**Synthesis of a Visibly Emissive 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine Scaffold with Large Stokes Shift and Live Cell Imaging**

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Synthesis of a Visibly Emissive 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine Scaffold with Large Stokes Shift and Live Cell Imaging

Ajay Kanungo, Dipendra Patra, Sanghamitra Mukherjee, Tridib Mahata, Prakas R. Maulik and Sanjay Dutta*†

Abstract. We have developed a novel fluorescent scaffold 4 which is a 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine derivative from the reaction between di-tert-butyl but-2-yneidoate and a quinoxaline molecule containing a dimethyl amine side tail in high yield. The synthesis of scaffold 4 involves sp3 C-N bond cleavage mechanism which is not very common. The scaffolds 4 is emissive in the visible range λem ~ (517-540) nm with large stokes shifts (5005-6378) cm⁻¹ in ethanol. Laser confocal microscopy of the live HepG2 cells treated with compound 4f shows that it can be used for live cell imaging in nanomolar concentrations.

Introduction

Cellular imaging with fluorescent molecules is an important field and currently different fluorescent dyes are developed and marketed for their extensive use in the biomedical applications. Particular interest are in the development of fluorescent compounds which absorb in the visible region with large stokes shift. We are currently interested in development of fluorescent molecules with large stokes shift which can target biological macromolecules and can be imaged inside cells. Quinoxaline compounds can be natural product derived or of synthetic origin and is an important class of heterocyclic compounds with lot of biological activities and pharmaceutical applications. Here our focus was to synthesize some novel and biologically active quinoxaline derivatives with fluorescent properties. Recently work done by Gemma et al shows that pyrroloimidazo) quinoxaline hydrazones can be used as fluorescent probes to target amyloid fibrils.

Scheme 1 Synthesis of quinoxaline derivatives 4.

Here we describe the synthesis, photophysical properties and biocompatibility of 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4, Scheme 1) compounds with a built in push-pull system. The fundamental concept of a push-pull system consists of an electron donating group in conjugation with an electron withdrawing group with an extended π system. We have synthesized the active fluorophore scaffold 4 from the reaction between N2-(alkyl/aryl)-N3-(3-(dimethylamino)propyl)-6-nitroquinoxaline-2,3-diamine system with di-tert-butyl but-2-yneidoate in (95-98)% yield as shown in Scheme 1.

Fig. 1 A. Structure of BMS-345541. B. Imidazo[1,5-a]quinoxaline. C & D. Structures of Pyrimidoquinoxaline-6-oxide derivatives. E. Structures of scaffold 4a-4h derivatives.

The scaffold 4 which is 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine is completely novel and has not been reported before. The scaffold 4 is structurally very similar to some very important bioactive scaffolds such as BMS-345541 which exhibits cytotoxic activities on melanoma cell lines and also acts as a selective inhibitor of IkB kinase (Fig 1). Imidazo[1,5-a]quinoxaline compounds are also found to act as irreversible BTK inhibitors which is used for the treatment of rheumatoid arthritis. The pyrroloquinoxaline (POX) derivatives show their high selectivity...
towards 5-HT₃ receptors and also show anti-proliferative activity in different cancerous cell lines. Pyrimidoquinolinozine 6-oxides show anti-neoplastic activities and are particularly active against hypoxic cells. These compounds are also used as anti-bacterial, anti-amoebic and anti-anaerobic agents.¹²,¹³

We have synthesized several derivatives of scaffold 4a-4h as shown in Fig. 1, where substitutions were made on the exocyclic amine functionality with alkyl, aliphatic amine, furan-2-ylmethyl, methyl pyridine, benzy, o-chlorobenzyl, p-methoxybenzyl and aromatic group. We also get scaffold 5 as another product in the reaction of N₂-(alkylaryl)-N3-(dimethylamino)propyl-6-nitroquinoline-2,3-diamine system with di-methylbut-2-ynedioate (Scheme 2). When we use aromatic amine instead of aliphatic or benzyl amines we get only scaffold 4 in high yield (60%). We also studied the photophysical properties (absorbance and fluorescence) of scaffold 4. Our studies shows that compounds 4a-4h are fluorescent and visibly emissive with large stokes shift. It is interesting to note that the nitro substituents generally quench the fluorescence¹⁴, where as our scaffold of 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinolin-5-amine is fluorescent with low quantum yield. We have also shown by laser confocal microscopy that live cell imaging of HepG2 cells can be performed with compound 4f in the nanomolar concentration.

