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In vitro activity of novel dual action MDR anthranilamide modulators with inhibitory activity at CYP-450

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Abstract—Synthesis and in vitro cytotoxicity assays of new anthranilamide MDR modulators have been performed to assess their inhibition potency of the P-glycoprotein (P-gp) transporter. The aromatic spacer group between nitrogen atoms (N^1 and N^2) in the known inhibitor XR9576 was replaced with a flexible alkyl chain of 2 to 6 carbon atoms in length. 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline and their open-chain *N*-methylhomoveratrylamine counterparts were shown to be potent P-gp inhibitors. The maximal inhibition was obtained when using an ethyl or propyl spacer. Several compounds were more potent than verapamil and intrinsically less cytotoxic than XR9576. In addition, in vitro metabolism studies of **23a** with a subset of human CYP-450 isoforms revealed that, unlike XR9576, **23a** inhibited CYP3A4, an enzyme that colocalizes with P-gp in the intestine and contributes to tumor cell chemoresistance by enhancing the biodisposition of anticancer drugs such as paclitaxel toward metabolism. In this context, **22a** might be a suitable candidate for further drug development.

1. Introduction

A major limitation to the successful chemotherapeutic treatment of cancer is the resistance, both natural and acquired, of tumor cells to cytotoxic drugs.[1] The overexpression of drug transport proteins is a major mechanism for this multiple drug resistance (MDR).[2] P-glycoprotein (P-gp) is a member of the ATP-binding cassette (ABC) family of membrane transporters that transfer substrates out of cells in an unidirectional fashion.[3] In tumor cells overexpressing P-gp, this results in reduced intracellular drug concentrations which in turn decrease the efficacy of a broad spectrum of antitumor drugs including 5-12-anthracyclinediones (e.g., daunorubicin), vinca alkaloids (e.g., vincristine), podophyllotoxins (e.g., etoposide), and taxus alkaloids (e.g., taxol).

P-gp is increasingly recognized as a key determinant in drug disposition given its expression in normal tissues exhibiting excretory functions such as liver, kidney, and intestine.[4] Expression of P-gp in such tissues results in reduced drug absorption from the gastrointestinal tract and enhanced drug elimination into bile and urine. Moreover, expression of P-gp in endothelial cell membranes of the blood–brain barrier prevents the diffusion of drugs into the central nervous system. Therefore, P-gp plays a key role in tumor chemoresistance via significant alterations of anticancer drugs' pharmacokinetics (e.g., decrease of bioavailability) and biodistribution. Furthermore, P-gp can act synergistically with cytochrome P450 (CYP) 3A4 to enhance drug metabolism and elimination.[5]

The inhibition of P-gp and other proteins related to MDR is of utmost importance in modern cancer chemotherapy. It is hoped that P-gp inhibitors could restore the therapeutic activity of anticancer agents. In that context, the MDR phenotype can be reversed in P-gp -positive cells by a large number of structurally heterogeneous molecules including notably calcium-channel blockers (e.g., verapamil)[6] immunomodulators (e.g., cyclosporin A, FK506)[7] cardiac antiarrhythmic agents (e.g., quinidine)[8] hypotensives (e.g., reserpine,[9]

