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Original article

Discovery of Tröger's base analogues as selective inhibitors against human breast cancer cell line: Design, synthesis and cytotoxic evaluation

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ABSTRACT

A library of structurally diverse Tröger's base analogues has been constructed *via* unusual amination of methylene bridge employing Vilsmeier–Haack conditions as well as by the incorporation of five and six membered heterocycles on the aromatic core of Tröger's base framework. The constructed structurally diverse frameworks were evaluated for their cytotoxic activities against a panel of three human cancer lines A549 (lung adenocarcinoma), MDAMB-231 (breast) and SK-N-SH (neuroblastoma). From the activity profile obtained, a redesign of Tröger's base analogues led to the construction of more potent molecular entities. The study led to development of a series of compounds with MDAMB-231 cell line specific cytotoxicity. Of the 30 compounds synthesized and evaluated, 7 compounds were found to possess cytotoxicity that is equivalent or better than standard drug doxorubicin against MDAMB-231 cell line while only one compound was found to be active against SK-N-SH cell line.

1. Introduction

Tröger's base, 2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,8]diazocine (Fig. 1), was first synthesized in 1887 by *Julius* Tröger by the reaction of *p*-toluidine with methylal [CH_2-(OCH_3)$_2$] in acidic conditions [1]. Its correct structure was determined by Spielman in 1935 and assigned as racemic [2]. Later a number of aromatic, heterocyclic Tröger's bases with various functional groups were synthesized by using different methylene donors such as paraformaldehyde, hexamethylenetetramine (HMTA), dimethoxymethane, or DMSO and by varying acidic media such as trifluoroacetic acid (TFA), HCl, or acetic acid [3]. The basic structure of Tröger's base features a central bicyclic aliphatic diazocine unit fused with two arene rings, oriented approximately at right angles to each other (V shaped) [4]. Tröger's base also has C_2-symmetry and chirality that is provided by the two stereogenic bridgehead nitrogen atoms (inability to invert) of the diazocine ring. The unique geometry of Tröger's base has been exploited for many applications in the field of supramolecular chemistry as molecular receptors [5], in development of molecular torsion balances [6], in asymmetric catalysis [7], and as chiral solvating agent [8].

Tröger's base structural features were utilized in the design and development of DNA minor grove binders (Fig. 2). Tröger's bases derived from heterocyclic aromatic amines like acridine [9], phenanthroline and proflavine [10] have shown good affinity for DNA. Among these, the geometry of acridine derived Tröger's base gives rise to a helix shape which can be similar or opposite to the helicity of DNA, resulting in enantioselective binding to calf-thymus B-DNA [9a] and hence serves as a molecular probe. Racemic Tröger's base containing phenanthroline units are known to cleave DNA in the presence of Cu^{2+} ions [11]. Proflavine type Tröger's base has also been used for enantiospecific binding to calf-thymus B-DNA sequences containing both A.T and G.C base pairs. Veale et al. reported the synthesis and fluorescence imaging studies of 1,8-naphthalimide analogues of Tröger's base and illustrated their rapid uptake by cancer cells [12]. However, limited studies have been reported on Tröger's base derivatives as anticancer agents [13].

Earlier, our group has reported the Vilsmeier–Haack reaction of Tröger's base analogues resulting in an unusual functionalization of methylene bridge of the diazocine unit [14]. In our continued interest in developing small molecules as cytotoxic agents [15], we have expanded our chemistry on Tröger's base scaffold in designing

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0223-5234/

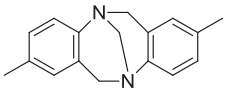


Fig. 1. Tröger's base.

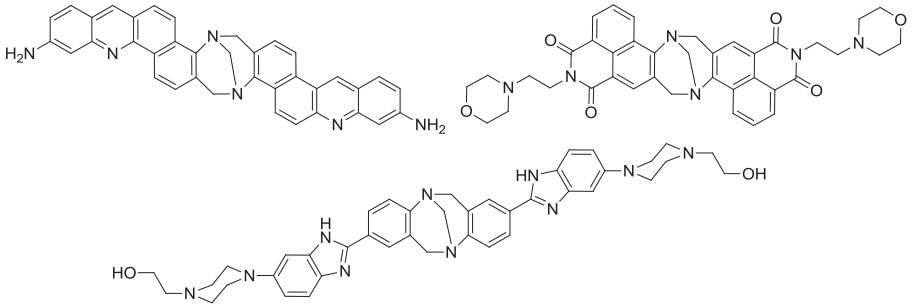


Fig. 2. Examples of biologically active Tröger's base analogues.

and synthesizing novel derivatives. In this context, we have incorporated small heterocyclic groups like morpholine, piperidine, imidazole, triazole, and tetrazole which are effective pharmacophores into methylene bridge or as substitutions on aromatic ring of Tröger's base and evaluated them for anticancer activity. In first phase, 18 compounds were synthesized. Based on their cytotoxic activity, a set of 12 new compounds have been synthesized that displayed improved efficacy against MDAMB-231 cell line.

2. Results and discussion

2.1. Chemistry

A series of methylene bridge substituted Tröger's base analogues with *N,N*-dimethylamino, *N*-alkyl-*N*-arylamino and *N*-cycloalkylamino functionalities have been synthesized. A general method for the synthesis of methylene bridge substituted Tröger's base derivatives that employs Vilsmeier–Haack conditions has been adopted. Accordingly, a series of Tröger's base analogues were prepared by the reaction of corresponding formamides with $POCl_3$ (Scheme 1). Target compounds were obtained in good yields.

The crucial precursor **9**, required for generation of various benzene substitutions has been synthesized from commercially available 4-nitrophenol in two steps i.e., by first reacting 4-nitrophenol with 1,2-dibromoethane, followed by reduction of $-NO_2$ group. 4-(2-Bromoethoxy)aniline thus obtained was converted into Tröger's base **9**, using classic Tröger's base formation conditions i.e., 37% formaldehyde solution in presence of HCl in EtOH. A series of small heterocyclic ring substituted Tröger's base analogues **11(a–i)** were prepared by the condensation of **9** with corresponding heteroaryl rings in presence of K_2CO_3 and acetonitrile solvent (Scheme 2). The products were obtained in 74–89% yield.

With a view to increase the diversity of the library, functionalization of the methylene bridge of newly designed analogues was taken up. The methylene bridge substitution of **9** was carried out under classic Vilsmeier–Haack conditions to afford **12** in 65% yields. The targeted compounds **13(a–c)** (Scheme 3) were obtained in good yield by the reaction of Tröger's base **12** with corresponding heterocycles in presence of K_2CO_3 and acetonitrile (Scheme 3). The new compounds thus synthesized are completely characterized by their spectral data before proceeding for biological evaluation.