Results and discussion

![Scheme 2 Synthesis of quinoxaline derivatives 4 and 5.](image)

Synthesis of quinoxaline derivatives 4 and 5: Synthesis of the quinoxaline analogues was achieved as by treating commercially available 4-nitro orthophenylenediamine with diethyl oxalate in 4N HCl under reflux to form corresponding compound 6-nitroquinoline-2,3(1H, 4H)-dione, followed by treatment with excess of POCl₃ and reflux at 110 °C to form 2,3-dichloro-6-nitroquinoline (1). Supporting info, Scheme S1)¹⁵ Compounds 2(a-h) were achieved by treating compound 1 with amines in 1:1 ratio in the presence of CaCO₃ at R.T. Subsequently, (2a-2h) under C-N coupling (Buchwald–Hartwig amination) using Pd(dba)₃ as a catalyst, XPhos as ligand and Cs₂CO₃ as base and amines afforded compounds 3a-3h. Compound 3 was treated with dimethylbut-2-ynedioate at 60 °C for 18 h in dioxane to afford compounds 4 and 5 with reasonable yields (Table 1).

We used a variety of substrates in the reaction of 3 with dimethylbut-2-ynedioate (DMAD). When R² is methyl, the yield of N-methyl-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinolin-5-amine (4a) is 22% with 58% yield of (5a). When R² is 1-methyl-4-propylpiperazine, the yield of N-[3-(4-methylpiperazin-1-yl)propyl]-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinolin-5-amine (4b) is 45% without the formation of (5b). When R² is substituted with furan-2-ylmethyl (4c), methyl pyridine (4d), benzyl (4e), o-chlorobenzyl (4f), p-methoxybenzyl (4g), the yield of (4c-4g, Table 1) varies from (25-31)% and of (5c-5e) from (52-58)%. When R² was substituted with p-anisole group we isolated 4h in 60% yield and without the formation of 5h. When the dimethylbut-2-ynedioate is switched to di-tert-butyl but-2-ynedioate we get only 4a, 4e in almost 95-99% isolated yield. This shows that the control of selective generation of one product is possible by switching the ester.

<table>
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<tr>
<th>Entry</th>
<th>R²</th>
<th>%Yield 4a-4h</th>
<th>%Yield 5a-5h</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>a = Me</td>
<td>22</td>
<td>58</td>
</tr>
<tr>
<td>2.</td>
<td>b =</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>c =</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>4.</td>
<td>d =</td>
<td>25</td>
<td>52</td>
</tr>
<tr>
<td>5.</td>
<td>e =</td>
<td>28</td>
<td>58</td>
</tr>
<tr>
<td>6.</td>
<td>f =</td>
<td>31</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>g =</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>h =</td>
<td>60</td>
<td>-</td>
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![Scheme 3 Plausible mechanism for the formation of 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinolin-5-amine (4) derivative.](image)
scheme 3. The initial nucleophilic attack is from the nitrogen of dimethylamine side tail on the alkyne carbon of the dimethyl but-2-ynedioate to form intermediate 3i which abstracts a proton from neighbouring quinoxaline amine to form intermediate 3ii. Then the quinoxaline nitrogen of intermediate 3ii attacks the α-carbon of the activated dimethylamine side chain causing a sp³ C-N bond cleavage. Similar sp³ C-N bond cleavage of activated dimethyl amine is shown by Li-Wen Xu et al.16

![Scheme 4](image)

Scheme 4. Plausible mechanism for the formation of scaffold 5.

The formation of compound 5A and 5B involves the initial nucleophilic attack of N2 nitrogen of the quinoxaline moiety on the carbonyl carbon of the dimethyl but-2-ynedioate group generating tetrahedral intermediate 3iii which expels alkoxide to generate species 3iv. Then the N3 nitrogen of the quinoxaline moiety attacks the β carbon of the alkyne-conjugated ester group to generate intermediate 3v and after subsequent proton transfer generates a mixture of geometrical isomers (E and Z) of 5. The E isomer is predominant as shown by crystal structure and NOESY data (Fig. 2B, supporting info). This also shows that if a bulky ester is used then the probability of nucleophilic attack at carbonyl carbon of the dialkyl but-2-ynedioate group will decrease due to the greater steric interaction. This is done by replacing small methyl groups of ester group with large and bulky tertiary butyl groups. When we carried out the reaction of di-tert-butyl but-2-ynedioate with 3a (alkyl) and 3e (benzyl), we get 95-99% formation of only 4a and 4e. This shows that the reaction can be steered to predominantly one product by changing the ester substituent of the acetylenedicarboxylate moiety. However this type reaction of a phenylenediamine moiety with acetylenic diester is reported previously with the generation 2-oxo-3-carbomethoxy methylene-1,2,3,4-tetrahydroquinoxaline compound.17, 18

