RSC Advances

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Journal:	*RSC Advances*
Manuscript ID:	RA-ART-07-2015-012960.R2
Article Type:	Paper
Date Submitted by the Author:	11-Aug-2015
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Journal Name

ARTICLE

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

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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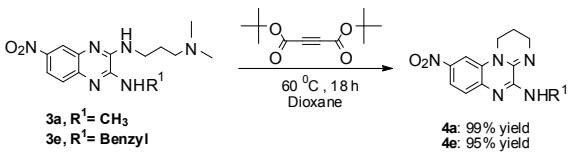
Abstract. We have developed a novel fluorescent scaffold **4** which is a 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine derivative from the reaction between di-*tert*-butyl but-2-ynedioate and a quinoxaline molecule containing a dimethyl amine side tail in high yield. The synthesis of scaffold **4** involves sp^3 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.[1,2] Particular interest are in the development of fluorescent compounds which absorb in the visible region with large stokes shift.[3,4] 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.[5] Here our focus was to synthesize some novel and biologically active quinoxaline derivatives with fluorescent properties. Quite recently work done by Gemma *et al* shows that pyrrolo(imidazo) quinoxalinehydrazones can be used as fluorescent probes to target amyloid fibrils.[6]



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.[7] 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-ynedioate in (95-98)% yield as shown in Scheme 1.

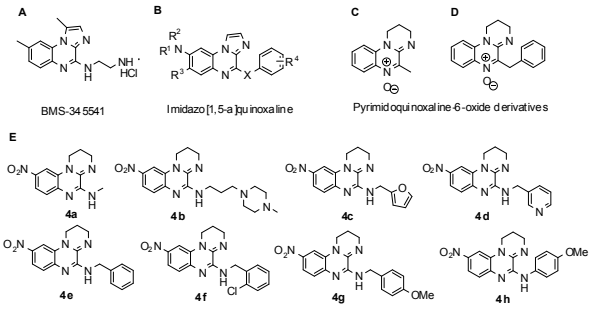


Fig. 1 A. Structure of BMS-345541. B. Imidazo[1,5-a]quinoxaline. C & D. Structures of Pyrimidoquinoxaline-6-oxide derivatives. E. Structures of scaffold **4a-4h** derivatives.

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).[8, 9, 10] Imidazo[1,5-a]quinoxaline compounds are also found to act as irreversible BTK inhibitors which is used for the treatment of rheumatoid arthritis.[11] The pyrroloquinoxaline (PQX) derivatives show their high selectivity

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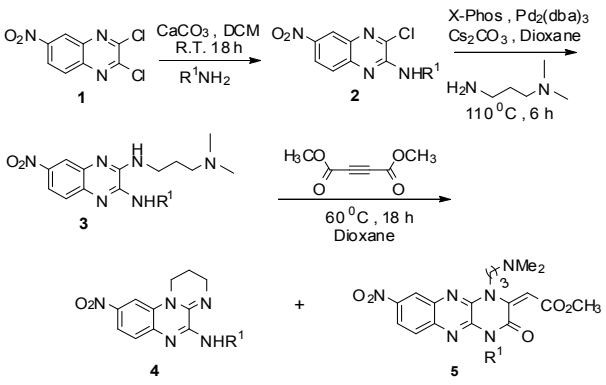
Electronic supplementary information (ESI) available: Spectral characterization and ^1H, and ^{13}C spectra. See DOI: 10.1039/x0xx00000x

towards 5-HT3R receptors and also show anti-proliferative activity in different cancerous cell lines. Pyrimidoquinoxaline 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 exocylic amine functionality with alkyl, alkyl amine, furan-2-ylmethyl, methyl pyridine, benzyl, *o*-chlorobenzyl, *p*-methoxybenzyl and aromatic group. We also get scaffold **5** as another product in the reaction of N2-(alkyl/aryl)-N3-(3-(dimethylamino)propyl)-6-nitroquinoxaline-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 fluorescence[14], where as our scaffold of 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine derivative 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

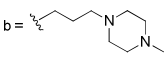
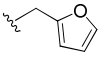
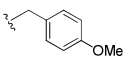
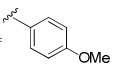


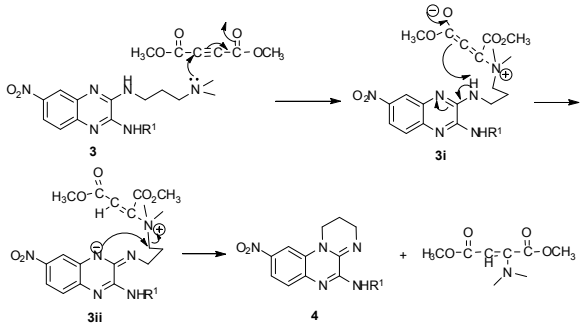
Scheme 2 Synthesis of quinoxaline derivatives **4** and **5**.

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-nitroquinoxaline-2,3(1H, 4H)-dione, followed by treatment with excess of $POCl_3$ and reflux at 110 ^{0}C to form 2,3-dichloro-6-nitroquinoxaline (**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 $CaCO_3$ at R.T. Subsequently, (**2a-2h**) under C-N coupling (Buchwald–Hartwig amination) using $Pd_2(dba)_3$ as a catalyst, XPhos as ligand and Cs_2CO_3 as base and amines afforded compounds **3a-3h**. Compound **3** was treated with dimethylbut-2-ynedioate at 60 ^{o}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 R^1 is methyl, the yield of N-methyl-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (**4a**) is 22% with 58% yield of (**5a**). When R^1 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]quinoxalin-5-amine (**4b**) is 45% without the formation of (**5b**). When R^1 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 R^1 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 1 Substrate scope for reaction of **3** with dimethylbut-2-ynedioate.