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piperazine analogues[10]), dihydropyridines (e.g., nicardipine)[11] and many others.[12] Consequently, clinical trials using therapeutically relevant drugs such as verapamil,[13] tamoxifen,[14] progesterone[15] and cyclosporin A[16] have been conducted albeit with very limited successes owing to their intrinsic toxicity and inability to achieve pharmacologically effective plasma levels. A second generation of P-gp modulators such as cyclosporin D analogues (e.g., PSC 833),[17] dexverapamil, biricodar (VX-710), GF120918,[18] and MS209 exhibited improved P-gp selectivity but remained unselective toward other ABC transporters leading to deleterious drug-drug interactions. More recently, a third generation of inhibitors such as XR9576 (tariquidar),[19,20] cyclopropyldibenzosuberane derivatives (LY335979),[21] and diarylimidazoles (OC144-093)[22] has been developed. The anthranilamide derivative XR9576 is able to reverse primary doxorubicin, vinblastine, and paclitaxel resistance in advanced breast cancer. Unfortunately, a recent phase II clinical trial on breast carcinoma and a phase III on lung carcinoma assessing the efficacy of tariquidar in combination with vinorelbine versus vinorelbine alone were discontinued due to a significant proportion of adverse events in the tariquidar cohort. In addition a third clinical trial testing paclitaxel/carboplatin/tariquidar in combination was also terminated due to poor response rates and toxicity.[23] Therefore, there are unmet needs for newer P-gp inhibitors with reduced intrinsic toxicity.

We report here the synthesis of new P-gp inhibitors based on anthranilamide derivatives such as XR9576 (Fig. 1). As aforementioned, it is known that anthranilamides abrogate cell chemoresistance at low nanomolar levels. While the anthranilamide nucleus tolerates a variety of substitutions[19] with modest reduction of potency, due to the potential toxicity of the anilide core,[20] we decided to investigate the replacement of the anilide linker and investigate the effect of the distance between N^1 and N^2 on the in vitro activity (Fig. 1). To this end, we replaced the aromatic linker by alkyl chains of increasing length. In addition, we synthesized conformationally flexible open A-ring analogues corresponding to the *N*-methylhomoveratrylamine derivatives (Fig. 2). All compounds were compared to verapamil (VRP) and XR9576. Herein we describe our efforts to identify new anthranilamide-based P-gp inhibitors.

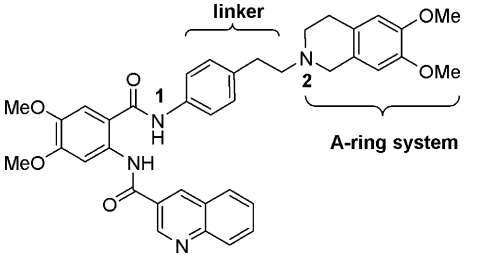


Figure 1. Structure of XR9576.

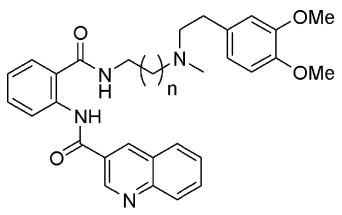
2. Chemistry

The preparation of the target compounds was achieved from commercially available veratrylamine **1a** (Scheme 1) and dimethoxytetrahydroisoquinoline **1b** (Scheme 2). The nucleophilic substitution of the secondary amines **1a** or **1b** and the chloronitriles **2**–**6** in the presence of base generates the corresponding aminonitriles **7a**–**11a** and **7b**–**11b** in good yields. Hydrogenation of **7a**–**11a** or **7b**–**11b** with Raney nickel at 40 psi produced the amines **12a**–**16a** and **12b**–**16b**, respectively. The synthesis of **17a**–**21a** or **17b**–**21b** was carried out by the acylation of isatoic anhydride with amines **12a**–**16a** or **12b**–**16b** in acetonitrile. Afterwards, anilines **17a**–**21a** or **17b**–**21b** were reacted with 3-quinoloyl chloride in the presence of Dowex 1X8 basic resin to produce compounds **22a**–**26a**.

An alternative synthetic approach to the synthesis of new anthranilamides such as compound **24b** was investigated (Scheme 2). The synthetic pathway of **24b** was identical except that the acylation reaction was performed using 2-nitrobenzoyl chloride instead of isatoic anhydride to produce the corresponding nitro derivative **19**. The nitro group was reduced by zinc metal in presence of ammonium chloride in refluxing methanol to give the aniline **19b** in 72% yields. Finally, the synthesis of **24b** was completed as by acylation of **19b** by 3-quinoloyl chloride using the basic resin Dowex 1X8 as an acid scavenger.