![Fig. 2](image)

Fig. 2 The ORTEP diagrams showing the X-ray determined molecular structures of compound 4c (left) and 5e (right) with 30% probability level of thermal ellipsoids.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Absorption λabs (nm)</th>
<th>Emission λem (nm)</th>
<th>% Φ</th>
<th>Brightness Φ X ε</th>
<th>Stokes Shift (Δν = νabs - νems) (cm⁻¹)</th>
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<tbody>
<tr>
<td>4a</td>
<td>400</td>
<td>537</td>
<td>1.15</td>
<td>143</td>
<td>6378</td>
</tr>
<tr>
<td>4b</td>
<td>403</td>
<td>517</td>
<td>1.58</td>
<td>204</td>
<td>5472</td>
</tr>
<tr>
<td>4c</td>
<td>400</td>
<td>524</td>
<td>1.45</td>
<td>155</td>
<td>5916</td>
</tr>
<tr>
<td>4d</td>
<td>402</td>
<td>532</td>
<td>1.12</td>
<td>186</td>
<td>6079</td>
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<tr>
<td>4e</td>
<td>402</td>
<td>534</td>
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<td>527</td>
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<tr>
<td>4g</td>
<td>403</td>
<td>540</td>
<td>1.90</td>
<td>203</td>
<td>6295</td>
</tr>
<tr>
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<td>417</td>
<td>527</td>
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<td>108</td>
<td>5005</td>
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</table>

Quantum yields were determined by using Coumarin-153 (Φ = 0.38) in ethanol as the reference standard (supporting info).19

![Fig. 3](image)

Fig. 3 Absorption spectra of 4a-4h (50 µM) in EtOH.
The photo-physical properties of the scaffolds 4a-4h were carried out as shown in Fig. 3, Fig. 4 and Table 2. Full absorbance spectrum of compounds (4a-4h) (Fig. S2, supporting info) show that there are two absorbance maximums, one around (270-293) nm and the other around (400-417) nm. It is interesting to note that simple alkyl/benzyl substituents (4a-4g) have \( \lambda_{\text{max}} \) around (400-403) nm, whereas when scaffold 4 is conjugated with p-anisole group, the \( \lambda_{\text{max}} \) shift by almost 17 nm. To study the fluorescence properties of the compounds (4a-4h) were excited at their respective \( \lambda_{\text{max}} \) as shown in Table 2, Fig. 4. The compounds are visibly emissive in the range of \( \lambda \text{em} \) (517-540) nm. The compounds (4a-4h) have \( \lambda_{\text{max}} \)5 around (517-540) nm. The compounds (4a-4h) have a large stokes shift (5005-6378 cm\(^{-1}\)) with low quantum yields (1-2)%.

Next we studied the cellular distribution of compound 4f by laser confocal microscopy of live HepG2 cells treated with 50 nM of compound 4f. Fig. 5 shows that after 1h incubation of live HepG2 cells with 4f (50 nM), 4f localized in the cytoplasm.

**Conclusions**

In summary, we have shown that a reaction between a quinoxaline molecule and dimethyl but-2-yndioate generates two diversity oriented fluorescent scaffolds 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine derivatives (4) and (E/Z)-methyl 2-(4-(alkyl/benzyl)-1-(dimethylamino)methyl)-4-nitro-3-oxo-3,4-dihydropyrazino[2,3-b]quinoxalin-2(1H)-yldiene)acetate (5). The synthesis of scaffold 4 is novel and involves sp\(^3\) C-N bond cleavage mechanism which is not shown earlier with quinoxaline compounds. We have also shown that the reaction can be steered to only scaffold 4 by changing to a bulky ester group. We have also synthesized different derivatives of scaffold 4 with alkyl, alky amine, benzyl and substituted benzyl and aromatic substituents.