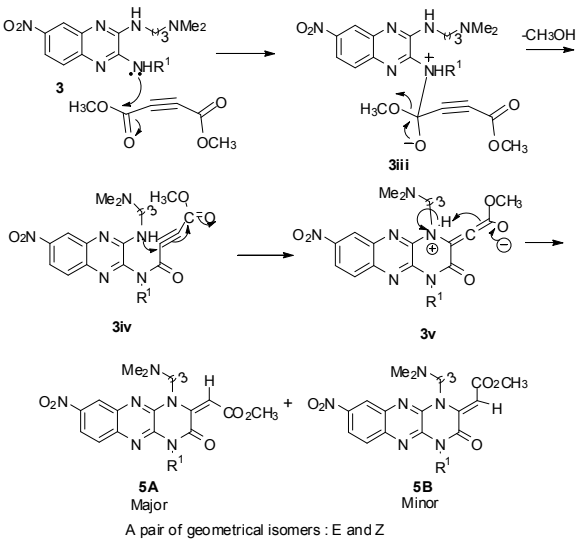
Entry.	R^1	%Yield (**4a – 4h**)	% Yield (**5a – 5h**)
1.	a = - Me	22	58
2.	b = 	45	-
3.	c = 	29	58
4.	d = 	25	52
5.	e = 	28	58
6.	f = 	31	-
7.	g = 	40	-
8.	h = 	60	-



Scheme 3 Plausible mechanism for the formation of 9-nitro-2, 3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (**4**) derivative.

The plausible mechanism for formation of 9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-aminederivative (**4**) is proposed in

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 sp^3 C-N bond cleavage. Similar sp^3 C-N bond cleavage of activated dimethyl amine is shown by Li-Wen Xu*et al*.[16]



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 and Fig. S1, 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-carbomethoxymethylene-1,2,3,4-tetrahydroquinoxaline compound.[17, 18]

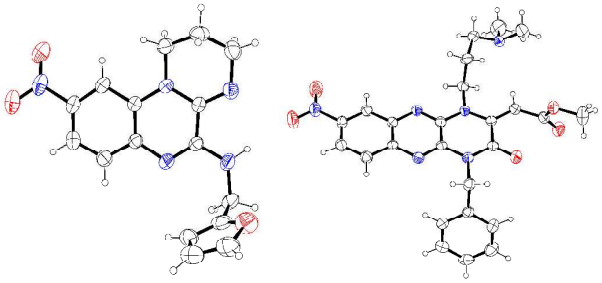


Fig. 2 The *ORTEP* diagrams showing the X-ray determined molecular structures of compound **4c** (left) and **5e** (right) with 30% probability level of thermal ellipsoids.

Table 2 Photophysical properties of compounds **4a-4h.**

Entry	Absorption λ_{abs}(nm)	ε(M^{-1} cm^{-1})	Emission λ_{em} (nm)	% Φ[b]	Brightness Φ X ε	Stokes Shift ($\Delta\bar{\nu}$ = $\bar{\nu}_{abs}$ - $\bar{\nu}_{ems}$) (cm^{-1})
4a	400	12462	537	1.15	143	6378
4b	403	12916	517	1.58	204	5472
4c	400	10722	524	1.45	155	5916
4d	402	16622	532	1.12	186	6079
4e	402	10684	534	1.65	176	6149
4f	400	13776	527	1.47	202	6025
4g	403	10712	540	1.90	203	6295
4h	417	10866	527	1.00	108	5005

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

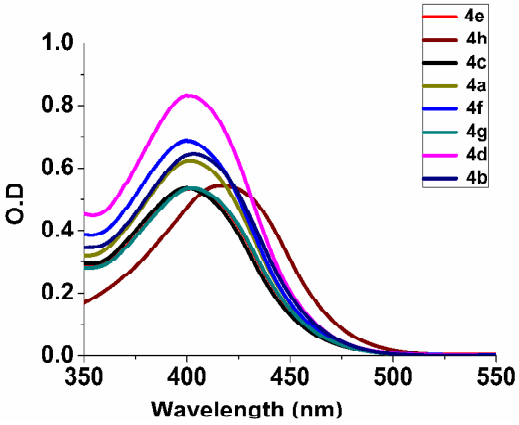


Fig. 3 Absorption spectra of **4a**-**4h** (50 μM) in EtOH.

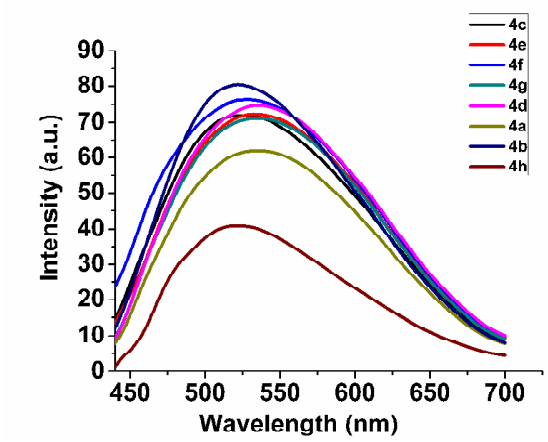


Fig. 4 Fluorescent spectra of **4a-4h** (50 µM) in EtOH (Slit: 10 nm, 10 nm) at their respective λ_{abs}.

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 λ_{max}s around (400-403) nm, whereas when scaffold **4** is conjugated with p-anisole group, the λ_{max} shift by almost 17 nm. To study the fluorescence properties of the compounds (**4a-4h**) were excited at their respective λ_{max}s as shown in Table 2, Fig. 4. The compounds are visibly emissive in the range of λ_{em}(517-540) nm. The compounds (**4a-4h**) have a large stokes shift (5005-6378 cm^{-1}) with low quantum yields (1 ~ 2)%.

