 0.25 mm thickness of silica gel (60F-254 Merck), and visualization was accomplished with UV light or I_2/ $KMnO_4$ staining. Melting points were uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker's Ascend 500MHz spectrophotometer operating at 500.3 MHz for 1H and 125.8 MHz for ^{13}C experiments; Spectra were recorded at 295 K in $CDCl_3$. Chemical shifts were calibrated to the residual proton and carbon resonance of the solvent, $CDCl_3$ (^1H δ 7.269; ^{13}C δ 77.0). The abbreviations used: singlet = s, doublet =d, triplet = t, quartet = q, double doublet = dd, multiplet = m, broad singlet = br s & broad signal = br. Mass spectra were recorded on Water Q-ToF-Micro Micromass.

UV-Vis and photoluminescence experiment

Electronic absorption spectra were recorded on a Varian Cary 100 UV-Vis spectrometer, in transmission mode. Diffuse reflectance spectra of the solid samples (on quartz plates) were recorded using the DRA-CA-30I sphered accessory and converted into absorption spectra using the Kubelka–Munk function. Steady-state fluorescence excitation and emission spectra were recorded on a Horiba Jobin Yvon Model FL3-22 Fluorolog spectrofluorimeter and Perkin Elmer spectrofluorometer (Model: LS55). An Absolute value of the fluorescence quantum yield of the solution and solid samples were determined using an integrating sphere and the PLQY Calculator v.3 software (Horiba Jobin Yvon).

General Procedure for the *ortho*-Azidobenzaldehydes 1

2-azidobenzaldehydes were prepared by using a protocol reported by Driver et al. in 2011 in one step using the reaction of commercially available 2-nitrobenzaldehydes with sodium azide in HMPA.[22] In a reaction vial dissolved 2-nitrobenzaldehyde (1.0 g, 6.62 mmol) in HMPA (10 ml) and sodium azide (0.90 g, 13.9 mmol) was added at 0 °C. The reaction was stirred at ambient temperature for overnight. Monitor the reaction on TLC after completion of the reaction, the mixture was diluted with ice water and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with water (3×30 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography as eluent EtOAc: hexanes to afford the desired product.

General Procedure for the Aryl sulphonyl hydrazide 3

Aryl sulfonyl hydrazides were prepared according to the literature procedure.[23] To a solution of an aryl sulfonyl chloride (2.0 mmol) in tetrahydrofuran (10 mL), was added hydrazine monohydrate (10 mmol) drop wise under nitrogen at 0 °C. After vigorous stirring for 30 min at 0 °C, the reaction mixture was diluted with ethyl acetate (40 mL) and washed with saturated brine (3 x 10 mL). The organic layer was dried over sodium sulfate, concentrated and added to hexane (12 mL) over 5 min. The mixture was filtered, and the collected solid was dried in vacuum and used as such without further purification.

General Procedure for the synthesis of 5

A 10 mL Schlenk tube equipped with a stir bar was charged with 2-Azidobenzaldehyde (1.0 equiv), isocyanide (1.2 equiv), $Pd(OAc)_2$ (7.5 mol%), 4 Å MS, 4-chlorosulfonyl hydrazide (1.0 equiv) in THF (2 ml) stirred at 70 °C in oil bath for 10 min.. The reaction tube was purged with argon. Then after aryne precursor (1.2 equiv) and KF/18-crown-6 (3/3 equiv) was added and reaction mixture was stirred at 70 °C for 12 h. This reaction was monitored by TLC. On completion of the reaction , the reaction was diluted with ethyl acetate (15 mL) and washed with water (10 mL). The organic layer was separated, dried on

Na_2SO_4, filtered and evaporated in vacuo. The crude product was purified by the column chromatography to afford the desired product.