The scaffold 4 is structurally very similar to some very important bioactive scaffolds such as BMS-345541, Imidazo[1,5-a]quinoxaline compounds pyrroloquinoxaline (PQX) derivatives and pyrimidoquinoxaline 6-oxides derivatives. The scaffold 5 is very similar in structure to some common flavin compounds. Electron rich arylddehyde can be oxidised quickly and selectively to the corresponding phenols with high yield by using \( \text{FIOH} \) catalysts. Vitamin B2, Riboflavin, Molybdoptereines, all contain the important flavin component which is biologically very significant.

We have also shown that the scaffold 4 is fluorescent and visibly emissive with large stokes shift and contain a built in push-pull system. We have also shown by laser confocal microscopy that live cell imaging of HepG2 cells can be performed with compound 4f in the nanomolar concentration. It is very important to note that compound 4 can be now modified to target different cellular components for live cell imaging.

**Experimental**

**Chemical and solvents:**

The reagents (chemicals) were purchased from Sigma, Alfa-aeasar, Acros and used without further purification. All solvents purchased from Rankem, Merck, Acros were distilled prior to use. Petroleum ether refers to fraction boiling in range of 60-80°C. Dichloromethane, dimethyl formamide were dried over calcium hydride. Acetonitrile and Anhydrous 1, 4-dioxane were purchased from Acros.

**Reactions:**

All reactions were carried out under nitrogen atmosphere and anhydrous condition unless otherwise mentioned. Analytical thin layer chromatography (TLC) was performed on silica Gel60F\(_{254}\) aluminium TLC sheets, for visualization of developed chromatogram was performed by UV absorbance or iodine exposure. For purification, column chromatography was performed using 100-200 mesh silica gel.

**Analysis:**

\(^1\)H and \(^13\)C-NMR spectra were recorded on a 300 MHz and 600 MHz (\(^1\)H), 75 MHz and 150 MHz (\(^13\)C) NMR spectrometer using tetratolylsilane (TMS) as internal standard. Chemical shifts (\( \delta \)) are given from TMS (\( \delta \)) in part per million (ppm) with reference to residual nuclei of deuterated solvent used. Coupling constant (J) are expressed in Hertz (Hz) and spin multiplication are given as s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet) and bs (broad). Mass spectra were performed using ESI, EI and FAB positive ionization mode.

**Instrumentation:**

\(^1\)H-NMR spectra were collected on a BRUKER DPX 300 MHz and 600 MHz. All EI HRMS were collected using a EI Mass spectrometer MS Station Jms-700, Jeol, Japan. All UV/Vis were recorded on a Jasco V-630 UV-VIS Spectrophotometer. Emission spectra were taken on Cary Eclipse Fluorescence Spectrophotometer. Unit cell determination and X-ray intensity data collection for all the compounds were performed on a BRUKER KAPPA APEX II CCD diffract meter at 296(2) K. Computer programs used: data collection.
3-chloro-N-(furan-2-ylmethyl)-6-nitroquinolin-2-amine (2c);

The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoline and furfurylamine. The crude product was purified by Column chromatography (SiO₂, eluting with 2 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (318 mg, 51 %);

HRMS (EI⁺) m/z: [M]+: 304.03427; 13C-NMR (300 MHz, CDCl₃); δ (ppm): 8.72 (d, J = 2.4 Hz, 1H), 8.40 (dd, J₁ = 9.3 Hz, 1H), 7.81 (d, J = 9.3 Hz, 1H), 7.43 (s, 1H), 6.39 (2H), 6.22 (brs, 1H), 4.84 (d, J = 5.4 Hz, 2H); 13C-NMR (75 MHz, CDCl₃); δ (ppm): 38.5, 108.5, 110.6, 124.1, 124.3, 126.9, 134.7, 142.0, 142.7, 144.2, 145.0, 148.8, 150.0.

3-chloro-6-nitro-N-(pyridin-3-ylmethyl)quinolin-2-amine (2d);

The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoline and 3-aza benzylamine. The crude product was purified by Column chromatography (SiO₂, eluting with 30 % ethyl acetate in pet ether) afforded the desired product as a yellow solid (310 mg, 48 %);

HRMS (EI⁺) m/z: [M]+: 315.05057; 13C-NMR (300 MHz, CDCl₃); δ (ppm): 9.60 (brs, 1H), 9.04 (d, J = 2.4 Hz, 1H), 8.78 (d, J = 2.7 Hz, 1H), 8.67 (s, 1H), 8.42-8.38 (m, 1H), 7.91 (s, 1H), 7.77 (d, J = 9.3 Hz, 1H), 7.28 (s, 1H), 4.98 (d, J = 6 Hz, 2H); 13C-NMR (75 MHz, CDCl₃); δ (ppm): 42.2, 123.6 (2C), 124.0, 126.5, 133.9, 134.1, 135.6, 140.8, 143.0, 145.0, 148.3, 149.3, 149.5.