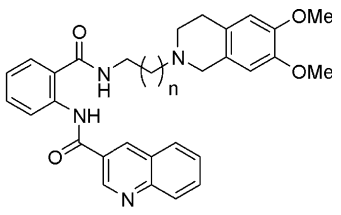
3. In vitro biological evaluation

The P-gp inhibition potency of compounds **22a**–**26a** and **22b**–**26b** was assessed using CEM/VLB_{500} human leukemia cells. The assay is based on the ability of the drug to revert the chemoresistance of these cells to daunorubicin



n = 1, 2, 3, 4, 5

22a-26a



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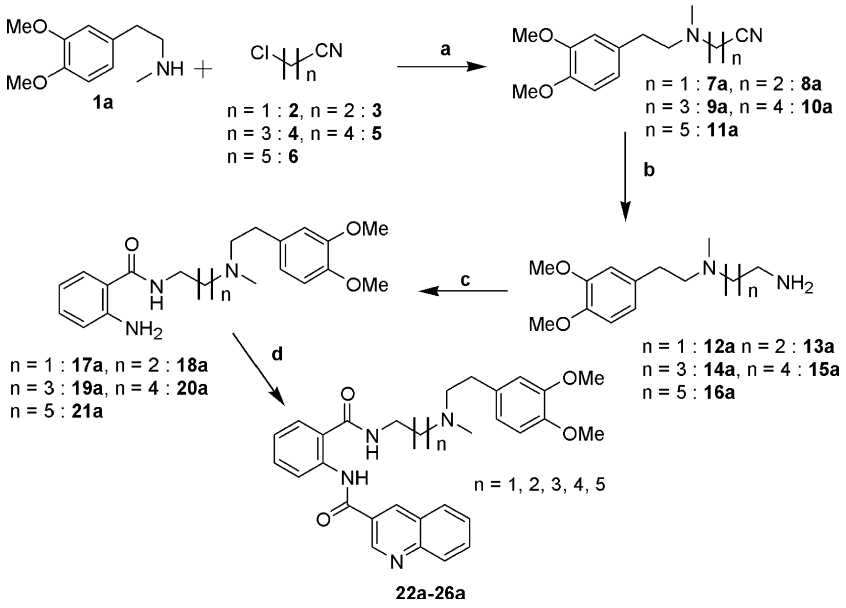
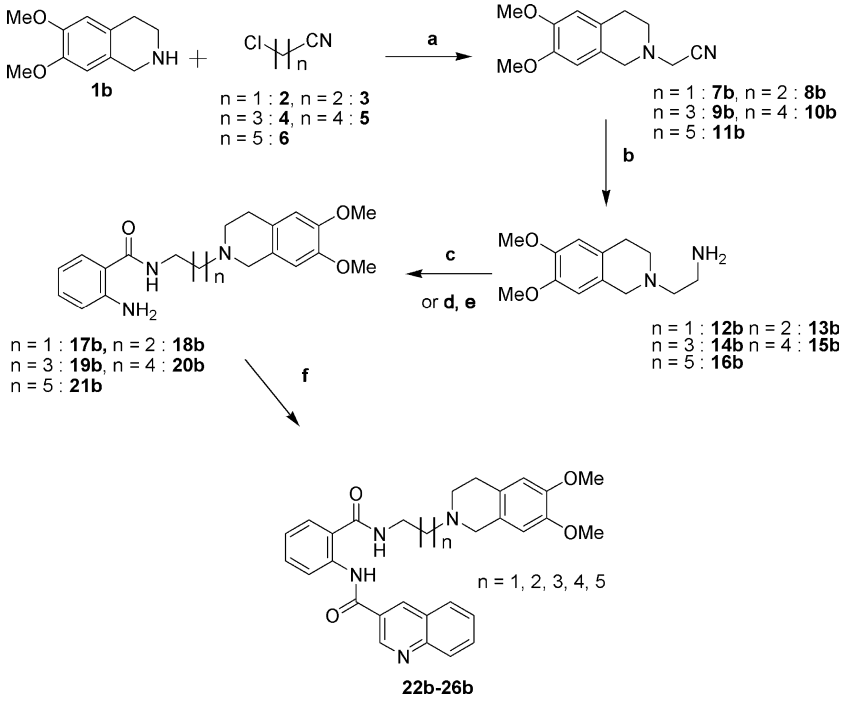
22b-26b