5aa: ***N-(tert-butyl)indazolo[2,3-c]quinazolin-6-amine*** The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.067 g (0.816 mmol) of t-butyl isocyanide **2a,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in1.0 mL of THF and 0.243 g (0.816 mmol) of 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4a**, KF(0.118 g, 2.04 mmol)/18- crown-6 (0.538 g, 2.04 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5aa** as a Light brown solid (0.167 g, 82%), mp 103-104 °C, R_f = 0.6 (5:95 EtOAc:hexanes, visualized by 254 nm UV light). ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.43 (dd, 1H, *J* = 8.0, 0.9 Hz), 8.40 (d, *J* = 8.6 Hz), 7.91 (d, 1H, *J* = 8.3 Hz), 7.86 (d, 1H, *J* = 7.9 Hz), 7.63-7.57 (m, 2H), 7.47 (t, 1H, *J* = 8.1*)*, 7.33 (td, 1H, *J* = 0.6, 7.5 Hz), 6.90 (s,1H), 1.73 (s, 9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 148.8, 142.3, 141.2, 132.7, 129.2, 128.9, 126.4, 123.5, 122.5, 122.1, 121.9, 117.5, 177.1, 116.9, 52.3, 29.0. HRMS (EI) calcd for $C_{18}H_{19}N_4$ ($M+H^+$) 291.1604 found 291.1616.

5ab: ***N-(2,4,4-trimethylpentan-2-yl)indazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.067 g (0.816 mmol) of 1,1,3,3 tetrabutyl methyl isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in 1.0 mL of THF and 0.243 g (0.816 mmol) of 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4a**, KF (0.118 g, 2.04 mmol)/18-crown-6 (0.538 g, 2.04 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5ab** as a Light brown solid (0.165 g, 70%), mp 105-106 °C, R_f = 0.4 (5:95 EtOAc : hexanes, visualized by 254 nm UV light). ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.43 (d, 1H, *J* = 7.7 Hz), 8.39 (d, 1H, *J* = 8.5 Hz), 7.91 (d, 1H, *J* = 8.8 Hz), 7.87 (d, 1H, *J* = 8.9 Hz, 7.63-7.56 (m, 2H), 7.45 (t, 1H, *J* = 7.3), 7.32 (t, 1H, *J* = 8.5), 7.02 (br s, 1H), 2.16 (s, 2H), 1.78 (s, 6H), 1.06 (s, 9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 148.8, 142.2, 141.0, 132.5, 129.2, 128.9, 126.3, 123.4, 122.5, 122.0, 121.9, 117.7, 117.0, 116.9, 56.1, 51.5, 31.8, 31.5, 29.0. HRMS (EI) calcd for $C_{22}H_{27}N_4$ ($M+H^+$) 347.223 found 347.2230.

5ac: ***N-(tert-butyl)-3-chloroindazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.552 mmol) of 2-azido-4-chloro-benzaldehyde **1b**, 9.28 mg of $Pd(OAc)_2$, 0.054 g (0.662 mmol) of *t*-butyl isocyanide **2a,** and 200 mg of powdered 4 Å molecular sieves followed by 0.103 g (0.607 mmol) 4-chlorophenylsulfonylhydrazide **3a** in 1.0 mL of THF and 0.196 g (0.607 mmol) of 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4a**, KF (0.096 g, 1.65 mmol)/18-crown-6 (0.435 g, 1.65 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5ac** as a Colorless oil (0.129 g, 72%), R_f = 0.6 (5:95 EtOAc : hexanes, visualized by 254 nm UV light). ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.35 (dd, 2H, *J* = 3.4, 8.5 Hz), 7.92 (d, 1H, *J* = 8.8 Hz), 7.85 (s, 1H), 7.60 (t, 1H, *J* = 7.9 Hz*)*, 7.43 (dd, 1H, *J* = 2.1, 8.5 Hz), 7.35 (t, 1H, *J* = 7.5 Hz), 6.96 (br s, 1H), 1.73 (s, 9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 148.8, 142.7, 142.3, 134.7, 132.1, 129.2, 125.8, 123.8, 123.5, 122.3, 122.2, 121.8, 117.6, 116.8, 115.6, 54.4, 28.9. HRMS (EI) calcd for $C_{18}H_{18}ClN_4$ ($M+H^+$) 325.1215 found 325.1210.