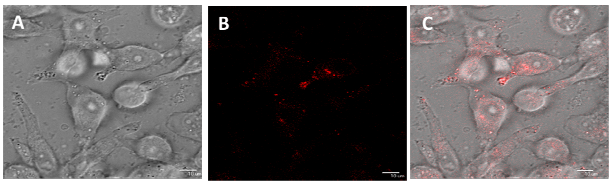


Fig. 5 Fluorescent image of live HepG2 cells after incubation with **4f**. (A) Brightfield, (B) 1h with **4f** (50 nM), (C) DIC merged. Excitation and emission wavelength for **4f** was at 405 nm and 542 nm respectively. Scale 10 µm.

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-ynedioate 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)-8-nitro-3-oxo-3,4-dihydropyrazino[2,3-b]quinoxalin-2(1H)-ylidene)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, alkyl 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 arylaldehydes can be oxidised quickly and selectively to the corresponding phenols with high yield by using FlOOH catalysts.[20] VitaminB2, Riboflavin, Molybdopterines, 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-aesar, 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^{0}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 tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are given from TMS (δ) 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 brs (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

and data reduction by BRUKER APEX II and SAINT; structure solution by SHELXS and SHELXT and structure refinement by SHELXL. Andor Spining Disk Live Cell Confocal Microscopy was done using Andor Ixon3 897 EMCCD, software IQ2.7.

Synthesis: General

General Procedure A: Formation of 6-nitro quinoxaline-2,3-(1H,4H)-dione[15]: 4-Nitrobenzene-1,2-diamine (32.64 mmol, 1 equivalent) and diethyl oxalate (48.96 mmol, 1.5 equivalent) were added slowly to 4M HCl (13 ml). After stirring for 15 min. at r.t., the reaction mixture was refluxed at 100 °C for 4h then cooled down to room temperature and kept for 20 min. The deposited grey colour solid was obtained by filtration and washed with water and dried under vacuum. Isolated yield - 85%.

General Procedure B: Formation of 2,3-dichloro-6-nitroquinoxaline[15]: 6-nitro quinoxaline-2,3(1H, 4H)-dione (24.15mmol, 1 equivalent) was slowly added to $POCl_3$ (15 ml) followed by N,N-dimethylformamide (DMF) (0.5 ml). Then the reaction mixture was refluxed at 110 °C for 3 h. After the consumption of the starting materials as indicated by TLC, the reaction mixture was cooled down to r.t., slowly poured into 300 ml of ice water and extracted with dichloromethane (3 times), washed with sodium bicarbonate, brine and combined organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The grey colour solid was obtained which was used without further purification. Isolated yield - 90%.

General Procedure C: Formation of monoaminated products via activated aromatic nucleophilic substitution (2a-2g): To a solution of 2,3-dichloro-6-nitroquinoxaline (**1**) (4.09 mmol, 1 equivalent) in dichloromethane (DCM) (20ml), $CaCO_3$ (11.45 mmol, 2.8 equivalent), dried benzyl amine (4.09 mmol, 1 equivalent) was added and the reaction mixture was stirred at r.t. for 18 h under nitrogen atmosphere. After the consumption of the starting materials as indicated by TLC, it was diluted with DCM (100ml), followed by extraction with water (50 ml), brine and combined organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The yellow colour solid was obtained which was purified over silica gel (100-200) in ethyl acetate-petroleum ether (2 %), afforded the yellowish solid mono-benzylated product (**2e**). Isolated yield - 50%.

3-chloro-N-methyl-6-nitroquinoxalin-2-amine (2a): The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoxaline and methylamine (40% w/w aq. solution). The crude product was purified by Column chromatography (SiO_2, eluting with 1 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (244 mg, 50 %); HRMS (EI^+) *m/z*: $[M]^+$: 238.02359, calcd: 238.02575; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.70 (d, *J* = 2.4 Hz, 1H), 8.38 (dd, J_1 = 2.7 Hz, J_2 = 9.3 Hz, 1H), 7.79 (d, *J* = 9.3 Hz, 1H), 6.00 (brs, 1H), 3.22 (d, *J* = 5.1 Hz, 3H); ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): δ (ppm): 28.4, 123.6, 124.0, 126.3, 133.5, 140.9, 142.7, 145.5, 150.2.

3-chloro-N-(3-(4-methylpiperazin-1-yl)propyl)-6-nitroquinoxalin-2-amine (2b): The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoxaline and 3-(4-methylpiperazin-1-yl)propan-1-amine. The crude product was purified by Column chromatography (SiO_2, eluting with 2 % methanol in chloroform) afforded the desired product as a yellowish solid (373 mg, 50 %); HRMS (EI^+) *m/z*: $[M]^+$: 364.14026, calcd: 364.14145; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.68 (d, *J* = 2.7 Hz, 1H), 8.37-8.31 (m, 2H), 7.69 (d, *J* = 9 Hz, 1H), 3.74-3.67 (m, 2H), 2.66-2.57 (m, 10H), 2.35 (s, 3H), 1.95-1.87 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 23.3, 43.2, 46.1, 53.5 (2C), 54.7 (2C), 58.6, 124.0, 124.3, 126.4, 134.2, 140.7, 143.4, 145.6, 149.6.

3-chloro-N-(furan-2-ylmethyl)-6-nitroquinoxalin-2-amine (2c): The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoxaline and furfurylamine. The crude product was purified by Column chromatography (SiO_2, 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, calcd: 304.03632; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.72 (d, *J* = 2.4Hz, 1H), 8.40 (dd, J_1 = 2.4 Hz, J_2 = 9.3 Hz, 1H), 7.81 (d, *J* = 9.3 Hz, 1H), 7.43 (s, 1H), 6.39 (s, 2H), 6.22 (brs, 1H), 4.84 (d, *J* = 5.4 Hz, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) : δ (ppm): 38.5, 108.5, 110.6, 124.1, 124.3, 126.9, 134.7, 140.2, 142.7, 144.2, 145.0, 148.8, 150.0.