Figure 2. Molecular structures of target molecules.



Scheme 1. Preparation of the *N*-methylhomoveratrylamine derivatives **22a**–**26a**. Reagents and conditions: (a) *Method A.* K_2CO_3 or Na_2CO_3, NaI, *t*-BuOH, reflux 24 h. *Method B.* Acetonitrile or DMF, K_2CO_3, reflux 24 h; (b) H_2, Raney Ni, 40 psi, 1.4 M NaOH/EtOH, 12 h; (c) isatoic anhydride, acetonitrile, reflux, 18 h; (d) 3-quinoline acid chloride, CH_2Cl_2, 0–25 °C, Dowex 1X8 resin (100–200 mesh), 30 h.



Scheme 2. Preparation of the tetrahydroisoquinoline derivatives **22b**–**26b**. Reagents and conditions: (a) *Method A.* K_2CO_3 or Na_2CO_3, NaI, *t*-BuOH, reflux 24 h; *Method B.* Acetonitrile or DMF, K_2CO_3, reflux 24 h; (b) H_2, Raney Ni, 40 psi, 1.4 M NaOH/EtOH, 12 h; (c) i—isatoic anhydride, acetonitrile, reflux, 18 h or (d) 2-nitrobenzoylchloride, DOWEX 1X8 resin (100–200 mesh), ethyl acetate (e) NH_4Cl, Zn, MeOH, reflux, 90 min. (f) 3-quinoline acid chloride, CH_2Cl_2, 0–25 °C, Dowex resin 1X8 (100–200 mesh), 30 h.

(DNR) and vinblastine (VBL). These cells exhibit a high level of P-gp overexpression. As depicted in Figure 3, CEM/VLB_{500} cells were more than 5500-fold resistant to VLB than their wild-type counterpart (CEM).

The ability to reverse MDR in resistant cells was determined by treating the cells with escalating concentrations (0–15 μM) of the inhibitor either alone or in the presence of vinblastine (100 nM) or daunorubicin (100 nM), which are both P-gp substrates. After three days of treatment, the GI_{50} of the inhibitor (Table 1) (concentration of inhibitor necessary to kill 50% of the cells) was determined using the resazurin assay.[24] Also, a 'difference score' (EC_{50}) based on the difference between the number of cells

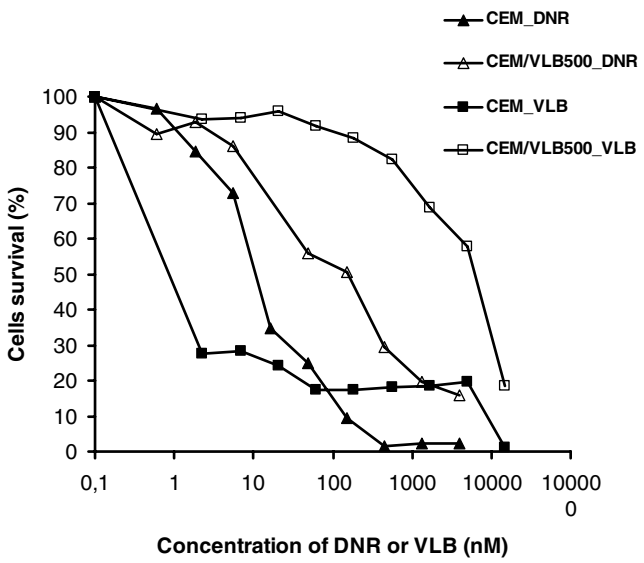


Figure 3. Comparison of cytotoxic activity of VLB and DNR against CEM and CEM/VLB$_{500}$ MDR cells.