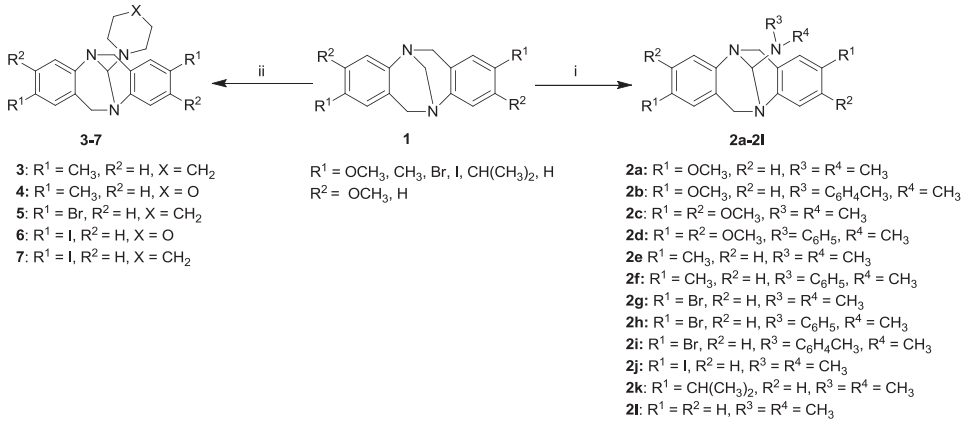
2.2. Pharmacology

In vitro cytotoxicity of all the synthesized Tröger's base derivatives was evaluated by MTT-micro cultured tetrazolium assay against a panel of three human cancer lines A549 (lung adenocarcinoma), MDAMB-231 (breast) and SK-N-SH (neuroblastoma). The IC_{50} values i.e., the effective concentration at which 50% growth of the cancer cells was inhibited, were calculated to evaluate the anticancer activities. All the experiments were carried out in triplicates. Activity results are shown in Tables 1 and 2.

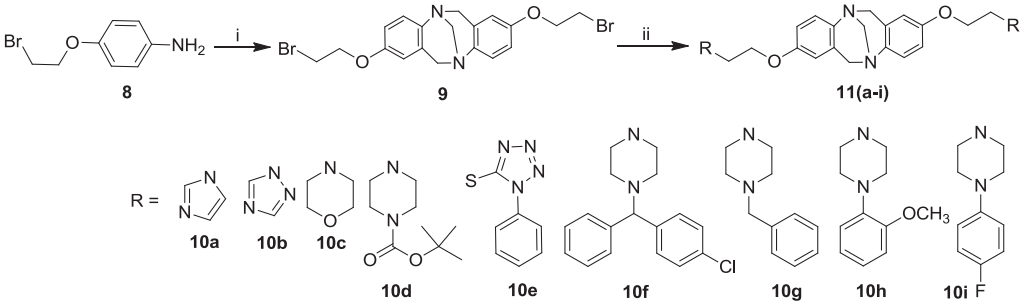
As shown in Table 1, the first phase of compounds showed moderate to significant cytotoxic activities on MDAMB-231 cell line. The cytotoxicity of these compounds was found to be dependent on the substituents present on the aromatic ring as well as on the methylene bridge of Tröger's base. Among the Tröger's base analogues with simple benzene ring substitutions it was found that methylene bridge substitutions such as *N,N*-dimethylamino, *N*-alkyl-*N*-arylamino and *N*-cycloalkylamino groups showed no enhancement of activity on MDAMB-231 cell line. However, in *N*-alkyl-*N*-arylamino substitutions, the activity was enhanced. Compound **2b** with *N*-methyl-*N*-*p*-tolyl amino group and compound **2h** with *N*-methyl-*N*-phenylamino group on methylene bridge were most potent with IC_{50} values of 10.72 ± 0.54 μM and 14.34 ± 0.19 μM, respectively. The other compounds in this series **2k**, **2e**, **2f**, **2c**, **3**, and **2d** have shown moderate inhibition with IC_{50} values 57.50 μM, 61.79 μM, 72.33 μM, 78.14 μM, 82.35 μM, and 86.76 μM respectively. No clear preference for benzene ring substitutions could be established but alkyl substitutions seem to have no benefit.

However, none of the compounds in this phase were active against A549 cell line. Among all the compounds, only compound **3** with piperidine as the bridge substituent and methyl groups on aromatic rings of Tröger's base has shown good inhibition against SK-N-SH cell line with an IC_{50} of 12.21 ± 0.13 μM. However, introduction of morpholine on bridge or changing the substituents on benzene ring did not improve the inhibition on this cell line.

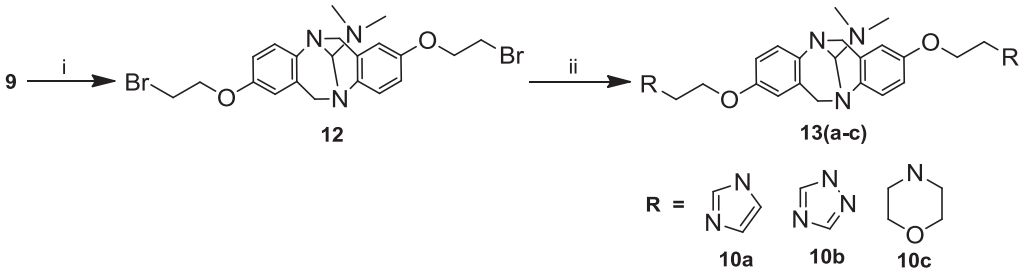
Selective inhibition of MDAMB-231 cell line in first phase of the synthesis has encouraged us to design molecules with increasing chain length on aromatic rings of Tröger's base and incorporation of heterocyclic units for the second phase. As shown in Table 2, compounds **11c**, **11d**, **11i**, **11h**, and **13c** have exhibited better cytotoxicity than the control compound doxorubicin against MDAMB-231 cell line with IC_{50} values of 4.29 μM, 4.79 μM, 5.15 μM, 4.82 μM and 5.80 μM, respectively. Increase in the chain length on aromatic rings of Tröger's base and incorporating heterocyclic units has indeed improved anticancer activity. Among the heterocycles introduced on the Tröger's base, five membered rings such as imidazole, triazole or tetrazole were not as effective as six membered rings like morpholine, piperidine or piperazine.



Scheme 1. Synthesis of methylene bridged Tröger's base analogues **2a**–**2l** and **3**–**7**. Reaction conditions: (i) DMF, $POCl_3$, 0 °C to r.t., 2–6 h; (ii) heterocyclic formamide, $POCl_3$, r.t., 4–6 h.



