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Articles

Synthesis of Heterocycle-linked Thioureas and Their Inhibitory Activities of NO Production in LPS Activated Macrophages

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A series of thioureas were synthesized as inhibitors of NO production in lipopolysaccharide-activated macrophages. We investigated the effect of lipophilic moiety and *N*-substituents of the thioureas on the activity. Phenoxazine and carbazole-containing derivatives revealed higher activity than indole-containing thioureas. The appropriate spacer between lipophilic tail and thiourea head and methyl substituent at N3 position of thiourea brought beneficial effect on the inhibition of NO production. Among prepared compounds, phenoxazine-containing derivative **2a** was the most potent with 2.32 μM of IC_{50} value. RT-PCR analysis suggested that the prepared thioureas inhibited NO production through the suppression of iNOS mRNA expression.

Key Words: Thiourea, Nitric oxide, iNOS

Introduction

Nitric oxide (NO) is a ubiquitous biological messenger involved in a variety of physiological and pathophysiological processes. NO is synthesized by three types of nitric oxide synthases (NOSs) that catalyze the oxidation of L-arginine to L-citrulline as a co-product. Three quite distinct isoforms of NOS have been identified. The diverse biological role of NO depends on which subtype of enzyme is involved, and how much amounts of NO is synthesized.[1] Two isoforms found in neuronal tissues (nNOS, type I) and vascular endothelium (eNOS, type III) are constitutive NOS (cNOS). cNOS is intermittently activated by transient elevation of intracellular calcium level and releases small amounts of NO. The physiological concentrations of NO produced by cNOS have roles in the regulation of blood pressure and neurotransmission.[2] The third isoform is inducible NOS (iNOS, type II) that can be induced by lipopolysaccharide (LPS) and various cytokines such as IFN-α, IL-1β, and TNF-α. The iNOS produces a large amount of NO sustained over a long period of time after the enzyme induction by cytokines or endotoxins.[3] Low concentrations of NO produced by iNOS possess beneficial roles in host defence mechanism against pathogens,[4] while overproduction of NO by iNOS has been implicated in the pathogenesis of numerous inflammatory diseases such as rheumatoid arthritis, cancer, and atherosclerosis. Therefore, the inhibition of NO could be a good strategy for the treatment of diseases accompanying the overproduction of NO. There have been substantial efforts in the pharmaceutical industry to discover potent, selective iNOS inhibitors.[5-8]

The best-known inhibitors of iNOS were analogs of L-arginine such as N^G-methyl-L-arginine (L-NMA)[9] and N^G-nitro-L-arginine (L-NNA)[10] which exhibit poor selectivity for iNOS over eNOS. Recently, various small molecules of iNOS inhibitors that are structurally distinct from arginine have been reported such as amidines,[11] isoquinolinamines,[12] and isothioureas.[13,14] But most of the inhibitors are neither potent nor NOS isoforms selective enough to be applied *in vivo*.

Our initial search for iNOS inhibitors focused on urea derivatives since urea,[15] thiourea,[16] and isothiourea[13,14,17] have been reported to inhibit iNOS expression and/or NO production. Previously, we have reported urea, thiourea and isothiourea derivatives as inhibitors of NO production in LPS activated macrophages.[18,19] We further investigated structure-activity relationship of thiourea derivatives on the inhibition of NO production. Herein, we report the design and synthesis of modified thioureas. Structural feature of the thioureas are composed of lipophilic tail, spacer and thiourea head. Modification of the lipophilic group, spacer and substituents at nitrogen of thiourea were furnished as depicted in Figure 1. Their inhibitory activities for the NO production were evaluated in LPS-activated macrophage cell culture system.

Experimental

The heterocycle-linked thioureas were prepared from appropriate lipophilic heterocyles such as indole, phenoxazine, and carbazole (Scheme 1). Linker was introduced by *N*-alkylation with iodoethanol and the hydroxyl group was mesylated to give compounds **6**. The resulted mesylates were treated with *p*-nitrophenol in the presence of NaH to afford corresponding nitro compounds **7**. Reduction of nitro group over 10% Pd/C under atmospheric pressure of hydrogen gas provided amines **8**, which

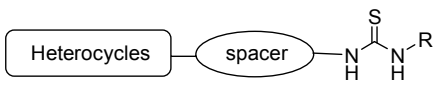
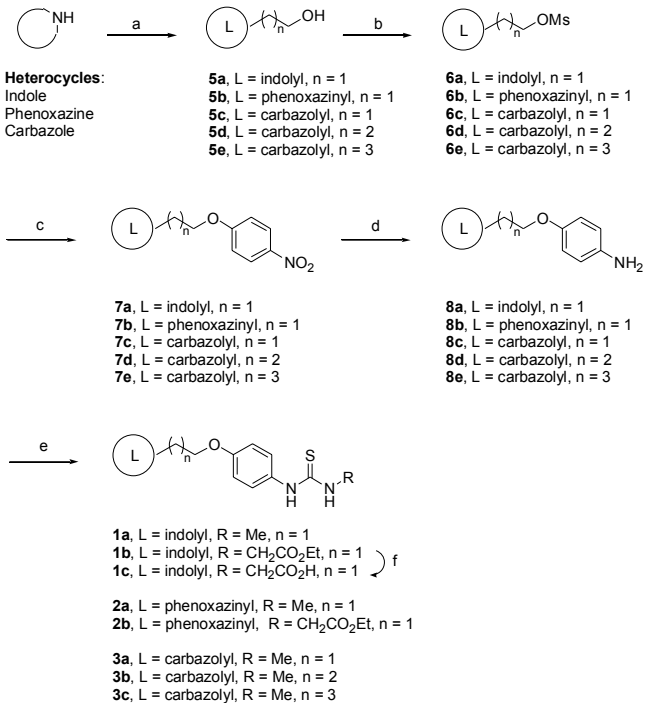
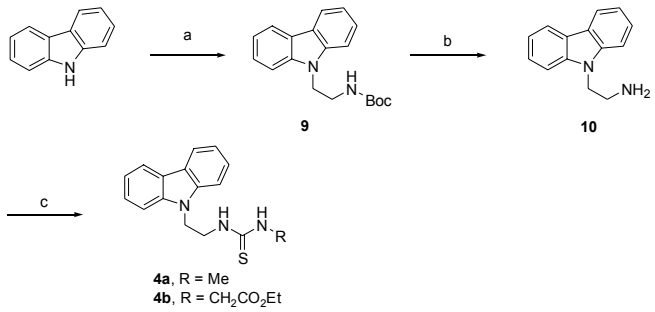


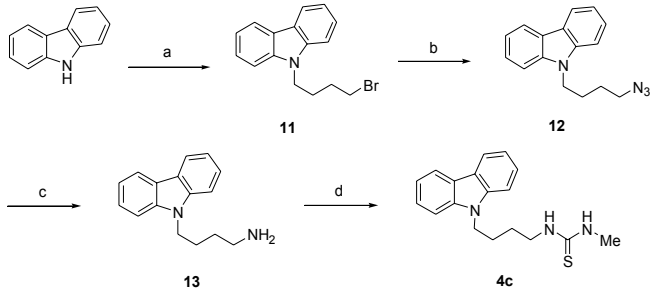
Figure 1. Representative structure of the prepared thioureas.