Table 1. Cytotoxic activity of new anthranilamides on CEM/VBL$_{500}$ cells

Compound	n[a]	CEM/VLB$_{500}$ GI$_{50}$ (nM)
22a	1	27707 ± 15909[c]
23a	2	29177 ± 13401[c]
24a	3	19063 ± 7858[c]
25a	4	17845 ± 10300[b]
26a	5	10201 ± 2147[d]
22b	1	42298 ± 4588[b]
23b	2	29339 ± 11787[b]
24b	3	26353 ± 4588[b]
25b	4	17210 ±4952[b]
26b	5	17060 ± 9197[b]
XR9576	—	11350 ± 3100[b]
VRP	—	>15,000

[a] Number of linker carbon atoms between the nitrogen atoms is $n + 1$.
[b] Average of six experiments performed in quadruplicate.
[c] Average of nine experiments performed in quadruplicate.
[d] Average of eight experiments performed in quadruplicate.

that survived in presence of the modulator alone (cytotoxicity of the inhibitor) minus the number of cells that survived in presence of the modulator and the cytotoxic agent[25] was calculated (Table 2).

4. In vitro CYP450 inhibition studies

Many drug–drug interactions are initiated by the metabolism of drugs by cytochrome P-450 (CYP). Eleven CYPs metabolizing xenobiotics namely CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1, and CYP 3A4/5 are expressed in a typical human liver. However, CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 appear to be involved in the metabolism, and the drug–drug interactions of commonly used drugs.[26] Our hit P-gp inhibitor **22a** along with XR9576 were examined in a CYP450 inhibition assay to evaluate their ability to

Table 2. Inhibition activity of new anthranilamide P-gp inhibitors on CEM/VBL$_{500}$ cell incubated in the presence of vinblastine or daunorubicin

Compound	n[a]	CEM/VLB$_{500}$–VLB EC$_{50}$ (nM)	CEM/VLB$_{500}$–DNR EC$_{50}$ (nM)[e]
22a	1	270 ± 84[b]	338 ± 27
23a	2	490 ± 90[b]	293 ± 92
24a	3	781 ± 177[b]	352 ± 121
25a	4	1345 ± 113[d]	843 ± 260
26a	5	904 ± 384[c]	539 ± 32
22b	1	899 ± 127[d]	1680 ± 200
23b	2	500 ± 147[d]	652 ± 152
24b	3	945 ± 346[d]	860 ± 24
25b	4	1274 ± 27[d]	741 ± 171
26b	5	1084 ± 255[d]	769 ± 376
XR9576	—	68 ± 40[c]	—
VRP	—	1041 ± 443[b]	799 ± 59

[a] Number of linker carbon atoms between the nitrogen atoms is $n + 1$.
[b] Average of four experiments performed in quadruplicate.
[c] Average of five experiments performed in quadruplicate.
[d] Average of two experiments performed in quadruplicate.
[e] Average of three experiments performed in quadruplicate.

Table 3. In vitro cytochrome-P450 inhibition assays of **22a** and XR9576

	Reference	Reference IC$_{50}$ (μM)	XR9576 IC$_{50}$ (μM)	**22a** IC$_{50}$ (μM)
CYP1A2	Furafylline	4.98	27.2	>100
CYP2A6	Tranylcypromine	0.98	>100	>100
CYP2B6	Tranylcypromine	10.3	>100	>100
CYP2C8	Quercetin	1.56	45.3	5.82
CYP2C9	Sulfaphenazole	0.56	7.1	2.08
CYP2C19	Tranylcypromine	8.74	>10	14.7
CYP2D6	Quinidine	0.0074	100	>100
CYP2E1	DDTC	17.1	>100	>100
CYP3A4/BFC	Ketoconazole	0.096	>100	1.62
CYP3A4/BQ	Ketoconazole	0.26	>100	7.75