Scheme 2. Synthesis of Tröger's base analogues **9** and **11a**–**11i**. Reaction conditions: (i) 37% HCHO solution, HCl, EtOH, 0 °C to r.t., 24 h; (ii) RH, K_2CO_3, CH_3CN, reflux, 24 h.



Scheme 3. Synthesis of *N,N*-dimethylamino crowned Tröger's base analogues **12** and **13(a–c)**. Reaction conditions: (i) DMF, $POCl_3$, 0 °C to r.t., 5 h; (ii) RH, K_2CO_3, CH_3CN, reflux, 24 h.

Morpholine (**11c**) substitution has brought about best inhibition of MDAMB-231 cell line. However, no distinct affect of the methylene bridge substitution was decipherable from the current study.

3. Conclusion

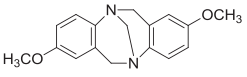
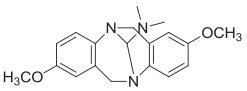
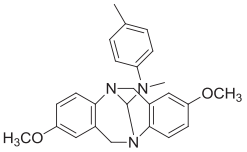
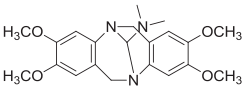
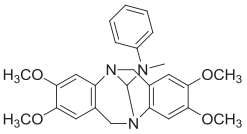
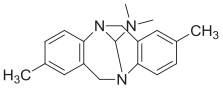
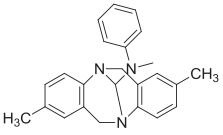
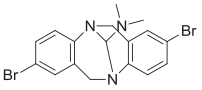
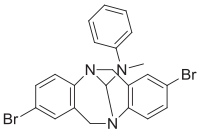
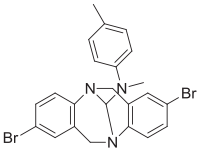
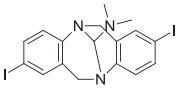
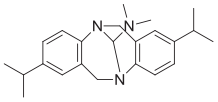
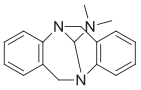
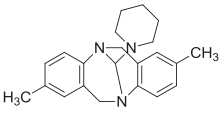
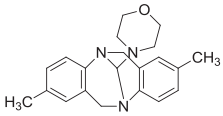
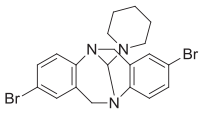
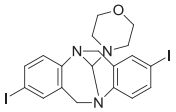
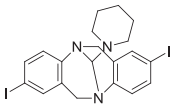
In summary, present work describes the synthesis of a series of Tröger's base analogues by extensive functionalization of both methylene bridge as well as benzene ring framework with various heterocycles in two phases. All the compounds synthesized in the first phase were tested for anticancer activity against A549, MDAMB-231 and SK-N-SH cancer cell lines. Several compounds from this phase showed selective activity against MDAMB-231 cell line while only compound **3** inhibited the growth of SK-N-SH cell line and none had any effect on A549 cell line. Several redesigned compounds synthesized in the second phase were effective inhibitors against MDAMB-231 cell line and better than doxorubicin. Methylene bridge functionalization either with *N,N*-dialkylamino or *N*-cycloalkylamino units has no influence on the cytotoxicity compared to *N*-alkyl-*N*-arylamino functionalization. Introduction of six membered heterocycles with a chain on aromatic rings of Tröger's base resulted in better activity compared to five membered heterocycles and the best activity was obtained with morpholine as the substituent. Improved and promising anticancer activity of the synthesized Tröger's base compounds provides the basis for further exploration of these molecules as therapeutics, specifically against breast cancer.

4. Experimental protocols

4.1. Materials and methods

All starting materials were obtained from the best known commercial sources and used as received. All reactions were carried out in anhydrous conditions. Organic layers were dried over anhydrous Na_2SO_4. Thin layer chromatography (TLC) was

Table 1
IC_{50} values of Tröger's base analogues **1a**, **2(a–l)** and **3–7** on human cancer cell lines.

Entry	Compound	IC_{50} values in μM		
		A549	SK-N-SH	MDAMB-231
1a		>100	>100	>100
2a		>100	>100	>100
2b		>100	>100	10.72 ± 0.54
2c		>100	>100	78.14 ± 0.97
2d		>100	>100	86.76 ± 8.86
2e		>100	>100	61.79 ± 0.30
2f		>100	>100	72.33 ± 0.38
2g		>100	>100	>100
2h		>100	>100	14.34 ± 0.19
2i		>100	>100	>100
2j		NT[a]	NT[a]	NT[a]
2k		>100	>100	57.5 ± 0.21
2l		>100	>100	>100
3		>100	12.21 ± 0.13	82.35 ± 3.68
4		>100	>100	>100
5		>100	>100	>100
6		>100	>100	>100
7		>100	>100	>100
	Doxorubicin	8.05 ± 0.37	8.5 ± 0.17	5.96 ± 0.13

[a] NT: not tested.

performed on Merck F_{254} precoated silica gel plates. Visualisation was accomplished with UV light and/or iodine. Column chromatography was performed on silica gel (60 × 120 mesh) on a glass column. Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on a FTIR spectrometer as KBr pellets or neat, ^1H and ^{13}C NMR spectra for analytical purpose were recorded in $CDCl_3$ on a Bruker instrument at 300 MHz, Avance instrument at 500 MHz and 75 MHz, respectively; chemical shifts are expressed in δ-scale downfield from TMS as an internal standard. Mass data (ESI) were recorded by quadruple mass spectrometry. HRMS data were obtained with the ESI ionization sources.

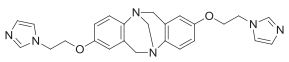
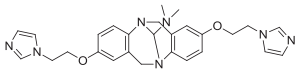
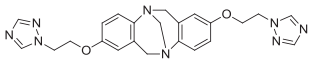
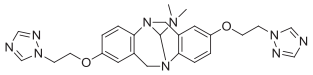
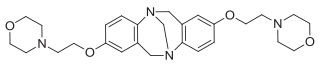
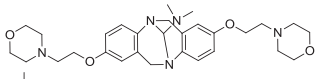
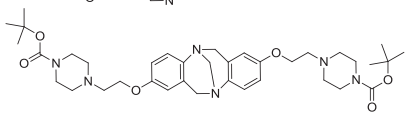
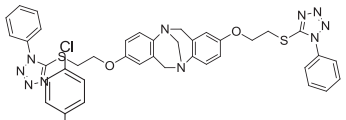
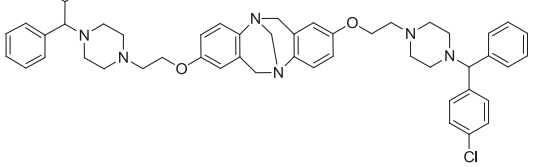
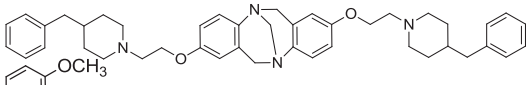
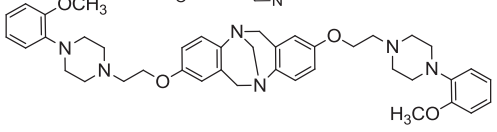
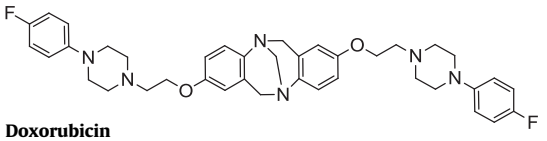
*4.2. General procedure for the synthesis of Tröger's bases **2a–2l** and **3–7***