3-chloro-6-nitro-N-(pyridin-3-ylmethyl)quinoxalin-2-amine (2d): The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoxaline and 3-aza benzylamine. The crude product was purified by Column chromatography (SiO_2, eluting with 30 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (310 mg, 48 %); HRMS (EI^+) *m/z*: $[M]^+$: 315.05057, calcd: 315.05230; ^{1}H-NMR (300 MHz, $CDCl_3$): 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); ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): 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-nitroquinoxalin-2-amine (2e): The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoxaline and benzylamine. The crude product was purified by Column chromatography (SiO_2, eluting with 2 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (322 mg, 50 %); HRMS (EI^+) *m/z*: $[M]^+$: 314.05716, calcd: 314.05705; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.67 (d, *J* = 2.7 Hz,1H), 8.35 (dd, J_1 = 2.7 Hz, J_2 = 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); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 45.7, 124.1, 124.3, 126.9, 128.0 (3C), 128.9 (2C), 134.8, 137.0, 140.3, 144.1, 145.2, 149.1.

3-chloro-N-(2-chlorobenzyl)-6-nitroquinoxalin-2-amine (2f): The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoxaline and 2-chloro benzylamine. The crude product was purified by Column chromatography (SiO_2, eluting with 2 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (350 mg, 49 %); HRMS (ESI^+) *m/z*: $[M+H]^+$: 349.0267, calcd: 349.0259; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.70 (d, *J* = 2.4 Hz, 1H), 8.38 (dd, J_1 = 2.4 Hz, J_2 = 9.3 Hz, 1H), 7.80 (d, *J* = 9.0 Hz, 1H), 7.55-7.28 (m, 5H), 6.44 (brs, 1H), 4.93 (d, *J* = 6.0 Hz, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 43.6, 124.1, 124.3, 126.9, 127.1, 129.4, 129.8, 130.6, 134.0, 134.5, 134.8, 140.3, 144.1, 145.0, 149.0.

3-chloro-N-(4-methoxybenzyl)-6-nitroquinoxalin-2-amine (2g): The title compound was prepared according to the general procedure C from 2,3-dichloro-6-nitroquinoxaline and 4-methoxy benzylamine. The crude product was purified by Column chromatography (SiO_2, eluting with 2 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (388 mg, 55%); HRMS (EI^+) *m/z*: $[M]^+$: 344.06754, calcd: 344.06762; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.71 (d, *J* = 2.7 Hz, 1H), 8.39 (dd, J_1 = 2.7 Hz, J_2 = 9.3 Hz, 1H), 7.79 (d, *J* = 9.3 Hz, 1H), 7.35 (d, *J* = 8.7 Hz, 2H), 6.92 (dd, J_1 = 2.1 Hz, J_2 = 6.9 Hz, 2H), 6.15 (d, *J* = 3.3 Hz, 1H), 4.76 (d, *J* = 5.4Hz, 2H), 3.82 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): 45.3, 55.3, 114.3

(2C), 124.1, 124.4, 126.8, 129.0, 129.5 (2C), 134.8, 140.3, 144.0, 145.2, 149.0, 159.4.

3-chloro-N-(4-methoxyphenyl)-6-nitroquinoxalin-2-amine (2h)[21]: The title compound was prepared by the reaction between 2,3-dichloro-6-nitroquinoxaline and 4-methoxyaniline in iso-propanol at r.t. for 3 h. The crude product was purified by Column chromatography (SiO_2, eluting with 2 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid; HRMS (ESI^+) *m/z*: $[M+Na]^+$: 353.0430, calcd: 353.0417; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.69 (d, *J* = 2.7 Hz, 1H), 8.38 (dd, J_1 = 2.7 Hz, J_2 = 9.0 Hz, 1H), 7.81-7.70 (m, 3H), 7.67 (brs, 1H), 7.00-6.95 (m, 2H), 3.85 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): 55.5, 114.3 (2C), 122.6 (2C), 124.2, 124.3, 127.2, 130.2, 135.2, 140.4, 144.5 (2C), 146.7, 157.1.

General Procedure D: Formation of di-aminated products by using Buchwald-Hartwig reaction (3a-3h): Cesium carbonate (3.82 mmol, 1.5 equivalent), XPhos (0.254 mmol, 0.1 equivalent), 3-dimethylaminopropyl-amine (3.82 mmol, 1.5 equivalent) were added to a solution of N-benzyl-3-chloro-6-nitroquinoxaline-2-amine (**2e**) (2.54mmol, 1 equivalent) in dry dioxane (20ml) and $Pd_2(dba)_3$ (0.127 mmol, 0.05 equivalent) were added at r.t. followed by purging with nitrogen. The reaction mixture was heated at 110°C under nitrogen atmosphere for 6 h. After the consumption of the starting materials as indicated by TLC, reaction mixture was cooled down to r.t. and diluted it with MeOH, then filtered through celite and concentrated to give dark brown oil, which was further purified over silica gel (100-200) in methanol-chloroform (2 %), afford the yellowish orange solid (**3e)**. Isolated yield - 85%.

N^3-(3-(dimethylamino)propyl)-N^2-methyl-6-nitroquinoxaline-2,3-diamine (3a): The title compound was prepared according to the general procedure D from 3-chloro-N-methyl-6-nitroquinoxalin-2-amine and N,N-dimethyl-propan-1,3-diamine. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloform) afforded the desired product as a orange solid (208 mg, 65%); HRMS (EI^+) *m/z*: $[M]^+$: 304.16537, calcd: 304.16477; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.43 (d, *J* = 2.7 Hz, 1H), 8.06 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H) , 7.59 (d, *J* = 9.0 Hz, 1H), 5.72 (brs, 1H), 3.68 (t, *J* = 5.7 Hz, 2H), 3.17 (d, *J* = 4.5 Hz, 3H), 2.74 (t, *J* = 6.0 Hz, 2H), 2.48 (s, 6H), 2.03-1.95 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 24.4, 28.4, 41.1, 44.8 (2C), 58.3, 118.4, 121.0, 125.4, 136.1, 142.1, 143.5, 145.1, 146.5.