BFC, 7-benzyloxy-4-trifluoromethylcoumarin; BQ, 7-benzyloxyquinoline; DDTC, diethyldithiocarbamic acid.

inhibit their catalytic activity. The assays are based on the capacity of a given drug to inhibit or to increase the metabolism of a specific substrate of the CYP yielding in a fluorescent metabolite. In that context, microsomes from an insect cell transfected with a baculovirus expressing individual human CYP450 subtypes were prepared.[27] The drug was incubated with the microsomes expressing a specific CYP. Afterwards, the CYP substrate was added and the kinetics of the substrate metabolism was established using fluorescence. Of interest, inhibition constants are substrate dependent for CYP3A4 and to avoid potential misinterpretation of the results two substrates (7-benzyloxy-4-trifluoromethylcoumarin and 7-benzyloxyquinoline) were used. The IC$_{50}$ are listed in Table 3.

5. Results and discussion

Our results have shown that compounds **22a**–**26a** and **22b**–**26b** exhibit potent MDR reversal activity. In addition, most of our compounds were at least as potent as

VRP as P-gp inhibitor. Three compounds **22a**, **23a**, and**23b** were more active than verapamil. Compound **22a** was 4-fold more potent than verapamil when tested in combination with VLB and twice as potent when combined with DNR. In addition **23a** and **23b** were twice as potent as verapamil with VLB. In combination with DNR, **23a** was about 3-fold more potent than verapamil and **23b** was equipotent to verapamil. The selection of a 'hit' or a 'lead compound' cannot be based solely on its ability to inhibit the activity of its target. Therefore, we have evaluated the cytotoxicity of the drug itself and its ability to be involved in drug–drug interactions through CYP inhibition or induction. These preliminary experiments aimed to determine if a drug candidate will potentially trigger deleterious effects in later animal experiments. As shown in Figure 4 and Table 1, our compounds exhibited minimal cytotoxicity at concentrations up to 10 μM while the evaluated XR9576 was significantly toxic at this concentration. At concentrations of up to 10 μM, **22a** is non-toxic alone, but killed cells in combination with vinblastine, while verapamil has half of the activity of **22a** and is toxic by itself.

Some general trends seem to emerge from the new compounds in terms of the effect of the modifications on the A-ring versus the P-gp inhibition. Indeed, the series containing the *N*-methylhomoveratrylamine group (**22a**–**26a**) are generally more active compared to the tetrahydroisoquinoline series (**22b**–**26b**). These results suggest that the flexibility of the linker between N^1 and N^2 might be important to the inhibitory activity. In addition, the distance between N^1 and N^2 may also influence the activity of the drug; the optimal activity being observed with an ethyl chain ($n = 1$). In the *N*-methylhomoveratrylamine series **22a**–**26a**, the activity steadily decreases as the number of carbon atoms in the linking arm increases to up to 5 atoms. The activity of **26a** with 6 carbon atoms distance between N^1 and N^2 is similar to the 4-carbon atom chain of compound **24a**. In the tetrahydroisoquinoline series, the most active compound bears a 3-carbon atom spacer ($n = 2$, **23b**). Similar trends are observed for both vinblastine and daunorubicin.