For compounds **2a–2l**, *N*,*N*-dimethylformamide (3 mmol) was cooled in an ice bath and phosphorous oxychloride (3 mmol) added drop wise. For compounds **3–7**, Vilsmeier–Haack reagent had to be formed at 70 °C. After formation of a yellow coloured paste, reaction mixture was reached to room temperature. Tröger's Base (1 mmol) dissolved in 10 mL of dry dimethylformamide/dichloromethane was added and the reaction mixture stirred at room temperature for 2–6 h. After completion of the reaction (monitored by TLC), reaction was quenched with saturated sodium carbonate solution. The aqueous layer was extracted with dichloromethane (3 × 25 mL), the combined organic extracts were washed with water (3 × 10 mL), dried and concentrated in vacuo. The crude product was purified by column chromatography using EtOAc/Hexane as eluent.

*4.2.1. Synthesis of (±)-2,8-dibromo-N-methyl-N-(p-tolyl)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocin-13-amine (**2i**)*

2,8-Dibromo-6,12-dihydro-5,11-methano-dibenzo[*b*,*f*][1,5]diazocine (500 mg, 1.32 mmol) in 15 mL of CH_2Cl_2 was added to Vilsmeier–Haack reagent prepared by reacting *N*-methyl-*N*-(*p*-tolyl) formamide (591 mg, 3.97 mmol) and $POCl_3$ (371 μL, 3.97 mmol),

Table 2
IC_{50} values of Tröger's base analogues **11(a–i)** and **13(a–c)** on MDAMB-231 cell line.

Entry	Compound name	IC_{50} values in μM on MDAMB-231
11a		42.62 ± 1.73
13a		>100
11b		>100
13b		40.35 ± 5.02
11c		4.29 ± 0.11
13c		4.79 ± 0.34
11d		5.15 ± 0.09
11e		>100
11f		57.86 ± 8.13
11g		64.08 ± 4.80
11h		5.80 ± 0.02
11i		4.82 ± 0.20
	Doxorubicin	5.96 ± 0.13

and stirred at room temperature for 5 h. Crude product was purified by column chromatography using 5% EtOAc in hexane as eluent afforded **2i** (433 mg, 66%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.29–7.24 (m, 2H), 7.07 (t, J = 8.0 Hz, 3H), 7.0 (s, 1H), 6.98–6.91 (m, 4H), 4.97 (s, 1H), 4.70 (d, J = 17.0 Hz, 1H), 4.44 (d, J = 17.0 Hz, 1H), 4.20 (d, J = 17.0 Hz, 1H), 3.76 (d, J = 16.0 Hz, 1H), 2.79 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 146.7, 143.7, 131.4, 130.7, 130.5, 130.4, 130.1, 129.3, 129.2, 129.1, 127.2, 126.9, 120.8, 116.8, 116.6, 84.9, 59.4, 50.7, 39.1, 20.7; IR (neat, cm^{-1}) 2963, 2865, 1474, 749; MS (ESI) m/z (%) 498 ([M+H], 100), HRMS (ESI) calcd for $C_{23}H_{22}Br_2N_3$ 498.01750, found 498.01830.

*4.2.2. Synthesis of (±)-(2,8-diiodo-N,N-dimethyl-6,12-dihydro-5,11-methano-dibenzo[b,f][1,5]diazocin-13-amine (**2j**)*

2,8-Diiodo-6,12-dihydro-5,11-methano-dibenzo[*b,f*][1,5]diazocine (500 mg, 1.05 mmol) in 8 mL of DMF was added to Vilsmeier–Haack reagent prepared by reacting DMF (246 μL, 3.16 mmol) and $POCl_3$ (296 μL, 3.16 mmol), and stirred at room temperature for 5 h. Crude product was purified by column chromatography using 5% EtOAc in hexane as eluent afforded **2j** (431 mg, 79%) as a white solid, mp 95–96 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.43 (dd, J = 13.3, 6.7 Hz, 2H), 7.24 (s, 1H), 7.18 (s, 1H), 6.85 (d, J = 7.79 Hz, 1H), 6.81 (d, J = 7.79 Hz, 1H), 4.57 (dd,

$J = 16.7, J = 6.8$ Hz, 2H), 4.12 (d, $J = 16.7$ Hz, 1H), 3.80 (d, $J = 13.3$ Hz, 2H), 2.37 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 136.2, 136.1, 135.3, 135.2, 131.0, 130.6, 127.6, 127.3, 89.6, 87.6, 58.7, 51.0, 41.3; IR (KBr, cm^{-1}) 2984, 2823, 1456, 750; MS (ESI) *m/z* (%) 518 ([M+H], 100), HRMS (ESI) calcd for $C_{17}H_{18}N_3I_2$ 517.95846, found 517.95809.

4.2.3. Synthesis of (±)-2,8-bromo-13-piperidin-1-yl-6,12-dihydro-5,11-methano-dibenzo[b,f][1,5]diazocine (**5**)

2,8-Dibromo-6,12-dihydro-5,11-methano-dibenzo[*b,f*][1,5]diazocine (498 mg, 1.31 mmol) in 15 mL of DCM was added to Vilsmeier–Haack reagent prepared by reacting piperidine-1-carbaldehyde (446 mg, 3.95 mmol) and $POCl_3$ (370 μL, 3.95 mmol) and stirred at room temperature for 6 h. Crude product was purified by column chromatography using 5% EtOAc in hexane as eluent afforded **5** (420 mg, 69%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.28–7.19 (m, 2H), 7.06–6.88 (m, 4H), 4.58 (d, $J = 16.6$ Hz, 2H), 4.12 (d, $J = 16.8$ Hz, 1H), 3.93 (s, 1H), 3.77 (d, $J = 16.4$ Hz, 1H), 1.55–1.19 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 147.6, 143.6, 131.0, 130.3, 130.0, 129.2, 129.1, 128.8, 127.2, 126.9, 116.5, 116.1, 88.3, 59.0, 51.2, 49.6, 25.6, 24.8; IR (neat, cm^{-1}) 2965, 2821, 1464, 747; MS (ESI) *m/z* (%) 462 ([M+H], 100), HRMS (ESI) calcd for $C_{20}H_{22}N_3Br_2$ 464.03315, found 464.01435.