N-benzyl-3-chloro-6-nitroquinolin-2-amine (2e);
The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoline and benzylamine. The crude product was purified by Column chromatography (SiO₂, eluting with 2 % ethyl acetate in pet ether) afforded the desired product as a yellow solid (322 mg, 50 %);

HRMS (EI⁺) m/z: [M]+: 314.05716; 13C-NMR (300 MHz, CDCl₃); δ (ppm): 8.67 (d, J = 2.7 Hz, 1H), 8.35 (dd, J₂ = 9.3 Hz, 1H, J₁ = 9.3 Hz, 1H), 7.76 (d, J = 9.3 Hz, 1H), 7.44-7.32 (m, 5H), 6.25 (brs, 1H), 4.83 (d, J = 5.7 Hz, 2H); 13C-NMR (75 MHz, CDCl₃); δ (ppm): 45.7, 124.1, 124.3, 126.9, 128.0 (3C), 128.9 (2C), 134.8, 137.0, 140.3, 141.4, 145.2, 149.1.

3-chloro-N-(2-chlorobenzyl)-6-nitroquinolin-2-amine (2f);
The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoline and 2-chlorobenzylamine. The crude product was purified by Column chromatography (SiO₂, eluting with 2 % ethyl acetate in pet ether) afforded the desired product as a yellow solid (344 mg, 50 %);

HRMS (EI⁺) m/z: [M]+: 325.05289; 13C-NMR (300 MHz, CDCl₃); δ (ppm): 8.70 (d, J = 2.4 Hz, 1H), 8.38 (dd, J₁ = 9.3 Hz, 1H, J₂ = 9.3 Hz, 1H), 6.00 (brs, 1H), 3.22 (d, J = 5.1 Hz, 3H); 13C-NMR (75 MHz, CDCl₃); δ (ppm): 28.4, 123.6, 124.0, 126.5, 133.9, 140.9, 141.5, 152.0.

3-chloro-N-methyl-6-nitroquinolin-2-amine (2g);
The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoline and methylamine (40 % w/w aq. solution). The crude product was purified by Column chromatography (SiO₂, eluting with 1 % ethyl acetate in pet ether) afforded the desired product as a yellow solid (373 mg, 50 %);

HRMS (EI⁺) m/z: [M]+: 344.06754; 13C-NMR (300 MHz, CDCl₃); δ (ppm): 7.81 (d, J = 2.7 Hz, 1H), 8.35 (dd, J₁ = 9.3 Hz, 1H, J₂ = 9.3 Hz, 1H), 7.79 (s, J₁ = 9.3 Hz, 1H), 6.44 (brs, 1H), 4.93 (d, J = 6.0 Hz, 2H); 13C-NMR (75 MHz, CDCl₃); δ (ppm): 43.6, 124.1, 124.3, 126.9, 127.1, 129.4, 129.8, 130.6, 134.0, 135.4, 138.0, 140.3, 141.4, 145.0, 149.0.

3-chloro-N-(4-methoxybenzyl)-6-nitroquinolin-2-amine (2h);
The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoline and 4-methoxybenzylamine. The crude product was purified by Column chromatography (SiO₂, eluting with 2 % ethyl acetate in pet ether) afforded the desired product as a yellow solid (388 mg, 55 %);

HRMS (EI⁺) m/z: [M]+: 344.06762; 13C-NMR (300 MHz, CDCl₃); δ (ppm): 8.71 (d, J = 2.7 Hz, 1H), 8.39 (dd, J₁ = 2.7 Hz, J₂ = 9.3 Hz, 1H), 7.79 (d, J = 9.3 Hz, 1H), 7.35 (d, J = 8.7 Hz, 2H), 6.92 (dd, J₁ = 2.1 Hz, J₂ = 6.9 Hz, 2H), 6.15 (d, J = 3.3 Hz, 1H), 4.76 (d, J = 5.4 Hz, 2H), 3.82 (s, 3H); 13C-NMR (75 MHz, CDCl₃); 45.3, 55.3, 114.3.