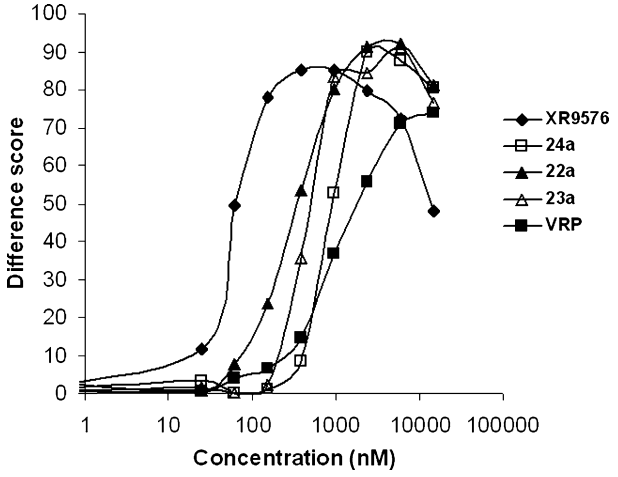


Figure 4. Comparison of the chemoresistance of **22a**, **23a**, **24a**, verapamil, and XR9576 in vinblastine treated CEM/VLB_{500} MDR cells. Difference score = (percent survival of cells treated with test agent alone) − (percent survival of cells treated with test compound and 100 nM vinblastine). The maximum variation of these data points was ±10%. See Section 7 for details.

Studies of the effect of **22a** and XR9576 on human CYP were also conducted using several human cytochrome P-450 enzymes, notably CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4, to determine the ability of these compounds to inhibit the metabolism of cytotoxic drugs thereby increasing their accumulation in cells. The results in Table 3 show that **22a** has a CYP inhibition profile significantly different from the one exhibited by XR9576. Indeed, **22a** inhibited CYP2C8 (5.82 μM), CYP2C9 (2.08 μM), CYP2C19 (14.7 μM), and CYP3A4 (1.62 for BFC and 7.75 μM for BQ) at μM level, while XR9576 inhibited CYP1A2, CYP2C8, and CYP2C9 at 27.2, 45.3 and 7.1 μM, respectively. These differences suggest that **22a** may have a greater impact on the metabolism of cytotoxic drugs such as paclitaxel, daunorubicin, vinblastine, and etoposide in comparison to XR9576. CYP3A4 accounts for approximately 25% of the total hepatic CYP enzymes and are responsible for the metabolism of the majority of cytotoxic drugs.[28] Fortuitously, P-gp shares a broad substrate overlap with CPY3A4 and is colocalized in the small intestine, which can also limit the gastrointestinal absorption of several drugs.[29] The apparent colocalization of CYP3A4 and P-gp could act synergistically to enhance drug metabolization via a recycling process in the gut. Therefore, an inhibitor of P-gp that is also an inhibitor of CYP3A4 might provide a therapeutical advantage by enhancing the bioavailability of the anticancer drugs through a decrease of its excretion and a decrease of its biotransformation. Several investigators reported that poor bioavailability of paclitaxel might be due to CYP and P-gp present in the gut wall.[30–32] To show the importance CYP450 inhibition in improving the absorption of paclitaxel, administration of known P-gp inhibitor LY335979 was shown to reduce paclitaxel clearance by approximately 25% in a recent study.[33] This improvement, although modest, is likely due to its weak inhibition of CYP3A4, as LY335979 is about 60-fold more selective for P-gp than for CYP3A4.[34] In our studies, **22a** inhibited both CYP3A4 and P-gp, while XR9576 inhibited only P-gp. Both **22a** and XR9576 may increase the accumulation of drugs in the cell, but **22a** may also enhance their bioavailability due to its inhibitory effect on CYP3A4 at μM levels.

There is increasing evidence suggesting that the presence of intratumoral enzymes, such as CYP450, may play an important role in the development of cancer chemoresistance.[35] Paclitaxel is a taxus derivative metabolized by CYP3A4. In a recent study on 23 patients having breast cancers, the analysis of CYP3A4 mRNA levels in tumor biopsies before docetaxel treatment revealed that patients with low CYP3A4 mRNA levels had significantly higher response rates compared to those with high CYP3A4 mRNA levels. This suggests that overexpression of CYP3A4 may provide a protective survival advantage to cancer cells by the inactivation of cytotoxic agents.