Scheme 1. Preparation of hetorocyle-linked phenylthiourea derivatives. Reaction conditions: (a) 2-Iodoethanol, NaH, DMF; (b) MsCl, TEA, DMF; (c) 4-Nitrophenol, NaH, DMF; (d) H_2, 10% Pd/C, THF; (e) SCNR, K_2CO_3, THF; (f) NaOH, THF/MeOH/H_2O.



Scheme 2. Preparation of carbazole-linked thiourea derivatives. Reaction conditions: (a) $Br(CH_2)_2NHBoc$, NaH, DMF; (b) TFA/MC=1:1; (c) **4a** : SCNMe, K_2CO_3, THF, **4b** : $SCNCH_2CO_2Et$, K_2CO_3, THF.



Scheme 3. Preparation of carbazole-linked thiourea derivatives. Reaction conditions: (a) 1,4-Dibromobutane, K_2CO_3, DMF; (b) NaN_3, DMF; (c) H_2, 10% Pd/C, THF; (d) SCNMe, K_2CO_3, THF.

were condensed with appropriate isothiocyanates to give the desired thioureas **1**-**3**.

Spacer-modified thioureas were also prepared to figure out the optimal size and type of spacer. Lipophilic moiety was directly connected to nitrogen of thiourea *via* methylene spacer without phenyl group. The preparation of directly *N*-substituted carbazolylethylthiourea is outlined in Scheme 2. *N*-alkylation of carbazole with Boc protected bromoethylamine and following deprotection of Boc group provided amine **10**. The obtained amine **10** was condensed with the appropriate isothiocyanates to give thioureas **4**. For the lengthening of spacer, carbazole was treated with 1,4-dibromobutane to afford compound **11**. Nucleophilic substitution of bromide **11** using NaN_3 gave azide **12**, which was reduced to amine **13**. Condensation of amine with methylisothiocyanate gave thiourea **4b**.

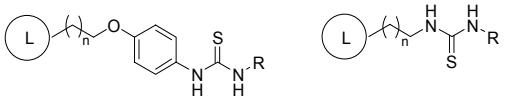
The inhibitory activity of NO production by prepared thioureas were determined by monitoring the amount of NO in cell culture media. In brief, murine macrophage cell line, RAW 264.7 cells were stimulated with 1 μg/mL of LPS in the presence or absence of samples for 20 h. The amounts of NO released into culture media were determined by the Griess method[20] in the form of nitrite.[21]

Results and Discussion

Our previous study with thiourea derivatives showed that the existence of suitable lipophilic moiety such as the carbazole group had beneficial effect on the inhibitory activity of NO production. We started with the SAR study of the lipophilic tail, and the results were summarized in Table 1. Activity of the thioureas was highly influenced by the modification of lipophilic moiety. When phenoxazine (**2a**) or carbazole (**3a**) moiety was replaced with indole (**1a**), the inhibitory activity toward NO production by **2a** or **3a** was decreased from 80% to 32% at 10 μM. Activity of other bicyclic benzoxazole-containing thiourea was as low as **1a** (data not shown). Regarding the N3 substituent of thioureas, the activities of methyl substituted derivatives were slightly higher than alkoxycarbonylmethyl substituted derivatives except **1b**. We next attempted to see the effect of spacer between lipophilic tail and thiourea head on the activity. Carbazole derivatives **3**, which have phenyl group in the spacer, were compared with compounds **4** in which the spacers were shortened by omitting the phenyl group. The activity of compounds **4** was lower than those of the corresponding compounds **3** having longer spacer. However, the activity of compounds **4a** was increased by homologation (**4b**) of the spacer with two methylene units. This result suggested that appropriate length of the spacer was necessary for the activity. Among the prepared thioureas, phenoxazine-containing derivative, **2a** was the most potent. IC_{50} values of compounds **2a** and **3a-c** were determined as 2.32, 3.29, 4.02 and 6.01 μM, respectively.

To elucidate the mechanism for the inhibition of NO production by thioureas, we examined the effects of **2a**, **2b**, **3b** and **3c** on the expression of iNOS mRNA in LPS-activated RAW 264.7 cells. At RT-PCR analysis,[22] the mRNA of iNOS was induced by the treatment of 1 μg/mL LPS for 6 hr. Curcumin was used as positive control in RT-PCR experiment. The treatment of **2a** and **3b** suppressed the expression of iNOS mRNA signi-

Table 1. Inhibitory activities of heterocycle-linked thioureas on the NO production in LPS-activated macrophages.



1, L = indolyl
2, L = phenoxazinyl
3, L = carbazolyl

4, L = carbazolyl

Compounds	R	n	Inh(%)[a] 10 μM	IC_{50} (μM)[b]
1a	Me	1	32	
1b	CH_2CO_2Et	1	43	
1c	CH_2CO_2H	1	6	
2a	Me	1	82	2.32 ± 0.29
2b	CH_2CO_2Et	1	41	15.17 ± 1.14
3a	Me	1	87	3.29 ± 0.55
3b	Me	2	77	4.02 ± 0.12
3c	Me	3	61	6.01 ± 0.17
4a	Me	1	23	31.62 ± 1.17
4b	CH_2CO_2Et	1	14	
4c	Me	3	50	13.04 ± 1.38

[a]Values mean the inhibition (%) of NO production of compounds relative to the LPS control (n = 3). [b]Values are means ± SD of three experiments.

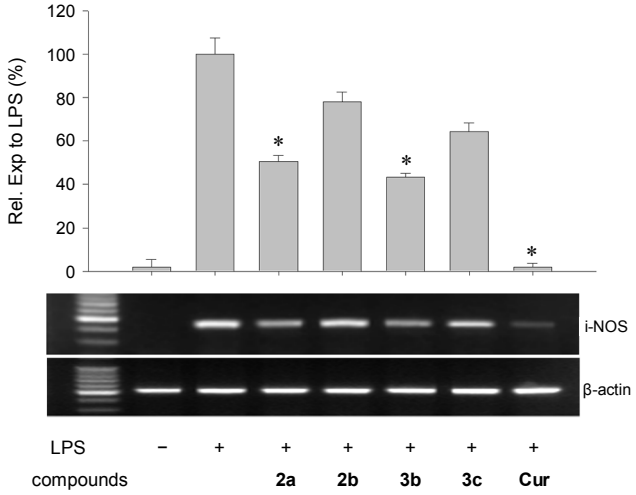


Figure 2. Effects of thioureas on the expression of iNOS mRNA in LPS-activated macrophages. RAW 264.7 cells were treated for 6 h with compounds **2a**, **2b**, **3b**, **3c** and curcumin (cur: as positive control)(10 μM) during LPS (1 μg/mL) activation. The mRNA levels of iNOS and β-actin were determined by RT-PCR from total RNA extracts. The relative iNOS mRNA levels were normalized with the respective amounts of β-actin. Values represent mean ± SD of three independent densitometric analyses of bands. *$p < 0.01$ indicate significant difference between LPS alone control and sample treatment.

ficantly at 10 μM (Figure 2). These results suggested that the inhibition of NO production by thiourea derivatives was due to the suppression of iNOS mRNA.