4.2.4. Synthesis of (±)-4-(2,8-diiodo-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocin-13-yl)morpholine (**6**)

2,8-Diiodo-6,12-dihydro-5,11-methano-dibenzo[*b,f*][1,5]diazocine (500 mg, 1.05 mmol) in 12 mL of DCM was added to Vilsmeier–Haack reagent prepared by reacting morpholine-1-carbaldehyde (364 mg, 3.16 mmol), and $POCl_3$ (296 μL, 3.16 mmol) and stirred at room temperature for 6 h Crude product was purified by column chromatography using 5% EtOAc in hexane as eluent afforded **6** (418 mg, 71%) as a light brown foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.44 (td, $J = 9.2$ Hz, $J = 1.7$ Hz, 2H), 7.27–7.17 (m, 2H), 6.81 (dd, $J = 15.7, 8.3$ Hz, 2H), 4.56 (dd, $J = 16.6, 9.1$ Hz, 2H), 4.12 (d, $J = 16.8$ Hz, 1H), 3.98 (s, 1H), 3.78 (d, $J = 16.6$ Hz, 1H), 3.70–3.56 (m, 4H), 2.86–2.60 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 136.3, 136.1, 135.4, 135.1, 131.2, 130.6, 127.6, 127.2, 88.0, 87.7, 87.4, 66.8, 58.9, 51.0, 49.3; IR (neat, cm^{-1}) 2923, 2850, 1471, 1262, 1029, 749; MS (ESI) *m/z* (%) 560 ([M+H], 100), HRMS (ESI) calcd for $C_{19}H_{20}N_3OI_2$ 559.96902, found 559.96965.

4.2.5. Synthesis of (±)-2,8-diiodo-13-(piperidin-1-yl)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**7**)

2,8-Diiodo-6,12-dihydro-5,11-methano-dibenzo[*b,f*][1,5]diazocine (500 mg, 1.05 mmol) in 12 mL of DCM was added to Vilsmeier–Haack reagent prepared by reacting piperidine-1-carbaldehyde (357 mg, 3.16 mmol), and $POCl_3$ (296 μL, 3.16 mmol) and stirred at room temperature for 6 h. Crude product was purified by column chromatography using 5% EtOAc in hexane as eluent afforded **7** (434 mg, 74%) as a light brown foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.42 (t, $J = 8.5$ Hz, 2H), 7.29–7.15 (m, 2H), 6.81 (dd, $J = 15.3, 8.5$ Hz, 2H), 4.56 (d, $J = 16.6$ Hz, 2H), 4.11 (d, $J = 16.6$ Hz, 1H), 3.92 (s, 1H), 3.75 (d, $J = 16.4$ Hz, 1H), 3.92 (s, 3H), 3.75 (d, $J = 16.6$ Hz, 1H), 1.61–1.18 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 148.2, 144.3, 136.0, 135.8, 135.2, 134.9, 131.4, 130.7, 127.5, 127.2, 88.0, 87.5, 87.1, 58.8, 51.0, 49.6, 25.5, 24.8; IR (neat, cm^{-1}) 2922, 2851, 1470, 1204, 747; MS (ESI) *m/z* (%) 558 ([M+H], 100), HRMS (ESI) calcd for $C_{20}H_{22}N_3I_2$ 557.98976, found 557.98983.

4.3. Synthesis of (±)-2,8-bis(2-bromoethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**9**)

To a mixture of 4-(2-bromoethoxy)aniline (5 gr, 23.2 mmol) in 20 mL ethanol was added 50 mL of 37% formaldehyde solution followed by 45 mL of concentrated HCl (slow addition) at 0 °C. After HCl addition, the reaction mixture was stirred at r.t. for 24 h. After completion of the reaction (monitored by TLC), the mixture was concentrated under reduced pressure until one half of the original volume remained and made basic with excess NH_4OH. The aqueous phase was extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (2 × 100 mL) and dried over Na_2SO_4. The solvents were removed under reduced pressure and purified by column chromatography using 30% ethyl acetate in hexane as eluent afforded **9** (2.93 g, 27%) as a white solid, mp 105–106 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.06 (d, $J = 8.7$ Hz, 2H), 6.75 (dd, $J = 2.6, 8.9$ Hz, 2H), 6.44 (d, $J = 2.5$ Hz, 2H), 4.64 (d, $J = 16.6$ Hz, 2H), 4.28 (s, 2H), 4.18 (t, $J = 6.4$ Hz, 4H), 4.07 (d, $J = 16.8$ Hz, 2H), 3.57 (t, $J = 6.2$ Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 154.4, 141.5, 128.7, 126.0, 114.6, 112.1, 68.0, 67.1, 58.8, 29.1; IR (neat, cm^{-1}) 2936, 2882, 1486, 1265, 1151, 1034, 750; MS (ESI) *m/z* (%) 469 ([M+H], 100), HRMS (ESI) calcd for $C_{19}H_{21}N_2O_2Br_2$ 466.99643, found 466.99695.

4.4. General procedure for the synthesis of Tröger's bases **11(a–i)**

Tröger's base **9** (1 mmol) was added to the mixture of amine (2.2 mmol) and K_2CO_3 (3 mmol) in 15 mL of acetonitrile. The reaction mixture was refluxed for 24 h. Acetonitrile was removed under reduced pressure. Water was added (30 mL), reaction mixture was extracted with dichloromethane (3 × 50 mL) and combined organic extracts were washed with water (3 × 20 mL). Solvent was removed under reduced pressure, dried over Na_2SO_4 and purified by column chromatography to afford the desired product.