5ad: ***N-(tert-butyl)-3-fluoroindazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.606 mmol) of 2-azido-4-fluoro-benzaldehyde **1c**, 9.28 mg (0.045) of $Pd(OAc)_2$, 0.060 g (0.727 mmol) of t-butyl isocyanide **2a,** and 200 mg of powdered 4 Å molecular sieves followed by 0.135 g (0.666 mmol) 4-chlorophenylsulfonylhydrazide **3a** in 1.0 mL of THF and 0.257 g (0.707 mmol) of 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4a**, KF (0.105 g, 1.81 mmol)/18-crown-6 (0.477 g, 1.81

mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5ad** as a White solid (0.138 g, 74%), mp 136-138 °C R_f = 0.63 (5:95 EtOAc : hexanes, visualized by 254 nm UV light).: ^{1}H NMR (δ ppm): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40-8.37 (m, 1H), 8.35 (d, 1H, *J* = 8.5 Hz), 7.91 (d, 1H, *J* = 8.8 Hz), 7.59 (t, 1H, *J* = 7.5 Hz), 7.51 (dd, 1H, *J* = 2.5, 10.4 Hz), 7.33 (t, 1H, *J* = 8.2 Hz), 7.21 (td, 1H, *J* = 8.6, 2.5 Hz), 6.96 (br s, 1H, N-*H*), 1.73 (s, 9H). ^{13}C{^{1}H}-NMR (126 MHz, $CDCl_3$) δ 163.2 (d, J_{C-F} = 248 Hz), 148.8, 143.2 (d, J_{C-F} = 12.6 Hz), 142.8, 132.3,129.1, 124.0 (d, J_{C-F} = 10 Hz) , 122.0, 121.8, 117.5, 116.5, 113.9, 112.1 ((d, J_{C-F} = 23.2 Hz), 111.7 (d, J_{C-F} = 21.4 Hz), 52.4, 28.9. HRMS (EI) calcd for $C_{18}H_{18}FN_4$ ($M+H^+$) 309.1510found 309.1520

5ae: ***3-bromo-N-(tert-butyl)indazolo[2,3-c]quinazolin-6-amine***. The general procedure was followed using 0.100 g (0.442 mmol) of 2-azido-4-bromo-benzaldehyde **1d**, 7.93 mg (0.033 mmol) of $Pd(OAc)_2$, 0.044 g (0.530 mmol) of t-butyl isocyanide **2a,** and 200 mg of powdered 4 Å molecular sieves followed by 0.082 g (0.486 mmol) 4-chlorophenylsulfonylhydrazide **3a** in 1.0 mL of THF and 0.157 g (o.530 mmol) of 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4a**, KF (0.076 g, 3 equiv)/18-crown-6 (0.348 g, 3 equiv) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5ad** as yellow oil (0.117 g, 72%), R_f = 0.5 (5:95 EtOAc : hexanes, visualized by 254 nm UV light).:^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.33 (d, 1H, *J* = 8.5 Hz), 8.26 (d, 1H, *J* = 8.5 Hz), 8.01 (s, 1H), 7.9 (d, 1H, *J* = 8.8 Hz*)*, 7.6 (t, 1H, *J* = 7.2 Hz), 7.5 (dd, 1H, *J* = 1.7, 8.5 Hz), 7.33 (t,1H, *J* = 8.1 Hz), 6.94 (br, s, 1H, N-*H*), 1.70 (s, 9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 148.8, 142.7, 142.4, 132.1, 129.2, 128.9, 126.5, 123.6, 122.8, 122.3, 121.8, 117.6, 116.9, 115.9, 52.4, 28.9. HRMS (EI) calcd for $C_{18}H_{18}BrN_4$ ($M+H^+$) 369.0710 found 369.0718.