Conclusion

We prepared a series of heterocycle-linked thiourea derivatives and evaluated their inhibitory activities of NO production in LPS-activated macrophages. Several thioureas showed promising inhibitory activities of NO production. The SAR study demonstrated that the modification of lipophilic moiety, *N*-substituents of the thioureas, and the spacer between lipophilic tail and thiourea head highly influenced the inhibitory activity of NO production. Among prepared compounds, phenoxazine-containing derivative **2a** was the most potent with 2.32 μM of IC_{50} value. RT-PCR analysis suggested that the prepared thioureas inhibited NO production through the suppression of iNOS mRNA expression. The result provided our thiourea derivatives might serve as good leads for the development of therapeutic agents for the management of diseases accompanying overproduction of NO.

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Supporting Information. It is available on request from the correspondence author (Raok Jeon, Phone: 82-2-710-9571, Fax: 82-2-715-9571, E-mail: rjeon@sookmyung.ac.kr).

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21. Cell culture and nitrite assay in LPS-activated RAW 264.7 cells- Cells in 10% fetal bovine serum (FBS)-DMEM medium, were plated in 48-well plates (1×10^5 cells/mL), and then incubated for 24 h. The cells were replaced with fresh media with 1% FBS, and then incubated for 20 h in the presence or absence of test compounds with LPS (1 μg/mL). NO production in each well was assessed by measuring the accumulated nitrite in culture supernatant. Culture media (100 μL) were incubated with Griess reagent (150 μL) for 10 min at room temperature in 96 well microplate. Absorbance at 570 nm was read using an ELISA plate reader. A standard calibration curve was prepared using sodium nitrite as a standard. A dose-response curve was prepared, and the results were typically expressed as IC_{50} values.
22. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of iNOS mRNA expression - RAW 264.7 cells (1.6×10^6 cells/60 mm dish) were stimulated for 6 h with LPS (1 μg/mL) in the absence or presence of test samples. Total RNA was isolated from cell pellet using an RNA isolation reagent (Trizol, Invitrogen, Carlsbad, CA). Two microgram of RNA was reverse transcribed into cDNA using reverse transcriptase (Invitrogen, Carlsbad, CA) and random hexamer (Cosmo, Seoul, Korea). The PCR samples, contained in the reaction mixture, were comprised of mixture buffer, dNTP, Taq DNA polymerase (Promega, Madison, WI) and primers (sense and antisense). The sense and antisense primers for iNOS were 5'-CCCTTCCGAAGTTTCTGGCAGCAG-3' and 5'-GGCTGTCAGAGCCTCGTGGCTTTGG-3', respectively. The sesnse and antisense primers for β-actin were 5'-TGTGATGGT GGGAATGGGTCAG-3' and 5'-TTTGATGTCACGCACGATT TCC-3', respectively. The PCR amplification was performed under following conditions; 25 cycles of denaturation at 94 ^{o}C for 30 s, annealing at 55 ^{o}C for 30 s and extension at 72 ^{o}C for 30 s, using thermal cycler (Gene Amp PCR system 2400, Applied Biosystems, Foster City, CA). The amplified PCR products were separated on a 1% agarose gel.

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Key Words:

Experimental

Materials. Most of the reagents and solvents were purchased from Aldrich chemicals and used without purification, with the following exceptions. Ethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl. Acetonitrile, methylene chloride, benzene, toluene, triethylamine, pyridine, dimethyl formamide, and diisopropylamine were distilled from calcium hydride under nitrogen atmosphere. Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco Laboratories (Detroit, MI). Lipopolysaccharide (LPS, *Escherichia coli*, 0127:B8), bovine serum albumin, sodium nitrite, naphthylethylene diamine, sulfanilamide, aminoguanidine, L-arginine, N-(1-naphthyl) ethylenediamine and N^G-monomethyl-L-arginine (L-NMMA) were obtained from Sigma Chemical Co. (St. Louis, MO). Anti-mouse iNOS polyclonal antibody was purchased from Transduction Laboratories (Lexington, KY) and anti-β-actin monoclonal antibody from Sigma Chemical Co. (St. Louis, MO). Flash column chromatography (FCC) was performed using silica gel 60 (230 - 400 mesh, Merck) with the indicated solvents. Thin-layer chromatography (TLC) was performed using Kieselgel 60 F_{254} plates (Merck). IR spectra were recorded on a JASCO FT/IR 430 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian YH 400 spectrometer as solutions in $CDCl_3$, CH_3OH-d_4 or DMSO-d_6. Chemical shifts are expressed in parts per million (ppm, δ) downfield from an internal standard, tetramethylsilane.

Synthesis of the compounds.

1-[4-(2-Indol-1-yl-ethoxy)-phenyl]-3-methylthiourea (1a): To a mixture of **8a** (70.2 mg, 0.28 mmol), K_2CO_3 (46.1 mg, 0.33 mmol) and anhydrous THF (5 mL) was added methylisothiocyanate (24.4 mg, 0.33 mmol). The mixture was stirred at rt for 8 h and then the reaction mixture was diluted and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, *n*-hexane/EtOAc = 2:1) to afford 69.4 mg (0.21 mmol, 77%) of the title compound **1a** as a white solid.: R_f = 0.39 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 3202, 2929, 1509, 1239, 1057, 905, 832; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.61 (d, 1H, *J* = 8.0 Hz), 7.38 (d, 1H, *J* = 8.0 Hz), 7.22 (t, 1H, *J* = 7.6), 7.17 (d, 1H, *J* = 2.8 Hz), 7.10 (t, 1H, *J* = 7.6 Hz), 7.02 (d, 2H, *J* = 8.8 Hz), 6.74 (d, 2H, *J* = 8.8 Hz), 6.49 (d, 1H, *J* = 2.8 Hz), 5.84 (br s, 1H), 4.50 (t, 2H, *J* = 4.8 Hz), 4.20 (t, 2H, *J* = 4.8 Hz), 3.37 (d, 1H, *J* = 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9, 157.6, 136.3, 128.9, 128.7, 127.8, 121.8, 121.3, 119.8, 115.9, 109.6, 101.9, 67.5, 45.9, 32.2.