4.4.1. Synthesis of (±)-2,8-bis(2-(1H-imidazol-1-yl)ethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**11a**)

Tröger's base **9** (201 mg, 0.431 mmol) was added to the mixture of imidazole (65 mg, 0.948 mmol) and K_2CO_3 (178 mg, 1.29 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11a** (160 mg, 84%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.54 (s, 2H), 7.08–6.95 (m, 6H), 6.69 (dd, $J = 2.5, 8.7$ Hz, 2H), 6.38 (d, $J = 2.5$ Hz, 2H), 4.61 (d, $J = 16.8$ Hz, 2H), 4.29–4.21 (m, 6H), 4.09 (t, $J = 5.0$ Hz, 4H), 4.03 (d, $J = 16.8$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 153.8, 140.9, 136.8, 128.3, 128.1, 125.4, 118.8, 113.9, 111.2, 66.9, 66.4, 58.1, 45.8; IR (neat, cm^{-1}) 2940, 1492, 1235, 1079, 747; MS (ESI) *m/z* (%) 443 ([M+H], 100), HRMS (ESI) calcd for $C_{25}H_{27}N_6O_2$ 443.21900, found 443.21689.

4.4.2. Synthesis of (±)-2,8-bis(2-(1H-1,2,4-triazol-1-yl)ethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**11b**)

Tröger's base **9** (192 mg, 0.412 mmol) was added to the mixture of triazole (63 mg, 0.906 mmol) and K_2CO_3 (171 mg, 1.24 mmol) in 15 mL of acetonitrile, refluxed for 24 h, followed by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11b** (146 mg, 80%) as a white solid, mp 161–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.16 (s, 2H), 7.91 (s, 2H), 7.02 (d, $J = 9.0$ Hz, 2H), 6.67 (dd, $J = 3.0, 9.0$ Hz, 2H), 6.36 (d, $J = 3.0$ Hz, 2H), 4.60 (d, $J = 17.0$ Hz, 2H), 4.49 (t, $J = 5.0$ Hz, 4H), 4.25 (s, 2H), 4.21 (t, $J = 5.0$ Hz, 4H), 4.02 (d, $J = 17.0$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 154.1, 151.8, 143.8, 141.5, 128.6, 125.9, 114.3, 111.7, 66.9, 65.6, 58.6, 49.0; IR (neat, cm^{-1}) 2937, 2845, 1493, 1269, 1147, 1046, 736; MS (ESI) *m/z* (%) 445 ([M+H], 100), HRMS (ESI) calcd for $C_{23}H_{25}N_8O_2$ 445.20950, found 445.20813.

4.4.3. Synthesis of (±)-2,8-bis(2-morpholinoethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**11c**)

Tröger's base **9** (202 mg, 0.433 mmol) was added to the mixture of morpholine (83 mg, 0.954 mmol) and K_2CO_3 (179 mg, 1.30 mmol)

in 15 mL of acetonitrile, refluxed for 24 h, followed by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11c** (183 mg, 88%) as a white solid, mp 125–126 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.05 (d, J = 8.7 Hz, 2H), 6.74 (dd, J = 2.8, 8.7 Hz, 2H), 6.43 (d, J = 2.6 Hz, 2H), 4.63 (d, J = 16.8 Hz, 2H), 4.28 (s, 2H), 4.06 (d, J = 16.8 Hz, 2H), 4.0 (t, J = 5.7 Hz, 4H), 3.71 (t, J = 4.53 Hz, 8H), 2.74 (t, J = 5.7 Hz, 4H), 2.53 (t, J = 4.53 Hz, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 155.1, 141.0, 128.5, 125.8, 114.4, 111.7, 67.1, 66.7, 65.8, 58.7, 57.5, 53.9; IR (neat, cm^{-1}) 2913, 2860, 1492, 1244, 1114, 775; MS (ESI) m/z (%) 481 ([M+H], 100), HRMS (ESI) calcd for $C_{27}H_{37}N_4O_4$ 481.28093, found 481.27918.

4.4.4. Synthesis of (±)-di-tert-butyl 4,4′-(((6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine-2,8-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(piperazine-1-carboxylate) (**11d**)

Tröger's base **9** (180 mg, 0.386 mmol) was added to the mixture of *tert*-butyl piperazine-1-carboxylate (158 mg, 0.849 mmol), and K_2CO_3 (160 mg, 1.16 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11d** (204 mg, 78%) as a white solid, mp 126–127 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.04 (d, J = 8.9 Hz, 2H), 6.74 (dd, J = 2.8 Hz, 2H), 6.43 (d, J = 2.8 Hz, 2H), 4.63 (d, J = 16.8 Hz, 2H), 4.28 (s, 2H), 4.06 (d, J = 16.8 Hz, 2H), 3.99 (t, J = 5.67 Hz, 4H), 3.42 (t, J = 4.91 Hz, 8H), 2.75 (t, J = 5.67 Hz, 4H), 2.48 (t, J = 4.91 Hz, 8H), 1.45 (s, 18H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 154.97, 154.54, 140.86, 128.47, 125.79, 114.30, 111.57, 79.51, 67.02, 65.74, 58.68, 57.05, 53.14, 28.28 ppm; IR (neat, cm^{-1}) 2973, 2936, 1692, 1493, 1244, 1170, 756; MS (ESI) m/z (%) 679 ([M+H], 100), HRMS (ESI) calcd for $C_{37}H_{55}N_6O_6$ 679.41776, found 679.41780.

4.4.5. Synthesis of (±)-2,8-bis(2-((1-phenyl-1H-tetrazol-5-yl)thio) ethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**11e**)

Trögers base **9** (200 mg, 0.429 mmol) was added to the mixture of 1-phenyl-1*H*-tetrazole-5-thiol (168 mg, 0.944 mmol) and K_2CO_3 (177 mg, 1.28 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11e** (230 mg, 81%) as a white solid, mp 73–74 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.54 (s, 10H), 7.05 (d, J = 8.7 Hz, 2H), 6.74 (dd, J = 2.6, 8.7 Hz, 2H), 6.44 (d, J = 2.8 Hz, 2H), 4.62 (d, J = 17.2 Hz, 2H), 4.32–4.25 (m, 6H), 4.05 (d, J = 17.2 Hz, 2H), 3.71 (t, J = 5.7 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 154.5, 153.9, 141.3, 133.4, 130.1, 129.8, 128.6, 126.0, 123.7, 114.5, 111.7, 67.1, 65.8, 58.7, 32.5; IR (neat, cm^{-1}) 2925, 1496, 1242, 1151, 1028, 761; MS (ESI) m/z (%) 663 ([M+H], 100), HRMS (ESI) calcd for $C_{33}H_{31}N_{10}O_2S_2$ 663.20674, found 663.20815.