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3-chloro-N-(4-methoxyphenyl)-6-nitroquinoxalin-2-amine (2b): The title compound was prepared according to the general procedure D from 3-chloro-N-(4-methoxyphenyl)-6-nitroquinoxalin-2-amine and N,N-dimethylpropan-1,3-diamine. The crude product was purified by Column chromatography (SiO₂; eluting with 3 % methanol in chloroform) afforded the desired product as a reddish solid (258 mg, 85%).

HRMS (FAB⁺) m/z: [M+H⁺]: 382.2056, calcd: 382.2066; 1H-NMR (300 MHz, CDCl₃): δ (ppm): 8.44 (d, J = 2.4 Hz, 1H), 8.06 (dd, J = 2.4 Hz, J = 9.0 Hz, 1H), 7.61 (d, J = 9.0 Hz, 1H), 7.49 (d, J = 4.8 Hz, 2H), 4.77 (d, J = 5.7 Hz, 2H), 2.68 (t, J = 5.4 Hz, 2H), 3.12 (m, 8H), 1.95-1.89 (m, 2H), 11C-NMR (75 MHz, CDCl₃): δ (ppm): 24.4, 28.4, 41.1, 44.8 (2C), 58.3, 118.4, 121.0, 121.5, 124.6, 131.1, 141.3, 143.6, 144.8, 145.1, 146.5.

N²-(dimethylamino)propyl-N¹-(4-methoxybenzyl)6-nitroquinoxalin-2,3-diamine (3f): The title compound was prepared according to the general procedure D from N-benzyl-3-chloro-6-nitroquinoxalin-2-amino and N,N-dimethylpropan-1,3-diamine. The crude product was purified by Column chromatography (SiO₂; eluting with 3 % methanol in chloroform) afforded the desired product as an orange solid (196 mg, 65 %). HRMS (EI⁺) m/z: [M⁺]: 380.1964, calcd: 380.1961; 1H-NMR (300 MHz, CDCl₃): δ (ppm): 8.44 (d, J = 2.4 Hz, 1H), 8.06 (dd, J = 2.4 Hz, J = 9.0 Hz, 1H), 7.61 (d, J = 9.0 Hz, 1H), 7.46-7.33 (m, 5H), 5.44 (brs, 1H), 4.76 (d, J = 4.8 Hz, 2H), 3.67 (t, J = 5.7 Hz, 2H), 2.63 (t, J = 5.7 Hz, 2H), 2.30 (s, 8H), 1.95-1.89 (m, 2H), 11C-NMR (75 MHz, CDCl₃): δ (ppm): 23.7, 42.3, 44.7 (2C), 46.1, 59.4, 118.3, 121.0, 125.6, 127.9, 128.8, 136.6, 138.1, 141.2, 143.6, 144.8, 145.1, 148.5, 149.5.

N²-(2-chlorobenzyl)-N¹-(4-methoxybenzyl)6-nitroquinoxalin-2,3-diamine (3g): The title compound was prepared according to the general procedure D from N-benzyl-3-chloro-6-nitroquinoxalin-2-amino and N,N-dimethylpropan-1,3-diamine. The crude product was purified by Column chromatography (SiO₂; eluting with 3 % methanol in chloroform) afforded the desired product as an orange solid (216 mg, 73 %). HRMS (EI⁺) m/z: [M⁺]: 430.28134, calcd: 430.28047; 1H-NMR (600 MHz, CDCl₃): δ (ppm): 8.44 (s, 1H), 8.06 (dd, J₁ = 2.4 Hz, J₂ = 9.0 Hz, 1H), 7.55 (d, J = 9.0 Hz, 1H), 6.70 (brs, 1H), 6.59 (brs, 1H), 3.69-3.68 (m, 4H), 2.59-2.50 (m, 10H), 2.33-2.31 (m, 9H), 1.91-1.89 (m, 4H), 11C-NMR (75 MHz, CDCl₃): δ (ppm): 24.9, 25.3, 40.5, 41.1, 44.9 (2C), 46.0, 53.1 (2C), 55.0 (2C), 57.0, 57.6, 118.3, 120.9, 121.5, 123.5, 142.2, 143.2, 145.0, 145.8.
N-3-(dimethylamino)propyl)-N'-4-(methoxyphenyl)-6-nitroquinoxaline-2,3-diamine (3h): The title compound was prepared according to the general procedure D from 3-chloro-N-(4-methoxyphenyl)-6-nitroquinoxaline-2,amine and N,N-dimethylpropan-1,3-diamine. The crude product was purified by Column chromatography (SiO$_2$, eluting with 3 % methanol in chloroform) afforded the desired product as a yellowish solid (216 mg, 60 %); HRMS (ESI$^+$) m/z: [M+Na]$^+$: 397.1980, calcld: 397.1988; 13$^C$-NMR (300 MHz, CDCl$_3$): $\delta$ (ppm): 38.9 (3C), 43.0, 44.0, 44.7, 45.1, 55.1, 56.6, 113.7 (2C), 118.7, 120.5, 125.1, 129.7 (2C), 130.2, 135.7, 141.9, 142.3, 144.7, 145.1, 158.8.