{3-[4-(2-Indole-1-ylethoxy)phenyl]thioureido} acetic acid ethyl ester (1b): To a mixture of **8a** (350 mg, 1.39 mmol), K_2CO_3 (288 mg, 2.08 mmol) and anhydrous THF (7 mL) was added ethylisothiocyanatoacetate (302 mg, 2.08 mmol). The mixture was stirred at rt for 5 h and then the reaction mixture was diluted and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, *n*-hexane/EtOAc = 2:1) to afford 300 mg (0.75 mmol, 54%) of the title compound **1b** as a yellow oil.: R_f = 0.55 (*n*-hexane/EtOAc = 2/1); IR (neat, cm^{-1}) 3355, 1613, 1463, 1312, 1139, 1024; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, 1H, *J* = 7.6 Hz), 7.40 (d, 1H, *J* = 8.0 Hz), 7.25-7.20 (m, 2H), 7.15-7.10 (m, 3H), 6.87 (d, 2H, *J* = 7.2 Hz), 6.52 (d, 1H, *J* = 3.2 Hz), 6.34 (br s, 1H), 4.54 (t, 2H, *J* = 4.4 Hz), 4.39 (s, 2H), 4.28 (t, 2H, *J* = 5.2 Hz), 4.19 (q, 2H, *J* = 7.2 Hz), 1.26 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.3, 169.8, 158.0, 136.2, 128.9, 128.8, 128.5, 127.7, 121.9, 121.3, 119.8, 116.1, 109.3, 102.0, 67.4, 61.9, 47.0, 45.8, 14.3.

{3-[4-(2-Indole-1-ylethoxy)phenyl]thioureido} acetic acid (1c): To a mixture of **1b** (50 mg, 0.13 mmol) in THF (1 mL), MeOH (1 mL) and H_2O (1 mL) was added NaOH (10.1 mg, 0.25 mmol). The mixture was stirred at room temperature for 2 h and then the reaction mixture was concentrated and neutralized by the addition of aqueous HCl solution. The mixture was extracted with ethyl acetate and washed with water and dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent; *n*-hexane/ethyl acetate/methanol = 1:1 v/v) to afford **1c** (18.6 mg, 0.050 mmol, 40%) of the title compound as pale yellow solid. R_f = 0.18 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 3291, 3052, 1592, 1463, 1315, 1238, 1063; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.1 (br s, 1H), 7.81 (br s. 1H), 7.55 (td, 2H, *J* = 7.6, 0.8 Hz), 7.42 (d, 1H, *J* = 3.2 Hz), 7.33 (d, 2H, *J* = 9.2 Hz), 7.16-7.12 (m, 1H), 7.04-7.00 (m, 1H), 6.82 (dd, 2H, *J* = 6.8, 2.4 Hz), 6.44 (dd, 2H, *J* =3.2, 0.8 Hz), 4.55 (t, 2H, *J* = 5.6 Hz), 4.26 (t, 2H, *J* = 5.6 Hz), 3.76 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.0, 172.36, 150.3, 136.6, 133.8, 129.7, 128.8, 125.6, 121.7, 121.0, 119.7, 114.8, 110.6, 101.4, 67.8, 50.0, 45.7.

1-Methyl-3-[4-(2-phenoxazin-10-ylethoxy)phenyl]thiourea

(2a): Reaction of **8b** (100 mg, 0.31 mmol) with methylisothiocyanate (34 mg, 0.47 mmol), K_2CO_3 (65 mg, 0.47 mmol) and anhydrous THF (4 mL), as described above, gave **2a** (83 mg, 0.21 mmol, 68%) as a orange solid.: R_f = 0.55 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 3387, 3211, 1540, 1508, 1490, 1464, 1376, 1273, 1042; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (br s, 1H), 7.07 (d, 2H, *J* = 8.8 Hz), 6.85 (dd, 2H, *J* = 6.8, 2.0 Hz), 6.76 (td, 2H, *J* = 7.6, 1.6 Hz), 6.64 (td, 2H, *J* = 7.6, 1.6 Hz), 6.59-6.00 (m, 4H), 5.83 (br s, 1H), 4.17 (t, 2H, *J* = 6.4 Hz), 3.94 (t, 2H, *J* = 6.4 Hz), 3.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.3, 158.0, 145.0, 133.2, 129.2, 128.1, 123.8, 121.6, 116.1, 115.8, 111.9, 64.3, 44.0, 32.3.

{3-[4-(2-Phenoxazin-10-ylethoxy)phenyl]thioureido} acetic acid ethyl ester (2b): Reaction of **8b** (100 mg, 0.31 mmol) with ethylisothiocyanatoacetate (68 mg, 0.47 mmol), K_2CO_3 (65 mg, 0.47 mmol) and anhydrous THF (4 mL), as described above, gave **2b** (83 mg, 0.21 mmol, 68%) as a orange solid.: R_f = 0.48 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 3326, 3213, 1738, 1592, 1508, 1490, 1273, 1220, 1029; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (br s, 1H), 7.19 (d, 2H, *J* = 8.8 Hz), 6.93 (d, 2H, *J* = 8.8 Hz), 6.80 (td, 2H, *J* = 7.6, 2.0 Hz), 6.71-6.62 (m, 6H), 6.39 (br s, 1H), 4.41 (s, 2H), 4.23-4.17 (m, 4H), 3.99 (t, 2H, *J* = 6.4 Hz), 1.27 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.4, 169.9, 158.1, 145.5, 133.2, 128.9, 127.8, 123.9, 121.7, 116.2, 115.8, 111.8, 64.1, 61.9, 47.1, 44.0, 14.3.

1-[4-(2-Carbazol-9-ylpropoxy)phenyl]-3-methylthiourea (3a): Reaction of **8c** (85.9 mg, 0.28 mmol) with methylisothiocyanate (24.9 mg, 0.34 mmol), K_2CO_3 (47.1 mg, 0.34 mmol) and anhydrous THF (5 mL), as described above, gave **3a** (72.2 mg, 0.19 mmol, 67%) as a white solid.: R_f = 0.22 (*n*-hexane/EtOAc = 1/1); IR (KBr pellet, cm^{-1}) 3378, 3194, 1729, 1538, 1507, 1456, 1321, 1041; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (br s, 1H), 8.10 (d, 2H, *J* = 7.6 Hz), 7.65 (d, 2H, *J* = 8.4 Hz), 7.42 (t, 2H, *J* = 7.2 Hz), 7.25 (br s, 1H), 7.17 (t, 2H, *J* = 8.0 Hz), 7.06 (d, 2H, *J* = 8.4 Hz), 6.72 (d, 2H, *J* = 6.8 Hz), 4.76 (t, 2H, *J* = 5.2 Hz), 4.29 (t, 2H, *J* = 5.2 Hz), 2.78 (d, 3H, *J* = 4.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.0, 156.1, 140.9, 132.4, 126.3, 122.9, 120.8, 120.8, 119.6, 115.1, 110.3, 67.4, 42.8, 31.1.