4.4.6. Synthesis of (±)-2,8-bis(2-(4-((4-chlorophenyl)(phenyl) methyl)piperazin-1-yl)ethoxy)-6,12-dihydro-5,11-methanodibenzo [b,f][1,5]diazocine (**11f**)

Tröger's base **9** (200 mg, 0.429 mmol) was added to the mixture of 1-((4-chloro)(phenyl)methyl)piperazine (270 mg, 0.944 mmol) and K_2CO_3 (178 mg, 1.28 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11f** (278 mg, 74%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.39–7.14 (m, 18H), 7.02 (d, J = 8.9 Hz, 2H), 6.71 (dd, J = 8.9, 2.6 Hz, 2H), 6.40 (d, J = 2.5 Hz, 2H), 4.61 (d, J = 16.8 Hz, 2H), 4.26 (s, 2H), 4.18 (s, 2H), 4.03 (d, J = 16.9 Hz, 2H), 3.97 (t, J = 5.7 Hz, 4H), 2.74 (t, J = 5.7 Hz, 4H), 2.62–2.30 (m, 14H), 1.78–1.69 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 155.1, 142.1, 141.3, 140.9, 132.4, 129.1, 128.6, 128.5, 127.8, 127.1, 125.9, 114.4, 111.6, 75.4, 67.1, 65.9, 58.8, 57.0, 53.7, 51.7; IR (neat, cm^{-1}) 2936, 2809, 1490, 1243, 1154, 757; MS (ESI) m/z (%) 879 ([M+H], 100), HRMS (ESI) calcd for $C_{53}H_{57}N_6O_2Cl_2$ 879.39146, found 879.39151.

4.4.7. Synthesis of (±)-2,8-bis(2-(4-benzylpiperidin-1-yl)ethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**11g**)

Tröger's base **9** (200 mg, 0.429 mmol) was added to the mixture of 1-benzylpiperazine (165 mg, 0.944 mmol) and K_2CO_3 (177 mg, 1.28 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11g** (176 mg, 76%) as a white solid, mp 115–116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.28–7.24 (m, 4H), 7.19–7.11 (m, 6H), 7.02 (d, J = 8.9 Hz, 2H), 6.71 (dd, J = 8.9, 2.9 Hz, 2H), 6.41 (d, J = 2.9 Hz, 2H), 6.61 (d, J = 2.9 Hz, 2H), 4.61 (d, J = 16.6 Hz, 2H), 4.23 (s, 2H), 4.04 (d, J = 16.8 Hz, 2H), 3.98 (t, J = 5.9 Hz, 4H), 2.95–2.90 (m, 4H), 2.70 (t, J = 5.9 Hz, 4H), 2.52 (d, J = 7.0 Hz, 4H), 2.00 (td, J = 2.3, 2.1 Hz, 6H), 1.65–1.58 (m, 4H), 1.36–1.28 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 155.2, 140.9, 140.5, 129.0, 128.5, 128.0, 125.8, 125.7, 114.4, 111.7, 67.1, 66.1, 58.8, 57.4, 54.2, 43.1, 37.6, 32.1; IR (neat, cm^{-1}) 2914, 2847, 1494, 1271, 1151, 1077, 741; MS (ESI) m/z (%) 657 ([M+H], 100), HRMS (ESI) calcd for $C_{43}H_{53}N_4O_2$ 657.41630, found 657.41725.

4.4.8. Synthesis of (±)-2,8-bis(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**11h**)

Tröger's base **9** (200 mg, 0.429 mmol) was added to the mixture of 1-(2-methoxyphenyl)piperazine (181 mg, 0.944 mmol) and K_2CO_3 (177 mg, 1.28 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11h** (251 mg, 85%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.05 (d, J = 8.7 Hz, 2H), 7.01–6.97 (m, 2H), 6.95–6.89 (m, 4H), 6.85 (dd, J = 1.1 Hz, 2H), 6.76 (dd, J = 2.7 Hz, 2H), 6.45 (d, J = 2.7 Hz, 2H), 4.64 (d, J = 16.8 Hz, 2H), 4.23 (s, 2H), 4.14–4.02 (m, 8H), 3.85 (s, 6H), 3.15–3.04 (m, 8H), 2.82 (t, J = 5.8 Hz, 4H), 2.79–2.71 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 155.1, 152.1, 141.1, 140.9, 128.5, 125.8, 122.8, 120.9, 118.1, 114.4, 111.7, 111.0, 67.1, 65.9, 58.8, 57.2, 55.2, 53.7, 50.4; IR (neat, cm^{-1}) 2931, 2867, 1496, 1239, 1055, 1024, 748; MS (ESI) m/z (%) 691 ([M+H], 100), HRMS (ESI) calcd for $C_{41}H_{51}N_6O_4$ 691.39663, found 691.39767.

4.4.9. Synthesis of (±)-2,8-bis(2-(4-(4-fluorophenyl)piperazin-1-yl) ethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocine (**11i**)

Tröger's base **9** (127 mg, 0.272 mmol) was added to the mixture of 1-(4-fluorophenyl)piperazine (108 mg, 0.599 mmol) and K_2CO_3 (113 mg, 0.817 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **11i** (134 mg, 74%) as a white solid, mp 154–155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.05 (d, J = 8.7 Hz, 2H), 6.98–6.93 (m, 4H), 6.89–6.84 (m, 8H), 6.76 (dd, J = 8.7, 2.7 Hz, 2H), 6.45 (d, J = 2.7 Hz, 2H), 4.64 (d, J = 16.8 Hz, 2H), 4.29 (s, 2H), 4.10–4.02 (m, 6H), 3.12 (t, J = 4.9 Hz, 8H), 2.82 (t, J = 5.3 Hz, 4H), 2.76–2.68 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 158.7, 155.6, 155.1, 147.8, 141.0, 128.6, 125.9, 117.9, 117.7, 115.6, 115.3, 114.5, 111.7, 67.2, 65.9, 58.8, 57.1, 53.5, 50.0; IR (neat, cm^{-1}) 2937, 2823, 1509, 1491, 1230, 1152, 825; MS (ESI) m/z (%) 667 ([M+H], 100), HRMS (ESI) calcd for $C_{39}H_{45}N_6O_2F_2$ 667.35666, found 667.35777.

4.5. Synthesis of (±)-2,8-bis(2-bromoethoxy)-N,N-dimethyl-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocin-13-amine (**12**)

Following the general procedure 4.2, Tröger's base **9** (1 g, 2.15 mmol) in 10 mL of DMF was added to Vilsmeier–Haack reagent prepared by reacting DMF (499 μL, 6.44 mmol), and $POCl_3$ (603 μL, 6.44 mmol) and stirred at room temperature for 5 h. Crude product was purified by column chromatography using 10% EtOAc in hexane as eluent afforded **12** (709 mg, 65%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.02 (dd, J = 9.1, 8.3 Hz, 2H), 6.73 (td, J = 3.0, 2.3 Hz, 2H), 6.43 (dd, J = 3.0, 2.3 Hz, 2H), 4.59 (d, J = 16.6 Hz, 1H), 4.55 (d, J = 16.6 Hz, 1H), 4.20–4.07 (m, 5H), 3.84–3.69 (m, 5H),

3.59–3.51 (m, 1H), 2.40 (s, 6H); 13C NMR (75 MHz, CDCl3) δ (ppm): 154.4, 154.2, 142.4, 138.2, 129.4, 129.2, 126.6, 126.3, 114.6, 114.3, 111.8, 111.7, 90.3, 68.1, 67.9, 59.5, 51.8, 41.4, 29.3, 29.2; MS (ESI) *m/z* (%) 512 ([M+H], 100), HRMS (ESI) calcd for $C_{21}H_{26}N_3O_2Br_2$ 512.05428, found 512.03601.