N^3-(3-(dimethylamino)propyl)-N^2-(3-(4-methylpiperazin-1-yl)propyl)-6-nitroquinoxaline-2,3-diamine (3b): The title compound was prepared according to the general procedure D from 3-chloro-N-(3-(4-methylpiperazin-1-yl)propyl)-6-nitroquinoxalin-2-amine and N,N-dimethyl-propan-1,3-diamine. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloform) afforded the desired product as a orange solid (216 mg, 73 %); HRMS (EI^+) *m/z*: $[M]^+$: 430.28134, calcd: 430.28047; ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm): 8.44 (s, 1H), 8.06 (dd, J_1 = 2.4 Hz, J_2 = 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); ^{13}C-NMR (75 MHz, $CDCl_3$): 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, 125.1, 135.9, 142.2, 143.2, 145.0, 145.8.

N^3-(3-(dimethylamino)propyl)-N^2-(furan-2-ylmethyl)-6-nitroquinoxaline-2,3-diamine (3c): The title compound was prepared according to the general procedure D from 3-chloro-N-(furan-2-ylmethyl)-6-nitroquinoxalin-2-amine and N,N-dimethyl-propan-1,3-diamine. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloform) afforded the desired product as a reddish solid (274 mg, 90 %); HRMS (EI^+) *m/z*: $[M]^+$: 370.17462, calcd: 370.17534; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.45 (d, *J* = 2.4 Hz, 1H), 8.33 (brs, 1H), 8.07 (dd, J_1 = 2.7 Hz, J_2 = 9.0 Hz, 1H), 7.61 (d, *J* = 9 Hz, 1H), 7.42 (s, 1H), 6.39 (d, *J* = 1.2 Hz, 1H), 5.49 (brs, 1H), 4.77 (d, *J* = 4.5 Hz, 2H), 3.68 (t, *J* = 5.7 Hz, 2H), 2.68 (t, *J* = 5.4 Hz, 2H), 2.31 (s, 6H), 2.08-1.89 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 23.8, 38.5, 42.1, 44.7 (2C), 59.2, 108.3, 110.7, 118.2, 121.0, 125.7, 136.6, 141.5, 142.3, 143.9, 145.0, 145.1, 151.2.

N^3-(3-(dimethylamino)propyl)-6-nitro-N^2-(pyridin-3-ylmethyl)quinoxaline-2,3-diamine (3d): The title compound was prepared according to the general procedure D from 3-chloro-6-nitro-N-(pyridin-3-ylmethyl)quinoxalin-2-amine and N,N-dimethyl-propan-1,3-diamine. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloform) afforded the desired product as a reddish solid (258 mg, 85%); HRMS (FAB^+) *m/z*: $[M+H]^+$: 382.20056, calcd: 382.1991; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.70 (s, 1H), 8.57 (d, *J* = 3.9 Hz, 1H), 8.48-8.45 (m, 1H), 8.12-8.05 (m, 2H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 1H), 7.32 (dd, J_1 = 4.8 Hz, J_2 = 7.8 Hz, 1H), 5.65 (brs, 1H), 4.81-4.76 (m, 2H), 3.67 (t, *J* = 5.7 Hz, 2H), 2.63 (t, *J* = 5.7 Hz, 2H), 2.30 (s, 6H), 1.95-1.89 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 25.1, 40.8, 42.9, 44.9 (2C), 58.0, 118.2, 120.9, 123.7, 125.5, 134.4, 136.3, 136.8, 141.5, 143.6, 144.8, 145.1, 148.5, 149.5.

N^2-benzyl-N^3-(3-(dimethylamino)propyl)-6-nitroquinoxaline-2,3-diamine (3e): The title compound was prepared according to the general procedure D from N-benzyl-3-chloro-6-nitroquinoxalin-2-amine and N,N-dimethyl-propan-1,3-diamine. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloform) afforded the desired product as an orange solid (196 mg, 65 %); HRMS (EI^+) *m/z*: $[M]^+$: 380.1964, calcd: 380.1961; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.44 (d, *J* = 2.4 Hz, 1H), 8.07 (dd, J_1 = 2.4 Hz, J_2 = 8.7 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), 2.65(t, *J* = 5.1 Hz, 2H), 2.17 (s, 6H), 1.95-1.89 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 23.7, 42.3, 44.7 (2C), 46.1, 59.4, 118.3, 121.0, 125.6, 127.9, 128.5, 128.8, 136.6, 138.1, 141.7, 143.9, 145.0, 145.3.

N^2-(2-chlorobenzyl)-N^3-(3-(dimethylamino)propyl)-6-nitroquinoxaline-2,3-diamine (3f): The title compound was prepared according to the general procedure D from 3-chloro-N-(2-chlorobenzyl)-6-nitroquinoxalin-2-amine and N,N-dimethyl-propan-1,3-diamine. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloform) afforded the desired product as a reddish solid (261 mg, 88 %); HRMS (EI^+) *m/z*: $[M]^+$: 414.15720, calcd: 414.15710; ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm): 8.45 (d, *J* = 2.4 Hz, 1H), 8.06 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H), 7.61 (d, *J* = 9 Hz, 1H), 7.58-7.27 (m, 5H), 5.50 (brs, 1H), 4.88 (dd, J_1 = 5.4 Hz, J_2 = 18 Hz, 2H), 3.67 (t, *J* = 5.4 Hz, 2H), 2.62 (d, *J* = 4.8 Hz, 2H), 2.22 (s, *6H*), 1.92-1.89 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 23.9, 42.2, 43.6, 44.9 (2C), 59.3, 118.3, 121.0, 125.7, 127.1, 129.3, 129.6, 131.2, 134.1, 135.6, 136.6, 141.6, 143.8, 145.0, 145.3.