General Procedure E: Formation of dihydropyrimido quinoxaline (4a-4h) and dihydropyrano quinoxaline derivatives (5a, 5c-5e):

1. The title compound was prepared according to the general procedure E from 3-chloro-N-(4-methoxyphenyl)-6-nitroquinoxaline-2,3-diamine and N,N-dimethylpropan-1,3-diamine. The crude product was purified by Column chromatography (SiO$_2$, eluting with 3 % methanol in chloroform) afforded the desired product as a redish solid (216 mg, 60 %); HRMS (ESI$^+$) m/z: [M+Na]$^+$: 397.1980, calcld: 397.1988; 13$^C$-NMR (300 MHz, CDCl$_3$): $\delta$ (ppm): 38.9 (3C), 43.0, 44.0, 44.7, 45.1, 55.1, 56.6, 113.7 (2C), 118.7, 120.5, 125.1, 129.7 (2C), 130.2, 135.7, 141.9, 142.3, 144.7, 145.1, 158.8.

N-3-(dimethylamino)propyl)-N'-4-(methoxyphenyl)-6-nitroquinoxaline-2,3-diamine (3h): The title compound was prepared according to the general procedure D from 3-chloro-N-(4-methoxyphenyl)-6-nitroquinoxaline-2,3-diamine and N,N-dimethylpropan-1,3-diamine. The crude product was purified by Column chromatography (SiO$_2$, eluting with 3 % methanol in chloroform) afforded the desired product as a yellowish solid (216 mg, 60 %); HRMS (ESI$^+$) m/z: [M+Na]$^+$: 397.1980, calcld: 397.1988; 13$^C$-NMR (300 MHz, CDCl$_3$): $\delta$ (ppm): 38.9 (3C), 43.0, 44.0, 44.7, 45.1, 55.1, 56.6, 113.7 (2C), 118.7, 120.5, 125.1, 129.7 (2C), 130.2, 135.7, 141.9, 142.3, 144.7, 145.1, 158.8.

N-benzyl-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinolin-5-amine (4g): The title compound was prepared according to the general procedure E from N-3-(dimethylamino)propyl)-N'-4-(methoxyphenyl)-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO$_2$, eluting with 6 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (20 mg, 31 %); HRMS (ESI$^+$) m/z: [M+Na]$^+$: 359.1224, calcld: 359.1232; 13$^C$-NMR (300 MHz, CDCl$_3$): $\delta$ (ppm): 19.6, 42.2, 42.8, 43.0, 105.9, 118.1, 123.5, 125.4, 130.3, 133.8, 135.8, 138.0, 139.2, 143.1, 148.8, 149.5.

N-(4-methoxypyridin-3-yl)-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinolin-5-amine (4d): The title compound was prepared according to the general procedure E from N-3-(dimethylamino)propyl)-N'-4-(methoxyphenyl)-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO$_2$, eluting with 6 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (15.5 mg, 25 %); HRMS (ESI$^+$) m/z: [M+Na]$^+$: 359.1243, calcld: 359.1232; 13$^C$-NMR (300 MHz, CDCl$_3$): $\delta$ (ppm): 19.6, 42.2, 42.8, 43.0, 105.9, 118.1, 123.5, 125.4, 130.3, 133.8, 135.8, 138.0, 139.2, 143.1, 148.8, 149.5.