1-[4-(3-Carbazol-9-ylpropoxy)phenyl]-3-methylthiourea (3b): Reaction of **8d** (150 mg, 0.47 mmol) with methylisothiocyanate (52 mg, 0.71 mmol), K_2CO_3 (131 mg, 0.71 mmol) and anhydrous THF (5 mL), as described above, gave **3b** (90 mg, 0.23 mmol, 49%) as a yellow solid.: R_f = 0.44 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 3202, 2947, 1595, 1509, 1453, 1326, 1242, 1052; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, 2H, *J* = 8 Hz), 7.78 (br s, 1H), 7.39-7.38 (m, 4H), 7.23-7.19 (m, 2H), 7.09 (d, 2H, *J* = 8.8 Hz), 6.84 (dd, 2H, *J* = 6.8, 2 Hz), 5.82 (br s, 1H), 4.53 (t, 2H, *J* = 6.4 Hz), 3.84 (t, 2H, *J* = 5.6 Hz), 3.10 (s, 3H), 2.34 (quintet, 2H, *J* = 6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.4, 158.3, 140.6, 128.8, 128.1, 125.9, 123.1, 120.6, 119.2, 116.1, 108.8, 65.1, 39.5, 32.3, 28.8.

1-[4-(4-Carbazol-9-ylbutoxy)phenyl]-3-methylthiourea (3c): Reaction of **8e** (200 mg, 0.61 mmol) with methylisothiocyanate (88 mg, 1.21 mmol), K_2CO_3 (167 mg, 1.21 mmol) and anhydrous THF (8 mL), as described above, gave **3c** (120 mg, 0.30 mmol, 49%) as a yellow solid.: R_f = 0.45 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 3196, 2940, 1595, 1509, 1452, 1327, 1240, 1052; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 2H, *J* = 7.6 Hz), 7.64 (br s, 1H), 7.48-7.40 (m, 4H), 7.25-7.22 (m, 2H), 7.09 (d, 2H, *J* = 8.8 Hz), 6.81 (dd, 2H, *J* = 6.8, 2.4 Hz), 5.80 (br s, 1H), 4.40 (t, 2H, *J* = 6.8 Hz), 3.90 (t, 2H, *J* = 6.0 Hz), 3.10 (s, 3H), 2.12-2.04 (m, 2H), 1.88-1.81 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.4, 158.6, 140.5, 128.5, 128.2, 125.9, 123.1, 120.7, 119.1, 116.0, 108.8, 68.0, 42.9, 32.3, 27.1, 26.0.

1-(2-Carbazol-9-ylethyl)-3-methylthiourea (4a): Reaction of carbazole **10** (100 mg, 0.48 mmol) with methylisothiocyanate (53 mg, 0.72 mmol), K_2CO_3 (100 mg, 0.72 mmol) and anhydrous THF (4 mL), as described above, gave **4a** (62 mg, 0.22 mmol, 46%) as a white solid.: R_f = 0.33 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 3373, 1596, 1484, 1326, 1204; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, 2H, *J* = 7.6 Hz), 7.46-7.44 (m, 4H), 7.25-7.21 (m, 2H), 5.88 (br s, 1H), 5.27 (br s, 1H), 4.63 (t, 2H, *J* = 5.6 Hz), 4.04 (br s, 2H), 2.47 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.9, 140.6, 126.2, 123.1, 120.6, 119.6, 109.0, 44.1, 42.1, 32.2.

[3-(2-Carbazol-9-ylethyl)thioureido]acetic acid ethyl ester (4b): Reaction of **10** (200 mg, 0.95 mmol) with ethylisothiocyanatoacetate (208 mg, 1.43 mmol), K_2CO_3 (197 mg, 1.43 mmol) and anhydrous THF (8 mL), as described above, gave **4b** (165 mg, 0.46 mmol, 49%) as a yellow oil.: R_f = 0.18 (*n*-hexane/EtOAc = 2/1); IR (neat, cm^{-1}) 3362, 1596, 1484, 1326, 1218, 1021; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, 2H, *J* = 8.0 Hz), 7.41-7.40 (m, 4H), 7.20-7.16 (m, 2H), 5.86 (s, 2H), 4.57 (t, 2H, *J* = 5.2 Hz), 4.10-4.08 (m, 2H), 3.96-3.91 (m, 4H), 1.16 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.4, 170.4, 140.6, 126.2, 123.0, 120.5, 119.5, 109.0, 61.9, 46.1, 43.7, 42.1, 14.2.

1-(4-Carbazol-9-ylbutyl)-3-methylthiourea (4c): Reaction of **13** (100 mg, 0.44 mmol) with methylisothiocyanate (48 mg, 0.66 mmol), K_2CO_3 (91 mg, 0.66 mmol) and anhydrous THF (4 mL), as described above, gave **4c** (52 mg, 0.17 mmol, 38%) as a white solid.: R_f = 0.20 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 3368, 1596, 1486, 1323, 1219; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 2H, *J* = 7.6 Hz), 7.44 (td, 2H, *J* = 8.0, 1.2 Hz), 7.40 (d, 2H, *J* = 8.0 Hz), 7.25-7.22 (m, 2H), 5.59 (br s, 1H), 5.40 (br s, 1H), 4.37 (t, 2H, *J* = 6.8 Hz), 3.39 (s, 2H), 2.84 (s, 3H), 1.99-1.91 (m, 2H), 1.61-1.59 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.2, 140.5, 126.0, 123.0, 120.7, 119.2, 108.9, 44.3, 42.7, 30.7, 27.1, 26.3.

2-Indolylethanol (5a): To a mixture of indole (1000 mg, 8.54 mmol), NaH (60% dispersion in mineral oil, 683 mg, 17.07 mmol) and anhydrous DMF (20 mL) was added 2-iodoethanol (2936 mg, 17.07 mmol). The mixture was stirred at 70 °C for 24 h and then the reaction mixture was diluted and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, *n*-hexane/EtOAc = 2:1) to afford 1100 mg (6.82 mmol, 82%) of the title compound **5a** as a brown oil.: R_f = 0.27 (*n*-hexane/EtOAc = 2/1); IR (neat, cm^{-1}) 3357, 2930, 1509, 1461, 1400, 1335, 1315, 1186, 1064, 862; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, 1H, *J* = 8.0 Hz), 7.33 (d, 1H, *J* = 8.4 Hz), 7.23 (t, 1H, *J* = 7.6 Hz), 7.14 (t, 1H, *J* = 7.6 Hz), 7.10 (d, 1H, *J* = 2.8 Hz), 6.51 (d, 1H, *J* = 3.2 Hz),

4.13 (t, 2H, J = 5.4 Hz), 3.76 (t, 2H, J = 5.4 Hz), 1.96 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.3, 128.9, 128.7, 121.9, 121.3, 119.8, 109.7, 101.5, 61.9, 48.8.

2-Phenoxazin-10-yl-ethanol (5b): Reaction of phenoxazine (1000 mg, 5.46 mmol) with 2-iodoethanol (1878 mg, 10.92 mmol), NaH (60% dispersion in mineral oil, 437 mg, 10.92 mmol) and anhydrous DMF (20 mL), as described above, gave **5b** (1053 mg, 4.63 mmol, 85%) as a black solid.: R_f = 0.26 (*n*-hexane/EtOAc = 3/1); IR (neat, cm^{-1}) 3180, 3063, 2915, 1491, 1463, 1353, 1325, 1272; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (td, 2H, J = 8, 2 Hz), 6.67-6.58 (m, 6H), 3.89 (t, 2H, J = 5.6 Hz), 3.72 (t, 2H, J = 5.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.0, 133.7, 123.8, 121.5, 115.7, 112.0, 59.4, 47.0.