N^3-(3-(dimethylamino)propyl)-N^2-(4-methoxybenzyl)-6-nitroquinoxaline-2,3-diamine (3g): The title compound was prepared according to the general procedure D from 3-chloro-N-(4-methoxybenzyl)-6-nitroquinoxalin-2-amine and N,N-dimethyl-propan-1,3-diamine. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloform) afforded the desired product as a reddish solid (271 mg, 91 %); HRMS (EI^+) *m/z*: $[M]^+$: 410.20544, calcd: 410.2066; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.44 (d, *J* = 2.4 Hz, 1H), 8.06 (dd, J_1 = 2.4 Hz, J_2

= 9 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 5.49 (brs, 1H), 4.69 (d, *J* = 4.5 Hz, 2H), 3.81 (s, 3H), 3.66 (t, *J* = 5.7 Hz, 2H), 2.65 (t, *J* = 5.4 Hz, 2H), 2.20 (s, 6H), 1.93-1.89 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$, 1 drop CD_3OD): δ (ppm): 25.0, 38.9, 44.0 (2C), 44.7, 55.1, 56.6, 113.7 (2C), 118.4, 120.5, 125.1, 129.7 (2C), 130.2, 135.7, 141.9, 143.2, 144.7, 145.1, 158.8.

N^3-(3-(dimethylamino)propyl)-N^2-(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-nitroquinoxalin-2-amine and N,N-dimethyl-propan-1,3-diamine. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloform) afforded the desired product as a reddish solid (216 mg, 60 %); HRMS (ESI$^+$) *m/z*: $[M+H]^+$: 397.1980, calcd: 397.1988; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.43 (brs, 1H), 8.06 (dd, J_1 = 2.7 Hz, J_2 = 9.0 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.61 (d, *J* = 9.0 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 3.83 (s, 3H), 3.73 (t, *J* = 5.7 Hz, 2H), 2.81 (d, *J* = 5.7 Hz, 2H), 2.53 (s, 6H), 2.07-2.02 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 24.0, 41.4, 44.7, 55.5 (2C), 58.4, 114.2 (2C), 118.5 , 120.8, 122.4 (2C), 126.1, 132.0, 136.6, 141.0, 143.0, 144.2, 145.1, 156.0.

General Procedure E: Formation of dihydropyrimido quinoxaline (4a-4h) and dihydropyrazino quinoxaline derivatives (5a, 5c-5e): Dimethyl acetylenedicarboxylate (0.789 mmol , 1 equivalent) was added to a solution of N^2-benzyl-N^3-(3-(dimethylamino)propyl)-6-nitroquinoxaline-2,3-diamine (0.789 mmol, 1 equivalent) in dry dioxane (7 ml). The reaction mixture was heated at 60^0C under nitrogen atmosphere for 18 h. After the consumption of the starting materials as indicated by TLC, reaction mixture was cooled down to r.t. and concentrated to obtain yellowish-orange solid. The products were purified by column chromatography by using 100-200 silica gel. The average isolated yield of Dihydropyrimido Quinoxaline and Dihydropyrazino Quinoxaline were 30 % and 55 % respectively.

N-methyl-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4a): The title compound was prepared according to the general procedure E from N^3-(3-(dimethylamino)propyl)-N^2-methyl-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 dark yellowish solid (19 mg, 22 %); HRMS (ESI$^+$) *m/z*: $[M+Na]^+$: 282.0960, calcd: 282.0967; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 7.96 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H), 7.86 (d, *J* = 2.1 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 7.06 (brs, 1H), 3.93 (t, *J* = 6 Hz, 2H), 3.63 (t, *J* = 5.4 Hz, 2H), 3.11 (d, *J* = 4.8 Hz, 3H), 2.13-2.05 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 19.7, 27.6, 42.8, 43.1, 105.8, 118.0, 124.9, 130.1, 138.0, 139.7, 142.5, 152.9.

N-(3-(4-methylpiperazin-1-yl)propyl)-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4b): The title compound was prepared according to the general procedure E from N^3-(3-(dimethylamino)propyl)-N^2-(3-(4-methylpiperazin-1-yl)propyl)-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 2 % methanol in chloroform) afforded the desired product as a reddish solid (40 mg, 45%); HRMS (ESI$^+$) *m/z*: $[M+H]^+$: 386.2323, calcd: 386.2304; ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm): 8.00 (brs, 1H), 7.95 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H), 7.85 (d, J = 2.4 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 3.94 (t, J = 6.0 Hz, 2H), 3.66-3.60 (m, 5H), 2.62 (brs, 5H), 2.56 (t, J = 6.6 Hz, 3H), 2.39 (s, 3H), 2.12-2.06 (m, 3H), 1.88-1.83 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 19.7, 25.2, 40.6, 42.7, 43.1, 45.9, 53.0 (2C), 54.7 (2C), 57.2, 105.7, 118.0, 124.7, 130.1, 138.0, 140.0, 142.3, 151.5.

N-(furan-2-ylmethyl)-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4c): The title compound was prepared according to the general procedure E from N^3-(3-(dimethylamino)propyl)-N^2-(furan-2-ylmethyl)-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 dark yellowish solid (25 mg, 29 %); HRMS (ESI$^+$) *m/z*: $[M+Na]^+$: 348.1091, calcd: 348.1073; ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm): 7.97 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H), 7.87 (d, *J* = 2.4 Hz, 1H), 7.43 (d, *J* = 9.0 Hz, 1H), 7.40-7.39 (m, 1H), 6.36-6.33 (m, 2H), 4.75 (d, *J* = 4.2 Hz, 2H), 3.94 (t, *J* = 6 Hz, 2H), 3.61 (t, *J* = 6.0 Hz, 2H), 2.12-2.08 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 19.6, 37.7, 42.8, 43.0, 105.8, 107.8, 110.4, 118.0, 125.3, 130.3, 137.9, 139.3, 142.3, 142.9, 150.8, 151.0.