5af: ***N-(tert-butyl)-3-(trifluoromethyl)indazolo[2,3-c]quinazolin-6-amine*** The general procedure was followed using 0.100 g (0.465 mmol) of 2-azido-4-trifluoro-benzaldehyde **1e**, 7.74 mg (0.034 mmol) of $Pd(OAc)_2$, 0.045 g (0.558 mmol) of t-butyl isocyanide **2a,** and 200 mg of powdered 4 Å molecular sieves followed by 0.086 g (0.511 mmol) 4-chlorophenylsulfonylhydrazide **3a** in 1.0 mL of THF and 0.164 g (0.558 mmol) of 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4a**, KF (0.080 g, 3 equiv)/18-crown-6 (0.368 g, 3 equiv) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5af** as a Yellow semisolid (0.108 g, 65%), R_f = 0.7 (5:95 EtOAc : hexanes, visualized by 254 nm UV light). ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.52 (d, 1H, *J* = 8.4 Hz), 8.41 (d, 1H, *J* = 8.6 Hz), 8.12 (s, 1H), 7.97 (d, 1H, *J* = 8.8 Hz*)*, 7.67-7.62 (m, 2H), 7.41 (dd, 1H, *J* = 6.8, 8.8 Hz), 7.01 (s, 1H), 1.75 (s, 9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 148.8, 142.9, 140.4, 131.6, 129.2, 123.8 ((q, J_{C-F} = 6.3, 10.0 Hz), 123.1, 122.8, 122.4, 119.5, 119.3 (q, J_{C-F} = 3.7, 7.3 Hz), 117.9, 52.5, 28.9. HRMS (EI) calcd for $C_{19}H_{18}F_3N_4$ ($M+H^+$) 359.1478 found 359.1475.

5ag: ***N-(tert-butyl)-2-chloroindazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.552 mmol) of 2-azido-5-chloro-benzaldehyde **1f**, 9.28 mg of $Pd(OAc)_2$, 0.054 g (0.662 mmol) of *t*-butyl isocyanide **2a,** and 200 mg of powdered 4 Å molecular sieves followed by 0.103 g (0.607 mmol) 4-chlorophenylsulfonylhydrazide **3a** in 1.0 mL of THF and 0.196 g (0.607 mmol) of 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4a**, KF (0.096 g, 1.65 mmol)/18-crown-6 (0.435 g, 1.65 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5ag** as a white solid (0.113 g, 63%), mp 136-138 °C Rf = 0.7 (5:95 EtOAc:hexanes, visualized by 254 nm UV light).: ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.37-8.35 (m, 2H), 7.93 (d, 1H, *J* = 8.8 Hz), 7.77 (d, 1H, *J* = 8.8 Hz), 7.62 (t, 1H, *J* = 7.4 Hz), 7.56 (dd, 1H, *J* = 2.3, 8.8 Hz), 7.38 (t, 1H, *J* = 7.5 Hz), 6.92 (br s,1H), 1.73 (s, 9H); 13C{1H}-NMR (125 MHz, CDCl3): 148.8, 142.4, 139,8, 131.4, 129.4, 129.1, 128.6, 127.8, 122.4, 121.8, 117.9, 117.7, 116.9, 52.3, 29.7; HRMS (EI) calcd for $C_{18}H_{18}ClN_4$ ($M+H^+$) 325.1215 found 325.1212.

5ah: ***3-chloro-N-(2,4,4-trimethylpentan-2-yl)indazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.552 mmol) of 2-azido-4-chloro-benzaldehyde **1b**, 9.28 mg of $Pd(OAc)_2$, 0.092 g (0.662 mmol) of 1,1,3,3 tetrabutyl methyl isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.103 g (0.607 mmol) 4-chlorophenylsulfonylhydrazide **3a** in 1.0 mL of THF and 0.196 g (0.607 mmol) of 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4a**, KF (0.096 g, 1.65 mmol)/18-crown-6 (0.435 g, 1.65 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5ah** as a yellow solid (0.144 g, 69 %), mp 115-116 °C , R_f = 0.4 (5:95 EtOAc : hexanes, visualized by 254 nm UV light)8.: ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.34-8.32 (m, 2H), 7.92 (d, 1H, *J* = 8.8 Hz), 7.84 (s, 1H), 7.61 (t, 1H, *J* = 7.1 Hz*)*, 7.42 (dd, 1H, *J* = 1,9, 8.5 Hz), 7.36 (t, 1H, *J* = 7.7 Hz), 7.06 (br s,1H), 2.61 (s, 2H), 1.78 (s, 6H) 1.07 (s, 9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 148.8, 142.7, 142.3, 134.6, 129.0, 125.8, 123.7, 123.4, 122.2, 121.8, 117.8, 116.8, 115.5, 56.1, 51.5, 31.8, 31.5, 29.4. HRMS (EI) calcd for $C_{22}H_{26}ClN_4$ ($M+H^+$) 381.1841 found 381.1830