2-Carbazol-9-ylethanol (5c): Reaction of carbazole (2.0 g, 11.96 mmol) with 2-iodoethanol (4.1 g, 23.92 mmol), NaH (60% dispersion in mineral oil, 956.9 mg, 23.92 mmol) and anhydrous DMF (20 mL), as described above, gave **5c** (1640 mg, 7.84 mmol, 65%) as a yellow oil.: R_f = 0.18 (*n*-hexane/EtOAc = 2/1); IR (neat, cm^{-1}) 3253, 3048, 2914, 1492, 1483, 1457, 1348, 1325; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (td, 2H, J = 7.6, 1.2 Hz), 7.44-7.39 (m, 4H), 7.22 (td, 2H, J = 7.6, 1.2 Hz), 4.38 (t, 2H, J = 5.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.9, 126.0, 123.1, 120.6, 119.4, 109.0, 61.6, 45.6.

3-Carbazol-9-ylpropan-1-ol (5d): Reaction of carbazole (1000 mg, 5.98 mmol) with 3-iodopropanol (2224 mg, 11.96 mmol), NaH (60% dispersion in mineral oil, 478 mg, 11.96 mmol) and anhydrous DMF (20 mL), as described above, gave **5d** (880 mg, 3.91 mmol, 65%) as a white solid.: R_f = 0.20 (*n*-hexane/EtOAc = 4/1); IR (neat, cm^{-1}) 3365, 3050, 2941, 1596, 1484, 1452, 1345, 1326; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, 2H, J = 8.0 Hz), 7.45-7.44 (m, 4H), 7.25-7.20 (m, 2H), 4.42 (t, 2H, J = 6.8 Hz), 3.56 (t, 2H, J = 6.0 Hz), 2.10-2.09 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.7, 125.9, 123.1, 120.6, 119.1, 108.9, 59.8, 39.4, 31.6.

Methanesulfonic acid 2-indol-1-ylethylester (6a): To a mixture of **5a** (1000 mg, 6.20 mmol), triethylamine (941 mg, 9.30 mmol) and anhydrous DMF (20 mL) was added methanesulfonyl chloride (853 mg, 7.44 mmol). The mixture was stirred at rt for 3 h and then the reaction mixture was diluted and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, *n*-hexane/EtOAc = 2:1) to afford 1037 mg (4.33 mmol, 70%) of the title compound **6a** as a red solid.: R_f = 0.62 (*n*-hexane/EtOAc = 1:1); IR (neat, cm^{-1}) 1513, 1463, 1352, 1174, 971, 906; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, 1H, J = 8.0 Hz), 7.32 (d, 1H, J = 8.0 Hz), 7.22 (dt, 1H, J = 7.4, 1.2 Hz), 7.13 (dt, 1H, J = 7.4, 1.2 Hz), 7.11 (d, 1H, J = 3.2 Hz), 6.52 (d, 1H, J = 3.2 Hz), 4.48 (t, 2H, J = 4.8 Hz), 4.45 (t, 2H, J = 4.8 Hz), 2.55 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.0, 128.9, 128.3, 122.3, 121.5, 120.2, 109.2, 102.6, 68.3, 45.5, 37.2.

Methanesulfonic acid 2-phenoxazin-10-ylethylester (6b): Reaction of **5b** (1000 mg, 5.46 mmol) with methanesulfonyl chloride (605 mg, 5.28 mmol), triethylamine (668 mg, 6.60 mmol) and anhydrous DMF (20 mL), as described above, gave **6b** (1040 mg, 3.41 mmol, 77%) as a green solid.: R_f = 0.66 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 2941, 1491, 1465, 1357, 1274, 1175, 1011, 915; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84-6.80 (m, 2H), 6.69-6.65 (m, 4H), 6.56 (d, 2H, J = 7.6 Hz), 4.41 (t, 2H, J = 6.8 Hz), 3.94 (s, 2H), 3.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.0, 132.6, 124.0, 122.1, 116.0, 111.7, 64.4, 43.6, 37.8.

Methanesulfonic acid 2-carbazol-9-ylethyl ester **(6c):** Reaction of **5c** (1.6 g, 7.84 mmol) with methanesulfonyl chloride (1.07 g, 9.36 mmol), triethylamine (1.58 g, 15.61 mmol) and anhydrous DMF (20 mL), as described above, gave **6b** (1960 mg, 6.78 mmol, 88%) as a yellow solid.: R_f = 0.59 (*n*-hexane/EtOAc = 1/1); IR (neat, cm^{-1}) 2922, 1487, 1458, 1351, 1172, 1013, 916; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, 2H, J = 8.0 Hz), 7.48-7.40 (m, 4H), 7.27 (t, 2H, J = 8.0 Hz), 4.61 (t, 2H, J = 4.8 Hz), 4.55 (t, 2H, J = 5.2 Hz), 2.49 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.7, 126.5, 122.9, 120.9, 119.8, 110.1, 69.1, 42.4, 37.1.

Methanesulfonic acid 3-carbazol-9-ylpropylester (6d): Reaction of **6c** (800 mg, 3.55 mmol) with methanesulfonyl chloride (488 mg, 4.26 mmol), triethylamine (539 mg, 5.33 mmol) and anhydrous DMF (15 mL), as described above, gave **6d** (1011 mg, 3.33 mmol, 94%) as a white solid.: R_f = 0.48 (*n*-hexane/EtOAc = 2/1); IR (neat, cm^{-1}) 2970, 1739, 1484, 1454, 1364, 1175, 1013, 934; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, 2H, J = 8.0 Hz), 7.48-7.38 (m, 4H), 7.25-7.21 (m, 2H), 4.44 (t, 2H, J = 6.8 Hz), 4.09 (t, 2H, J = 5.6 Hz), 2.81 (s, 3H), 2.33-2.27 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4, 126.1, 123.2, 120.7, 119.5, 108.7, 67.3, 39.0, 37.3, 28.7.

1-[2-(4-Nitrophenoxy)ethyl]-*1H*-indole (7a): To a mixture of 4-nitrophenol (828 mg, 5.96 mmol), NaH (60% dispersion in mineral oil, 254 mg, 6.35 mmol) and anhydrous DMF (20 mL) was added mesylate **6a** (950 mg, 3.97 mmol). The mixture was stirred at 70 °C for 24 h and then the reaction mixture was diluted and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, *n*-hexane/EtOAc = 3:1) to afford 800 mg (2.83 mmol, 71%) of the title compound **7a** as a yellow solid.: R_f = 0.65 (*n*-hexane/EtOAc = 2:1); IR (neat, cm^{-1}) 1592, 1509, 1337, 1231, 1173, 1110, 844; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, 2H, J = 8.0 Hz), 7.57 (d, 1H, J = 8.0 Hz), 7.33 (d, 1H, J = 8.4 Hz), 7.19-7.05 (m, 3H), 6.80 (d, 2H, J = 8.4 Hz), 6.46 (d, 1H, J = 3.3 Hz), 4.51 (t, 2H, J = 5.2 Hz), 4.29 (t, 2H, J = 5.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 141.6, 136.5, 129.7, 128.8, 126.5, 121.8, 121.0, 119.7, 115.7, 110.6, 101.6, 68.6, 45.4.