9-nitro-N-(pyridin-3-ylmethyl)-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4d): The title compound was prepared according to the general procedure E from N^3-(3-(dimethylamino)propyl)-6-nitro-N^2-(pyridin-3-ylmethyl)quinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 8 % 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, calcd: 359.1232; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.66 (s, 1H), 8.54 (d, *J* = 4.8 Hz,1H), 7.96 (dd, J_1 = 2.4 Hz, J_2 = 8.7 Hz,1H), 7.87 (d, *J* = 2.4 Hz,1H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 2H), 7.30 (brs, 1H), 4.76 (s, 2H), 3.94 (t, *J* = 6.3 Hz, 2H), 3.60 (t, *J* = 5.7 Hz, 2H), 2.13-2.05 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (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-benzyl-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4e): The title compound was prepared according to the general procedure E from N^2-benzyl-N^3-(3-(dimethylamino)propyl)-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 5 % ethyl acetate in pet ether) afforded the desired product as a yellowish solid (24.7 mg, 28 %); HRMS (ESI$^+$) *m/z*: $[M+Na]^+$: 358.1276, calcd: 358.1280; ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm): 7.97 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz, 1H), 7.87 (d, *J* = 2.4 Hz, 1H), 7.42-7.29 (m, 6H), 4.76 (d, *J* = 5.4 Hz, 2H), 3.95 (t, *J* = 6.0 Hz, 2H), 3.61 (t, *J* = 6.0 Hz, 2H) , 2.12-2.08 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 19.7, 42.8, 43.0, 44.8, 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.

N-(2-chlorobenzyl)-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4f): The title compound was prepared according to the general procedure E from N^2-(2-chlorobenzyl)-N^3-(3-(dimethylamino)propyl)-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*: $[M+Na]^+$: 392.0886, calcd: 392.0890; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 7.95 (dd, J_1 = 2.4 Hz, J_2 = 8.7 Hz, 1H), 7.85 (d, *J* = 2.4 Hz, 1H), 7.48-7.38 (m, 4H), 7.25-7.23 (m, 1H), 4.85 (d, *J* = 5.4 Hz, 2H), 3.93 (t, *J* = 6.3 Hz, 2H), 3.61 (t, *J* = 5.7 Hz, 2H), 2.12-2.05 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 19.6, 42.4, 42.8, 43.0, 105.8, 118.0, 125.3, 126.9, 128.9, 129.5, 130.0, 130.3, 133.7, 135.5, 137.9, 139.4, 142.8, 151.0.

N-(4-methoxybenzyl)-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4g): The title compound was prepared according to the general procedure E from N^3-(3-(dimethylamino)propyl)-N^2-(3-(4-methoxyphenyl)propyl)-6-

nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 14 % ethyl acetate in pet ether) afforded the desired product as a yellowish-orange solid (35 mg, 40 %); HRMS (ESI^+) *m/z*: $[M+H]^+$: 366.1566, calcd: 366.1566; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 7.96 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H), 7.86 (d, *J* = 2.1Hz, 1H), 7.41(d, *J* = 8.7 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 4.67 (d, *J* = 5.4 Hz, 2H), 3.93 (t, *J* = 6 Hz, 2H), 3.80 (s, *3H*), 3.58 (t, *J* = 5.4 Hz, 2H), 2.12-2.04 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 19.7, 42.8, 43.1, 44.3, 55.3, 105.8, 114.0 (2C), 118.0, 125.1, 129.4 (2C), 130.0, 130.3, 138.0, 139.7, 142.7, 151.0, 159.0.

N-(4-methoxyphenyl)-9-nitro-2,3-dihydro-1H-pyrimido[1,2-a]quinoxalin-5-amine (4h): The title compound was prepared according to the general procedure E from N3-(3-(dimethylamino)propyl)-N2-(4-methoxyphenyl)-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 14 % ethyl acetate in pet ether) afforded the desired product as a yellowish-orange solid (26 mg, 60 %); HRMS (ESI^+) *m/z*: $[M+H]^+$: 352.1408, calcd: 352.1410; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 9.07 (brs, 1H), 7.97 (dd, J_1 = 2.4 Hz, J_2 = 8.7 Hz, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.83-7.80 (m, 2H), 7.47 (d, *J* = 8.7 Hz, 1H), 6.95-6.92 (m, 2H), 3.96 (t, J = 6.0 Hz, *2H*), 3.83 (s, 3H), 3.67 (t, J = 5.7 Hz, *2H*), 2.16-2.09 (m, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm): 19.2, 42.5, 42.6, 55.1, 105.6, 113.7, 118.0, 121.3, 125.6, 129.9, 131.2, 137.8, 138.5, 142.9, 147.3, 155.7.

(E)-methyl 2-(1-(3-(dimethylamino)propyl)-8-nitro-3-oxo-4-(pyridin-3-ylmethyl)-3,4-dihydropyrazino[2,3-b]quinoxalin-2(1H)-ylidene)acetate (5a): The title compound was prepared according to the general procedure E from N^3-(3-(dimethylamino)propyl)-N^2-methyl-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloroform) afforded the desired product as a yellowish solid (88.5 mg, 58 %); HRMS (ESI^+) *m/z*: $[M+Na]^+$: 437.1533, calcd: 437.1549; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.64 (d, *J* = 2.1 Hz, 1H), 8.27 (dd, J_1 = 2.4 Hz, J_2 = 9 Hz, 1H), 7.91 (d, *J* = 9Hz, 1H), 6.06 (s, 1H), 4.24 (t, *J* = 7.2 Hz, 2H), 3.90 (s, 3H), 3.69 (s, 3H), 2.44 (t, *J* = 6.3 *Hz*, 2H), 2.30 (s, 6H), 2.00-1.905 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 23.1, 28.3, 42.8, 45.4 (2C), 52.6, 56.5, 107.0, 120.5, 122.5, 128.2, 133.0, 137.5, 138.63, 139.3, 140.3, 146.7, 157.2, 167.8.