4.6. General procedure for the synthesis of Tröger's bases **13(a–c)**

Tröger's base **12** (1 mmol) was added to the mixture of amine (2.2 mmol) and K_2CO_3 (3 mmol) in 15 mL of acetonitrile. The reaction mixture was refluxed for 24 h. Acetonitrile was removed under reduced pressure. Water was added (30 mL), reaction mixture was extracted with dichloromethane (3 × 50 mL) and combined organic extracts were washed with water (3 × 20 mL). Solvent was removed under reduced pressure, dried over Na_2SO_4 and purified by column chromatography to afford the desired product.

4.6.1. Synthesis of (±)-2,8-bis(2-(1H-imidazol-1-yl)ethoxy)-N,N-dimethyl-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocin-13-amine (**13a**)

Tröger's base **12** (200 mg, 0.393 mmol) was added to the mixture of imidazole (59 mg, 0.864 mmol) and K_2CO_3 (163 mg, 1.18 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **13a** (154 mg, 80%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.54 (s, 2H), 7.06–6.94 (m, 6H), 6.67(td, *J* = 2.8, 2.6 Hz, 2H), 6.37 (dd, *J* = 2.6 Hz, 2H), 4.60–4.49 (m, 2H), 4.29–4.20 (m, 4H), 4.13–4.04 (m, 5H), 3.80–3.71 (m, 2H), 2.38 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 155.0, 154.9, 142.0, 137.8, 129.3, 129.1, 126.4, 126.2, 114.4, 114.0, 111.5, 111.3, 90.4, 66.8, 65.9, 65.7, 59.6, 57.6, 53.9, 51.8, 41.4; IR (neat, cm^{-1}) 2923, 2852, 1461, 1279, 1079, 771; MS (ESI) *m/z* (%) 486 ([M+H], 100), HRMS (ESI) calcd for $C_{27}H_{32}N_7O_2$ 486.26120, found 486.26251.

4.6.2. Synthesis of (±)-2,8-bis(2-(1H-1,2,4-triazol-1-yl)ethoxy)-N,N-dimethyl-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocin-13-amine (**13b**)

Tröger's base **12** (200 mg, 0.393 mmol) was added to the mixture of triazole (59 mg, 0.864 mmol) and K_2CO_3 (163 mg, 1.18 mmol) in 15 mL of acetonitrile, purified by column chromatography in EtOAc as eluent afforded **13b** (154 mg, 80%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.17 (s, 2H), 7.91 (d, *J* = 2.3 Hz, 2H), 6.99 (dd, *J* = 9.1 Hz, 2H), 6.66 (tdt, *J* = 3.0, 2.3 Hz, 2H), 6.36 (dd, *J* = 3.0, 2.3 Hz, 2H), 4.59–4.45 (m, 6H), 4.24–4.02 (m, 5H), 3.79–3.71 (m, 2H), 2.37 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 154.1, 154.0, 151.9, 151.8, 143.7, 142.6, 129.2, 126.6, 126.4, 114.4, 114.1, 111.7, 111.4, 90.2, 65.8, 65.7, 59.4, 51.7, 49.2, 41.3; IR (neat, cm^{-1}) 2925, 2837, 1472, 1258, 1035, 736; MS (ESI) *m/z* (%) 488 ([M+H], 100), HRMS (ESI) calcd for $C_{25}H_{30}N_9O_2$ 488.25170, found 488.25169.

4.6.3. Synthesis of (±)-N,N-dimethyl-2,8-bis(2-morpholinoethoxy)-6,12-dihydro-5,11-methanodibenzo[b,f][1,5]diazocin-13-amine (**13c**)

Tröger's base **12** (200 mg, 0.393 mmol) was added to the mixture of morpholine (59 mg, 0.864 mmol) and K_2CO_3 (163 mg, 1.18 mmol) in 15 mL of acetonitrile, purified by column chromatography in MeOH:EtOAc (1:9) as eluent afforded **13c** (160 mg, 78%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.00 (dd, *J* = 9.065 Hz, 2H), 6.71 (td, *J* = 3.022 Hz, 2H), 6.42 (d, *J* = 3.022 Hz, 2H), 4.56 (dd, *J* = 16.618 Hz, 2H), 4.11 (d, *J* = 16.618 Hz, 1H), 4.03–3.95 (m, 4H), 3.83–3.59 (m, 10H), 2.79–2.68 (m, 4H), 2.61–2.45 (m, 8H), 2.39 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 155.0, 154.9, 142.0, 137.8, 129.3, 129.1, 126.4, 126.2, 114.4, 114.0, 111.5, 111.3, 90.4, 66.8, 65.9, 65.7, 59.6, 57.6, 53.9, 51.8, 41.4, 29.7 ppm; IR (neat, cm^{-1}) 2923, 2853, 1493, 1240, 1116, 1059, 755; MS (ESI) *m/z* (%) 524 ([M+H], 100), HRMS (ESI) calcd for $C_{29}H_{42}N_5O_4$ 524.32313, found 524.32244.

4.7. Cytotoxicity against three cell lines

Cytotoxicity of synthesized compounds was tested against three cell lines, human lung cancer cell line (A549), a human breast cancer cell line (MDAMB-231) and a human neuroblastoma cell line (SK-N-SH) by MTT-micro cultured tetrazolium assay method [16]. All the experiments were carried out in triplicates. All the three types of cell lines were seeded to flat bottom 96 well plates (10,000 cells/100 μL) and cultured in the medium containing 10% serum, incubated for 24 h in a 5% CO_2 humid chamber so that the cells adhere to the surface.

3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was dissolved in PBS at 5 mg/mL and sterile filtered. Different concentrations of the compounds were added to the adhered cells. After 48 h MTT solution (10 μL per well) was added to the culture plate. Cells were further incubated in the CO_2 chamber for 2 h. Following this, media was removed and 100 μL of DMSO was added. Absorbance was measured at 562 nm in a multimode micro plate reader (Tecan GENios).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.044.

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