N-(4-methoxypyridin-3-yl)-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinolin-5-amine (4d): The title compound was prepared according to the general procedure E from N-3-(dimethylamino)propyl)-N'-4-(methoxyphenyl)-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO$_2$, eluting with 6 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (20 mg, 31 %); HRMS (ESI$^+$) m/z: [M+Na]$^+$: 359.0886, calcld: 359.0890; 13$^C$-NMR (300 MHz, CDCl$_3$): $\delta$ (ppm): 19.6, 42.2, 42.8, 43.0, 105.8, 118.0, 125.2, 127.5, 128.0 (2C), 128.7 (2C), 130.3, 138.0 (2C), 139.6, 142.8, 151.0.
The crude product was purified by Column chromatography (SiO₂, eluting with 14% ethyl acetate in pet ether) afforded the desired product as a yellowish-orange solid (26 mg, 60%); HRMS (ESI⁺): [M+H⁺] = 481.1834, calcld: 481.1549; 1H-NMR (300 MHz, CDCl₃): δ (ppm): 8.62 (d, J = 2.1 Hz, 1H), 8.27 (dd, J = 2.4 Hz, J = 9 Hz, 1H), 7.93 (d, J = 9 Hz, 1H), 7.57 (d, J = 6.6 Hz, 2H), 7.33-7.28 (m, 3H), 6.05 (s, 1H), 5.54 (s, 2H), 4.22 (t, J = 7.2 Hz, 2H), 2.29 (s, 6H), 1.98-1.91 (m, 2H); 13C-NMR (75MHz, CDCl₃): δ (ppm): 23.0, 42.9, 44.4, 45.4 (2C), 52.6, 56.5, 107.6, 120.7, 122.6, 123.5, 128.2, 131.4, 133.0, 137.3, 137.8, 139.7, 149.3, 149.7, 151.1, 157.0, 167.7.

(E)-methyl 2-(4-benzyl-1-(3-(dimethylamino)propyl)-8-nitro-3-oxo-3,4-dihydropyrazino[2,3-b]quinoxalin-2(1H)-ylidene)acetate (5e): The title compound was prepared according to the general procedure D from N'-benzyl-N'-[3-(dimethylamino)propyl]-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO₂, eluting with 2% methanol in chloroform) afforded the desired product as a yellowish solid (75 mg, 58%); HRMS (ESI⁺): [M+H⁺] = 491.2043, calcld: 491.2043; 1H-NMR (300 MHz, CDCl₃): δ (ppm): 8.62 (d, J = 2.1 Hz, 1H), 8.27 (dd, J = 2.4 Hz, J = 9 Hz, 1H), 7.93 (d, J = 9 Hz, 1H), 7.57 (d, J = 6.6 Hz, 2H), 7.33-7.28 (m, 3H), 6.05 (s, 1H), 5.54 (s, 2H), 4.22 (t, J = 7.2 Hz, 2H), 2.29 (s, 6H), 1.98-1.91 (m, 2H); 13C-NMR (75MHz, CDCl₃): δ (ppm): 23.0, 42.9, 44.4, 45.4 (2C), 52.6, 56.5, 107.6, 120.7, 121.0, 122.5, 127.9, 128.2, 129.5, 129.6, 133.2, 133.3, 135.6, 137.6, 138.7, 140.1, 146.8, 157.0, 167.7.

Photophysics: Acetonitrile, methanol and dioxane of spectroscopic grades were purchased from Spectrum, Fischer Scientific and Merck, respectively. All spectroscopy samples were prepared from concentrated DMSO stock solutions, so all samples contains 0.5 v % DMSO. Absorption spectra were measured on a Jasco V-630 UV-VIS Spectrophotometer and corrected for the blank. The sample temperature was maintained at 20 °C with the help of thermostat. Emission spectra were taken on Cary Eclipse Fluorescence Spectrophotometer. The sample temperature was maintained at 20 °C with the help of thermostat. Quantum yields were determined by using Coumarin-153 (ϕ = 0.38 in ETOH) as the reference standard using the following equation:

\[
\varphi = \frac{I_{ref}}{I_{s}} \times \frac{O.D.}{O.D. ref} \times \frac{\eta_{ref}}{\eta_{s}}
\]

Here \( \varphi \), \( I_{ref} \) and \( I_{s} \) stand for quantum yield, integrated emission intensity, optical density at λex and refractive index (\( \eta_{ref} = 1.36 \)). Sample and reference are denoted by s and ref, respectively.

Andor spining dish live cell confocal microscopy: Human liver cancer cell (HepG2) was propagated in DMEM containing 10% heat inactivated FBS and 1% Pen Strep at 37°C and 5% CO₂. Cells were seeded into confocal dish (22mm) (5X10⁴ f/w). Cells were incubated for 1h at 37°C in 5% CO₂. After 1h, excitation and emission wavelength for 4f were 405 and 542 nm respectively.

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Notes and references

Synthesis and live cell imaging of a novel fluorescent scaffold which is emissive in the visible range with large stokes shifts.