(E)-methyl 2-(1-(3-(dimethylamino)propyl)-4-(furan-2-ylmethyl)-8-nitro-3-oxo-3,4-dihydropyrazino[2,3-b]quinoxalin-2(1H)-ylidene)acetate (5c): The title compound was prepared according to the general procedure E from N^3-(3-(dimethylamino)propyl)-N^2-(furan-2-ylmethyl)-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 2 % methanol in chloroform) afforded the desired product as a yellowish solid (75 mg, 58 %); HRMS (ESI^+) *m/z*: $[M+H]^+$: 481.1839, calcd: 481.1836; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.64 (d, *J* = 2.4 Hz, 1H), 8.28 (dd, J_1 = 2.4 Hz, J_2 = 9 Hz, 1H), 7.95 (d, *J* = 9 Hz, 1H), 7.31 (s, 1H), 6.48-6.46 (m, 1H), 6.30 (d, *J* = 1.8 Hz, 1H) , 6.05 (s, 1H), 5.56 (s, 2H), 4.23 (t, *J* = 6.9 Hz, 2H), 3.90 (s, 3H), 2.43 (t, *J* = 6.3 Hz, 2H), 2.29 (s, 6H), 1.99-1.83 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 23.0, 37.3, 42.9, 45.4 (2C), 52.6, 56.4, 107.2, 110.2, 110.4, 120.5, 122.5, 128.2, 133.1, 137.7, 138.4, 138.7, 140.1, 142.3, 146.8, 148.7, 156.5, 167.6.

(E)-methyl 2-(1-(3-(dimethylamino)propyl)-8-nitro-3-oxo-4-(pyridin-3-ylmethyl)-3,4-dihydropyrazino[2,3-b]quinoxalin-2(1H)-ylidene)acetate (5d): The title compound was prepared according to the general procedure D from N^3-(3-(dimethylamino)propyl)-6-nitro-N^2-(pyridin-3-ylmethyl)quinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 3 % methanol in chloroform) afforded the desired product as a dark yellowish solid (67 mg, 52 %); HRMS (ESI^+) *m/z*: $[M+Na]^+$: 514.1843, calcd: 514.1815; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.90 (s, 1H), 8.63 (d, *J* = 1.8 Hz, 1H), 8.51 (d, *J* = 3.6 Hz, 1H), 8.28 (dd, J_1 = 2.4 Hz, J_2 = 9.3 Hz, 1H), 7.96-7.89 (m, 2H), 7.27-7.23 (m, 1H), 6.10 (s, 1H), 5.54 (s, 2H), 4.23 (t, *J* = 6.6 Hz, 2H), 3.90 (s, 3H), 2.43 (t, *J* = 6.3 Hz, 2H), 2.29 (s, 6H), 1.94 (t, *J* = 6.9 Hz, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm): 23.0, 42.1, 43.0, 45.4 (2C), 52.6, 56.4, 107.6, 120.7, 122.6, 123.5, 128.2, 131.4, 133.0, 137.3, 137.8, 138.7, 139.9, 146.9, 149.3, 151.1, 157.1, 167.6.

(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^2-benzyl-N^3-(3-(dimethylamino)propyl)-6-nitroquinoxaline-2,3-diamine and dimethyl acetylenedicarboxylate. The crude product was purified by Column chromatography (SiO_2, eluting with 2 % methanol in chloroform) afforded the desired product as a yellowish solid (75 mg, 58 %); HRMS (ESI^+) *m/z*: $[M+H]^+$: 491.2024, calcd: 491.2043; ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm): 8.62 (d, *J* = 2.1 Hz, 1H), 8.27 (dd, J_1 = 2.4 Hz, J_2 = 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), 3.90 (s, 3H), 2.42 (t, *J* = 6.6 Hz, 2H), 2.29 (s, 6H), 1.98-1.91 (m, 2H); ^{13}C-NMR (75MHz, $CDCl_3$): δ (ppm): 23.0, 42.9, 44.4, 45.4 (2C), 52.6, 56.5, 107.1, 120.5, 122.5, 127.9, 128.2, 128.5, 129.5, 129.6, 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 Spectrochem, 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[19]:

$$\varphi_s = (I_s/I_{ref}).(O.D._{ref}/O.D.s).(n_s/n_{ref})^2.\varphi_{ref}$$

Here Φ, I, O.D. and *n* stand for quantum yield, integrated emission intensity, optical density at λex and refractive index (n_{EtOH} = 1.36). Sample and reference are denoted by *s* and *ref*, respectively.

Andor spining disk 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% CO_2.Cells were seeded into confocal dish (22mm) ($5X10^6$ cells) cultured overnight at 37°C and 5% CO_2. **4f** was dissolved in DMSO and added to the media at an indicated final concentration of 50 nM. Then the cells were incubated for 1h at 37°C in 5% CO_2 and imaged using Andor Spining Disk Live Cell Confocal Microscopy and visualized for another 1h. Excitation and emission wavelength for **4f** was 405 and 542 nm respectively.

Acknowledgements

S.D gratefully acknowledges support from CSIR-IICB (Start-up grant) and CSIR-New Delhi (Network Project BIO-DISCOVERY,

Grant No- BSC-0120). A.K and D.P thank CSIR and T.M thanks UGC, India for a fellowship and P.R.M. for a CSIR emeritus scientistship. SD thanks Dr. Partha Chakrabarti and Dr. Nakul C Maiti for providing assistance with the fluorimeter. S.D thanks Dr. Ramalingam Natarajan for providing helpful discussion with the manuscript.

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