10-[2-(4-Nitrophenoxy)ethyl]-*10H*-phenoxazine (7b): Reaction of mesylate **6b** (1000 mg, 3.27 mmol) with 4-nitrophenol (683 mg, 4.91 mmol), NaH (60% dispersion in mineral oil, 209 mg, 5.23 mmol) and anhydrous DMF (20 mL), as described above, gave **7b** (965 mg, 2.77 mmol, 85%) as a red solid.: R_f = 0.46 (*n*-hexane/EtOAc = 5/1); IR (neat, cm^{-1}) 3066, 2950, 1593, 1510, 1465, 1339, 1274, 1259; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, 2H, J = 7.2, 2 Hz), 6.93 (dd, 2H, J = 7.2, 2.0 Hz), 6.81 (td, 2H, J = 7.6, 2.0 Hz), 6.71-6.60 (m, 6H), 4.28 (t, 2H, J = 6.0 Hz), 4.02 (t, 2H, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 145.1, 142.1, 133.1, 126.2, 123.9, 121.8, 115.9, 114.7, 111.8, 64.7, 43.8.

9-[2-(4-Nitrophenoxy)ethyl]-9*H*-carbazole (7c): Reaction

of mesylate **6c** (1.96 g, 6.78 mmol) with *p*-Nitrophenol (1.04 g, 7.45 mmol), NaH (60% 325.5 mg, 8.14 mmol) and anhydrous DMF (20 mL), as described above, gave **7c** (1350 mg, 4.06 mmol, 60%) as a yellow solid.: R_f = 0.56 (*n*-hexane/EtOAc = 2/1); IR (neat, cm^{-1}) 3022, 2915, 1593, 1510, 1458, 1334, 1261; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, 4H, *J* = 8.8, 1.6 Hz), 7.47 (dd, 4H, *J* = 2.8, 0.8 Hz), 7.22-2.26 (m, 2H), 6.79 (dd, 2H, *J* = 8.0, 1.2 Hz), 4.74 (td, 2H, *J* = 5.6, 2.0 Hz), 4.42 (td, 2H 6.0, 1.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 141.5, 140.8, 126.5, 126.4, 122.8, 120.9, 119.6, 115.5, 110.2, 68.1, 42.4.

9-[3-(4-Nitrophenoxy)propyl]-*9H*-carbazole (7d): Reaction of mesylate **6d** (950 mg, 3.13 mmol) with 4-nitrophenol (653 mg, 4.70 mmol), NaH (60% dispersion in mineral oil, 200 mg, 5.01 mmol) and anhydrous DMF (20 mL), as described above, gave **7d** (890 mg, 2.57 mmol, 82%) as a yellow solid.: R_f = 0.40 (*n*-hexane/EtOAc = 4/1); IR (neat, cm^{-1}) 2938, 1738, 1593, 1510, 1453, 1261; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08-8.03 (m, 4H), 7.37-7.30 (m, 4H), 7.20-7.16 (m, 2H), 6.74 (dd, 2H, *J* = 6.8, 2 Hz), 4.46 (t, 2H, *J* = 6.4 Hz), 3.79 (t, 2H, *J* = 5.6 Hz), 2.32-2.26 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 141.8, 140.5, 126.1 (126.10, 126.06), 123.1, 120.7, 119.4, 114.6, 108.7, 65.5, 39.3, 28.6.

4-(2-Indole-1-ylethoxy)phenylamine (8a): A soluion of **7a** (750 mg, 2.65 mmol) in anhydrous THF (15 mL) was reacted with hydrogen over 10% Pd/C (75 mg, 10 % wt) under atmospheric pressure at rt for 20 h. The reaction mixture was filtered through a Celitr pad, and the filtrate concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, *n*-hexane/EtOAc = 2:1) to afford 550 mg (2.18 mmol, 82%) of the title compound **8a** as a black solid.: R_f = 0.11 (*n*-hexane/EtOAc = 2:1); IR (neat, cm^{-1}) 3336, 1652, 1596, 1534, 1236; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, 1H, *J* = 7.6 Hz), 7.41 (d, 1H, *J* = 8.0 Hz), 7.28-7.23 (m, 2H), 7.13 (t, 1H, *J* = 7.6 Hz), 6.70 (d, 2H, *J* = 6.4 Hz), 6.61 (d, 2H, *J* = 8.8 Hz), 6.53 (d, 1H, *J* = 2.8 Hz), 4.50 (t, 2H, *J* = 5.6 Hz), 4.23 (t, 2H, *J* = 5.2 Hz), 3.43 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.6, 140.7, 136.3, 128.8, 128.6, 121.7, 121.2, 119.6, 116.5, 116.0, 109.4, 101.7, 67.8, 46.0.

4-(2-Phenoxazin-10-ylethoxy)phenylamine (8b): Reaction of **7b** (900 mg, 2.58 mmol) with 10% Pd/C (90 mg, 10% wt) and anhydrous THF (15 mL), as described above, gave **8b** (610 mg, 1.92 mmol, 74%) as a red-brown solid.: R_f = 0.07 (*n*-hexane/EtOAc = 3/1); IR (neat, cm^{-1}) 3434, 3358, 1627, 1591, 1510, 1489, 1273, 1234; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80-6.76 (m, 2H), 6.74 (dd, 2H, *J* = 6.8, 2.0 Hz), 6.68-6.60 (m, 8H), 4.10 (t, 2H, *J* = 6.8 Hz), 3.91 (t, 2H, *J* = 6.8 Hz), 3.37 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.8, 145.1, 140.7, 133.4, 123.9, 121.4, 116.6, 115.9, 115.7, 111.9, 64.3, 44.2.

4-(2-Carbazol-9-ylethoxy)phenylamine (8c): Reaction of **7c** (1.71 g, 5.13 mmol) with 10% Pd/C (90 mg, 10% wt), anhydrous MeOH (30 mL) and anhydrous DMF (15 mL), as described above, gave **8c** (836.3 mg, 2.77 mmol, 56%) as a pale yellow solid.: R_f = 0.47 (*n*-hexane/EtOAc = 1/2); IR (neat, cm^{-1}) 3357, 3356, 1652, 1596, 1534, 1236; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (d, 2H, *J* = 7.6 Hz), 7.61 (d, 2H, *J* = 8.0 Hz), 7.41 (t, 2H, *J* = 8.0 Hz), 7.16 (t, 2H, *J* = 8.0 Hz), 6.46 (dd, 2H, *J* = 8.8, 2.0 Hz), 6.39 (dd, 2H, *J* = 4.8, 3.2 Hz), 4.68 (t, 2H, *J* = 5.6 Hz), 4.59 (br s, 2H), 4.16 (t, 2H, *J* = 5.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.1, 143.3, 140.9, 126.3, 122.8, 120.8, 119.5, 115.8, 115.5, 110.3, 67.5, 42.9.