5ba: ***N-(tert-butyl)-10,11-dimethoxyindazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.067 g (0.816 mmol) of t-butyl isocyanide **2a,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in1.0 mL of THF and 0.292 g (0.816 mmol) of 4,5-dimethoxy-2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4b**, (0.118 g, 2.04 mmol)/18-crown-6 (0.538 g, 2.04 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5ba** as a Colorless oil (0.180 g, 76%), R_f = 0.35 (5:95 EtOAc:hexanes, visualized by 254 nm UV light). ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.27 (d, 1H, *J* = 7.8 Hz), 7.96 (d, 1H,*J* = 8.1 Hz), 7.56 (t, 1H, *J* = 8 Hz), 7.49 (s, 1H) 7.41 (t, 1H, a*J* = 7.8 Hz*)*, 7.16 (s,1H), 6.70 (br s, 1H), 4.09 (s, 3H), 4.03 (s, 3H), 1.70 (s,9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 153.4, 147.9, 145.9, 142.4, 141.3, 131.7, 128.8, 126.4, 123.0, 122.1, 117.2, 110.8, 99.0, 95.6, 56.2, 56.1, 52.1 29.1. HRMS (EI) calcd for $C_{20}H_{23}N_4O_2$ ($M+H^+$) 351.1816found 351.1826

5bb:***10,11-dimethoxy-N-(2,4,4-trimethylpentan-2-yl)indazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.067 g (0.816 mmol) of 1,1,3,3 tetrabutyl methyl isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in1.0 mL of THF and 0.292 g (0.816 mmol) of 4,5-dimethoxy-2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4b**, (0.118 g, 2.04 mmol)/18-crown-6 (0.538 g, 2.04 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5bb** as a white solid (0.193 g, 70%), mp 105-106 °C, R_f = 0.4 (5:95 EtOAc : hexanes, visualized by 254 nm UV light). ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.29 (d, 1H, *J* = 7.3 Hz), 7.81 (d, 1H, *J* = 7.9 Hz), 7.58 (t, 1H, *J* = 8.3 Hz), 7.53 (s, 1H) 7.43 (t, 1H, *J* = 7.1 Hz*)*, 7.19 (s,1H), 6.81 (br s, 1H), 4.11 (s, 3H), 4.06 (s, 3H), 2.16(s, 2H), 1.77 (s, 6H) 1.07 (s,9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 153.3, 147.9, 145.9, 142.2, 141.3, 131.6, 128.7, 126.4, 122.9, 122.0, 117.2, 110.8, 99.0, 95.8, 56.2, 56.0, 55.8,.51.6, 31.5, 29.5. HRMS (EI) calcd for $C_{24}H_{31}N_4O_2$ ($M+H^+$) 407.2442found 407.2437

5bc+5bc': ***N-(tert-butyl)-12-methylindazolo[2,3-c]quinazolin-6-amine*** **+** ***N-(tert-butyl)-9-methylindazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.067 g (0.816 mmol) of t-buty isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in1.0 mL of THF and 0.254 g (0.816 mmol) of 4-methyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4c**, KF (0.118 g, 2.04 mmol)/18-crown-6 (0.538 g, 2.04 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded an inseparable mixture of **5bc** and **5bc'** (in approx. 1:1.4 ratio) as a Light

Yellow solid, mp 107-108 °C (0.150 g, 73%), R_f = 0.55 (5:95 EtOAc:hexanes, visualized by 254 nm UV light). Major isomer: ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.43 (d, 1H, *J* = 7.9 Hz), 8.23 (d, 1H, *J* = 8.5 Hz), 7.85 (d, 1H,, *J* = 8.2 Hz), 7.63-7.58 (m, 1H), 7.47-7.43 (m, 1H), 7.36 (d,1H, *J* = 6.7 Hz), 7.26 (t, 1H, *J* = 7.0 Hz), 6.96 (br s, 1H), 2.78 (s, 3H), 1.76 (s, 9H), ^{13}C-NMR (125 MHz, $CDCl_3$): ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.0, 142.4, 141.3, 132.7, 131.8, 129.0, 127.8, 126.4, 123.3, 122.4, 122.2, 119.3, 117.3, 116.6, 52.2, 29.0, 17.4. Minor isomer: ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.85 (d, 1H, *J* = 8.3 Hz), 7.85 (d, 1H, *J* = 8.2 Hz), 7.75 (d, 1H, *J* = 8.7 Hz), 7.63-7.58 (m, 1H), 7.47-7.43 (m, 1H), 7.41 (td, 1H, *J* = 1, 7.2 Hz), 7.08 (d, 1H, *J* = 6.7 Hz), 7.01 (br s, 1H), 3.17 (s, 3H), 1.75 (s, 7H); ^{13}C-NMR (125 MHz, $CDCl_3$): ^{13}C{^{1}H}-NMR (126 MHz, $CDCl_3$) δ 149.8, 142.5, 141.9, 134.1, 128.86, 128.85, 126.8, 124.8, 123.9, 122.7, 117.9, 116.6, 115.5, 52.2, 29.1, 17.4. HRMS (EI) calcd for $C_{19}H_{21}N_4$ ($M+H^+$) 305.1761 found 305.1759

5bd + 5bd': ***10-methoxy-N-(2,4,4-trimethylpentan-2-yl)indazolo[2,3-c]quinazolin-6-amine + 11-methoxy-N-(2,4,4-trimethylpentan-2-yl)indazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.067 g (0.816 mmol) of 1,1,3,3 tetrabutyl methyl isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in1.0 mL of THF and 0.267 g (0.816 mmol) of 4-methoxy 2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4d**, KF (0.118 g, 2.04 mmol)/18-crown-6 (0.538 g, 2.04 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded a inseparable mixture of **5bd** and **5bd'** (in approx. 1:1.1 ratio) as a Colorless oil (0.166 g, 65%), R_f = 0.6 (5:95 EtOAc : hexanes, visualized by 254 nm UV light). Due to the equimolar inseperable mixture of both isomers, we reported ^{1}H and ^{13} spectra as a mixture of both isomers. ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.37 (d, 1H, *J* = 7.9 Hz), 8.34 (d, 1H,*J* = 7.7 Hz), 8.25 (d, 1H, *J* = 9.1 Hz), 7.84-7.80 (m, 3H), 7.62-7.57 (m, 2H), 7.56 (d,1H, *J* = 1.9 Hz), 7.47-7.41 (m, 2H), 7.32 (dd, 1H, *J* = 2.3, 9.4 Hz), 7.14 (d, 1H, *J* = 1.9 Hz), 7.01 (dd, 1H, *J* = 2.1, 9.2 Hz), 6.89 (br s, 1H), 6.87 (br s, 1H), 4.02 (s, 3H), 3.97 (s, 3H), 2.16 (s, 4H), 1.77 (s, 12H), 1.07 (s, 18H); ^{13}C{^{1}H}-NMR (126 MHz, $CDCl_3$) δ 160.8, 155.3, 150.4, 145.4, 142.2, 141.7, 140.9, 139.3, 132.7, 131.4, 129.2, 128.6, 126.42, 126.37, 123.5, 123.0, 122.9, 122.5, 122.0, 119.1, 117.5, 116.8, 116.7, 116.6, 114.1, 114.0, 112.2, 98.5, 94.5, 55.9, 55.7, 55.4, 51.5, 51.5, 31.9, 31.8, 31.5, 29.7, 29.4 HRMS (EI) calcd for $C_{23}H_{29}N_4O$ ($M+H^+$) 377.2336 found 377.2339.