4-(3-Carbazol-9-ylpropoxy)phenylamine (8d): Reaction of **8c** (800 mg, 2.31 mmol) with 10% Pd/C (80 mg, 10% wt) and anhydrous THF (20 mL), as described above, gave **8d** (560 mg, 1.77 mmol, 84%) as a black solid.: R_f = 0.18 (*n*-hexane/EtOAc = 2/1); IR (neat, cm^{-1}) 3440, 3359, 1596, 1509, 1220; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, 2H, *J* = 8 Hz), 7.45-7.39 (m, 4H), 7.23-7.19 (m, 2H), 6.73 (dd, 2H, *J* = 6.4, 2.4 Hz), 6.63 (dd, 2H, *J* = 6.8, 2.4 Hz), 4.54 (t, 2H, *J* = 6.8 Hz), 3.85 (t, 2H, *J* = 5.6 Hz), 2.33-2.27 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.0, 140.6, 140.3, 125.9, 123.0, 120.5, 119.1, 116.6, 115.9, 108.8, 65.3, 39.6, 29.1.

(2-Carbazol-9-ylethyl)carbamic acid tert-butylester (9): Reaction of carbazole (500 mg, 2.99 mmol) with $Br(CH_2)_2NHBoc$ (1005 mg, 4.49 mmol), NaH (60% dispersion in mineral oil, 191 mg, 4.78 mmol) and anhydrous DMF (10 mL), as described above, gave **9** (540 mg, 2.09 mmol, 70%) as a white solid.: R_f = 0.37 (*n*-hexane/EtOAc = 7/1); IR (neat, cm^{-1}) 3339, 1738, 1596, 1484, 1365, 1055; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, 2H, *J* = 7.6 Hz), 7.46-7.40 (m, 4H), 7.23 (td, 2H, *J* = 8.0, 1.2 Hz), 4.57 (br s, 1H), 4.46 (t, 2H, *J* = 5.6 Hz), 3.53 (t, 2H, *J* = 5.6 Hz), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 140.7, 126.0, 123.1, 120.6, 119.4, 108.8, 79.8, 42.6, 40.0, 28.6.

2-Carbazol-9-ylethylamine (10): A solution of **9** (600 mg, 1.93 mmol) in MC (3 mL) was added TFA (3 mL). The mixture was stirred at rt for 2 h and then the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, MeOH) to afford 350 mg (1.66 mmol, 86%) of the title compound **10** as a yellow oil.: R_f = 0.05 (MeOH); IR (neat, cm^{-1}) 3051, 1675, 1485, 1138; ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (d, 2H, *J* = 8.0 Hz), 7.56 (d, 2H, *J* = 8.4 Hz), 7.48 (td, 2H, *J* = 6.8, 1.2 Hz), 7.26-7.23 (m, 2H), 4.66 (t, 2H, *J* = 6.8 Hz), 3.40 (t, 2H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 140.4, 126.0, 123.4, 120.2, 119.6, 108.2, 40.0, 38.0.

9-(4-Bromobutyl)-*9H*-carbazole (11): Reaction of carbazole (1000 mg, 5.98 mmol) with 1,4-dibromobutane (1811 mg, 8.97 mmol), K_2CO_3 (1653 mg, 11.96 mmol) and anhydrous DMF (20 mL), as described above, gave **11** (550 mg, 1.82 mmol, 30%) as a white solid.: R_f = 0.50 (*n*-hexane/EtOAc = 7/1); IR (neat, cm^{-1}) 1594, 1467, 1197, 1022; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, 2H, *J* = 8.0, 0.8 Hz), 7.47-7.43 (m, 2H), 7.37 (d, 2H, *J* = 8.0 Hz), 7.24-7.21 (m, 2H), 4.31 (t, 2H, *J* = 6.8 Hz), 3.34 (t, 2H, *J* = 6.8 Hz), 2.06-1.98 (m, 2H), 1.91-1.84 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.5, 126.0, 123.1, 120.7, 119.2, 108.8, 42.4, 33.4, 30.5, 27.9.

9-[4-(4-Nitrophenoxy)butyl]-*9H*-carbazole (12): Reaction of **11** (500 mg, 1.65 mmol) with 4-nitrophenol (345 mg, 2.48 mmol), NaH (60% dispersion in mineral oil, 106 mg, 2.64 mmol) and anhydrous DMF (10 mL), as described above, gave **12** (475 mg, 1.32 mmol, 80%) as a yellow solid.: R_f = 0.42 (*n*-hexane/EtOAc = 3/1); IR (neat, cm^{-1}) 2970, 2949, 1739, 1592, 1508, 1365, 1261; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13-8.09 (m, 4H), 7.48-7.44 (m, 2H), 7.39 (d, 2H, *J* = 7.6 Hz), 7.23 (td, 2H, *J* = 6.8, 1.2 Hz), 6.80 (dd, 2H, *J* = 6.8, 2 Hz), 4.38 (t, 2H, *J* = 6.8 Hz), 3.93 (t, 2H, *J* = 6.0 Hz), 2.11-2.04 (m, 2H), 1.88-1.81 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 141.6,

140.5, 126.1, 125.9, 123.1, 120.7, 119.2, 114.5, 108.8, 68.5, 42.8, 27.0, 25.9.

4-(4-Carbazol-9-ylbutoxy)phenylamine (13): Reaction of **12** (400 mg, 1.11 mmol) with 10% Pd/C (40 mg, 10% wt) and anhydrous THF (10 mL), as described above, gave **13** (290 mg, 0.88 mmol, 79%) as a yellow solid.: R_f = 0.20 (*n*-hexane/EtOAc = 2/1); IR (neat, cm^{-1}) 3435, 3357, 1626, 1596, 1510, 1233; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, 2H, *J* = 7.6 Hz), 7.43-7.39 (m, 2H), 7.34 (d, 2H, *J* = 8.4 Hz), 7.21-7.17 (m, 2H), 6.63 (dd, 2H, *J* = 6.8, 2.0 Hz), 6.51 (dd, 2H, *J* = 6.8, 2 Hz), 4.26 (t, 2H, *J* = 6.8 Hz), 3.75 (t, 2H, *J* = 6.0 Hz), 3.23 (br s, 2H), 2.00-1.92 (m, 2H), 1.73-1.67 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.3, 140.7, 140.4, 126.0, 123.2, 120.7, 119.1, 116.7, 115.9, 109.0, 68.4, 43.0, 27.4, 26.2.