5be + 5be': *9-methyl-N-(2,4,4-trimethylpentan-2-yl)indazolo[2,3-c]quinazolin-6-amine + 12-methyl-N-(2,4,4-trimethylpentan-2-yl)indazolo[2,3-c]quinazolin-6-amine*. The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.067 g (0.816 mmol) of 1,1,3,3 tetrabutyl methyl isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in1.0 mL of THF and 0.254 g (0.816 mmol) of 4-methyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4b**, KF (0.118 g, 2.04 mmol)/18-crown-6 (0.538 g, 2.04 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded an inseparable mixture of **5be** and **5be'** (in approx. 1:4 ratio) as a Yellow oil (0.168 g, 69%), R_f = 0. 63 (5:95 EtOAc : hexanes, visualized by 254 nm UV light). Major isomer: ^{1}H NMR (δ ppm): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, 1H, *J* = 7.9 Hz), 8.24 (d, 1H, *J* = 8.5 Hz), 7.85 (d, 1H, *J* = 8.1 Hz), 7.62-7.58 (m, 1H), 7.47-7.43 (m, 1H), 7.35 (d, 1H, *J* = 6.7 Hz), 7.26 (t, 1H, *J* = 8.2 Hz), 7.14 (br s, 1H), 2.77 (s, 3H), 2.15 (s, 2H), 1.82 (s, 6H), 1.12 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.9, 142.3, 141.3, 132.5, 131.7, 128.9, 127.6, 126.4, 123.2, 122.4, 121.1, 119.3, 117.3, 116.6, 55.9, 52.3, 31.8, 31.6, 29.7, 29.2, 17.2. Minor isomer: ^{1}H NMR (δ ppm): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (d, 1H, *J* = 8.2 Hz), 7.82 (d, 1H, *J* = 8.1 Hz), 7.76 (d, 1H, *J* = 8.7 Hz), 7.62-7.58 (m, 1H), 7.47 – 7.43 (m, 1H), 7.40 (t, 1H, *J* = 7.2 Hz), 7.11 (br s, 1H), 7.08 (d, 1H, *J* = 6.7 Hz), 3.18 (s, 2H), 2.18 (s, 1H), 1.79 (s, 6H), 1.07 (s, 9H).; ^{13}C{^{1}H}-NMR (126 MHz, $CDCl_3$) δ 149.8, 142.4, 139.3, 134.0, 131.7, 128.8, 128.7,

126.8, 124.8, 123.8, 122.6, 117.9, 116.8, 115.6, 55.9, 51.4, 31.9, 31.5, 29.5, 29.4, 26.4. HRMS (EI) calcd for $C_{23}H_{29}N_4$ ($M+H^+$) 361.2387 found 361.2380.

5bf: ***N-(tert-butyl)benzo[5,6]indazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.067 g (0.816 mmol) of tertbutyl methyl isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in1.0 mL of THF and 0.283 g (0.816 mmol) of 3-(trimethylsilyl)naphthalen-2-yl trifluoromethanesulfonate **4d**, KF (0.118 g, 3 equiv)/18-crown-6 (0.538 g, 3 equiv) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5bf** as a yellow solid (0.164 g, 71%), mp 228-230 °C, R_f = 0.6 (5:95 EtOAc : hexanes, visualized by 254 nm UV light). ^{1}H NMR (δ ppm): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.06 (s, 1H,), 8.71 (d, 1H, *J* = 8.1 Hz), 8.44 (s, 1H), 8.07 (d, 1H, *J* = 8.5 Hz), 8.00 (d, 1H, *J* = 8.6 Hz), 7.93 (d, 1H, *J* = 8.15 Hz), 7.68 (t, 1H, *J* = 7.1 Hz), 7.58 (t, 1H, *J* = 7.9), 7.44 (t, 1H, *J* = 6.8 Hz) 7.35 (t, 1H, *J* = 7.9 Hz), 7.08(br s, 1H), 1.77 (s, 9H). ^{13}C{^{1}H}-NMR (126 MHz, $CDCl_3$) δ 147.1, 142.3, 141.2, 134.5, 132.7, 129.6, 129.2, 129.1, 128.2, 126.6, 125.8, 123.8, 122.3, 121.1, 118.7, 117.3, 113.0, 52.4, 28.9. HRMS (EI) calcd for $C_{22}H_{21}N_4$ ($M+H^+$) 341.1761 found 341.1758.

5bg: ***3-chloro-10,11-dimethoxy-N-(2,4,4-trimethylpentan-2-yl)indazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.552 mmol) of 2-azido-4-chloro-benzaldehyde **1b**, 9.28 mg of $Pd(OAc)_2$, 0.092 g (0.662 mmol) of 1,1,3,3 tetrabutyl methyl isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.103 g (0.607 mmol) 4-chlorophenylsulfonylhydrazide **3a** in 1.0 mL of THF and 0.217 g (0.607 mmol) of 4,5-dimethoxy-2-(trimethylsilyl)phenyl trifluoromethanesulfonate **4b**, KF (0.096 g, 1.65 mmol)/18-crown-6 (0.435 g, 1.65 mmol) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5bg** as a light yellow solid (0.155 g, 64%) mp 145-146 °C R_f = 0.6 (5:95 EtOAc : hexanes, visualized by 254 nm UV light).: ^{1}H NMR (δ ppm): (500 MHz, $CDCl_3$), 8.18 (d, 1H, *J* = 8.5 Hz), 7.79 (s, 1H), 7.44 (s, 1H), 7.38 (dd, 1H, *J* = 2.1, 8.4 Hz*)*, 7.17 (s, 1H), 6.88 (br s), 4.10 (s, 3H), 4.06 (s, 3H), 2.13 s, 2H) 1.75 (s, 6H) 1.07 (s, 9H); ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$): 153.5, 148.3, 146.0, 142.7, 134.1, 131.0, 125.7, 123.2, 122.9, 115.6, 110.8, 98.7, 95.9, 56.2, 56.0, 56.0, 31.8, 31.5, 29.5. HRMS (EI) calcd for $C_{24}H_{29}ClN_4O_2$ ($M+H^+$) 441.2052 found 441.2045

5bh: ***N-(2,4,4-trimethylpentan-2-yl)benzo[5,6]indazolo[2,3-c]quinazolin-6-amine.*** The general procedure was followed using 0.100 g (0.680 mmol) of 2-azidobenzaldehyde **1a**, 11 mg (0.051 mmol) of $Pd(OAc)_2$, 0.113 g (0.816 mmol) of 1,1,3,3 tetrabutyl methyl isocyanide **2b,** and 200 mg of powdered 4 Å molecular sieves followed by 0.127 g (0.748 mmol) 4-chlorophenylsulfonylhydrazide **3a** in1.0 mL of THF and 0.283 g (0.816 mmol) of 3-(trimethylsilyl)naphthalen-2-yl trifluoromethanesulfonate **4d**, KF (0.118 g, 3 equiv)/18-crown-6 (0.538 g, 3 equiv) added and stirred at 70 °C for 12 h. Purification by column chromatography on the bed of silica gel using hexane as eluent afforded **5bh** as a yellow solid (0.180 g, 67 %), mp 210-211 °C, R_f = 0.7 (5:95 EtOAc : hexanes, visualized by 254 nm UV light). ^{1}H NMR (δ ppm): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (s, 1H,), 8.68 (d, 1H, *J* = 7.2 Hz), 8.45 (s, 1H), 8.06 (d, 1H, *J* = 8.5 Hz), 7.99 (d, 1H, *J* = 8.6 Hz), 7.93 (d, 1H, *J* = 8.1 Hz), 7.70 (dt, 1H, *J* = 1.2, 8.3 Hz), 7.58 (t, 1H, *J* = 8.0), 7.43 (t, 1H, *J* = 6.7 Hz) 7.36 (t, 1H, *J* = 7.6 Hz), 7.19 (br s, 1H), 2.21 (s, 2H) 1.83 (s, 6H) 1.03 (s, 9H). ^{13}C{^{1}H}-NMR (126 MHz, $CDCl_3$) δ 147.1, 142.3, 141.2, 134.4, 132.5, 129.5, 129.1, 129.0, 128.2, 126.6, 123.7, 123.5, 122.3, 121.1, 118.7, 117.3, 113.9, 56.1, 51.6, 31.9, 31.7, 29.4. HRMS (EI) calcd for $C_{26}H_{29}N_4$ ($M+H^+$) 397.2387 found 397.2397.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at.

Detailed optimization, mechanistic study, photophysical study, cell imaging study, copies of 1H and 13C NMR spectra of the products and X-ray crystallographic data (PDF).

X-ray crystallographic data for **5ba** compound (CIF)

Accession Codes

CCDC 1588727 contain the supplementary crystallographic data for this paper.

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Notes

The authors declare no competing financial interest.

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