6. Conclusions

In this work, we have demonstrated that the *N*-methylhomoveratrylamine series **22a**–**26a** exhibits a more potent P-gp inhibition than the tetrahydroisoquinoline series **22b**–**26b**. These results suggest that the flexibility of the linker arm between N^1 and N^2 might be an important factor modulating the potency. We have also shown that a two- or three-carbon atom linker between N^1 and N^2 seems optimal for P-gp inhibition. Compound **22a** was the most potent of the series of molecules that were prepared; it was four times more active than verapamil, but four times less active than XR9576. Although less active than XR9576 as P-gp inhibitor, compound **22a** has been shown to be less intrinsically cytotoxic than VRP and XR9576. In addition, **22a** has a different CYP inhibition profile compared to XR9576. In contrast to XR9576, **22a** inhibited CYP 3A4, which is known to colocalize with P-gp and that is a major impediment to chemotherapy. Compound **22a** might be a therapeutically interesting molecule that is able to improve the bioavailability and half-life of a number of anticancer drugs such as paclitaxel through inhibition of P-gp and bioinactivation through metabolism, respectively.

7. Experimental

7.1. Cell lines and cell culture

CEM and CEM/VLB_{500} human lymphoma cells were kindly provided by Dr. W. T. Beck (Department of Biopharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago). Both cell lines were grown in RPMI 1640 medium supplemented with 2.0 mM glutamine and 10% fetal bovine serum (Hyclone, Road Logan, Utah). Cells were cultured in a moisture-saturated atmosphere at 37 °C in 5% CO_2.

7.2. MDR inhibition assay

Cells were plated in 96-well microtiter plates at 1×10^4 cells/well in media with and without vinblastine or daunorubicin (100 nM). Then, anthranilamide modulators dissolved in DMSO were added at escalating concentrations ranging from 0.025 to 15 μM to the cells. The final concentration of DMSO in each well was maintained at maximum 0.5%. The plates were incubated for 3 days. The cell survival was assessed using the resazurin assay.[24] Briefly, cells were washed three times with 200 μL of PBS solution. Fifty microliters of a PBS solution containing 20% resazurin in RPMI-1640 were added. Cell survival was calculated from fluorescence (excitation, 485 nm; emission, 590 nm) measured with a FL 600 Reader (Bio-Tek Instruments). Cytotoxicity was expressed as the concentration of the drug required to inhibit cell growth by 50% (GI_{50}). Values are the means of at least three independent determinations. The values were electronically processed to determine 'difference scores,' that is defined as percent survival of cells treated with test agent alone minus percent survival of cells treated with test compound and 100 nM vinblastine. All experiments were performed in quadruplicate. The maximum variation of these data point was ±10%. The difference score was plotted in function of the concentration of the modulator to determine an EC_{50} that represents the concentration that is necessary to revert chemoresistance by 50%.[37]

7.3. CYP450 inhibition assay

A general description of the assays follows, with enzyme-specific parameters listed separately below for each CYP subtype. The CYP450 inhibition assays were conducted using microsomes (Supersomes®, BD GENTEST, Woburn, MA) prepared from insect cells, each expressing a specific CYP subtype (CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4) expressed from the corresponding human CYP cDNA using a baculovirus expression vector. The microsomes also incorporate supplemental cDNA-expressed human reductase and/or cytochrome b5, as these enzymes stimulate the activity of the CYPs, allowing for a reduction in the amount of enzyme required per reaction (BD GENTEST). The assays monitored, via fluorescence detection, the formation of a fluorescent metabolite following incubation of the microsomes with a specific CYP substrate. Two CYP substrates were tested for CYP3A4 (7-benzyloxy-4-trifluoromethylcoumarin and 7-benzyloxyquinoline), as this enzyme has been shown to exhibit complex inhibition kinetics[36] Reactions (0.2 mL) are performed in 96-well microtiter plates at 37 °C in the presence of an NADPH regenerating system [β-nicotinamide adenine dinucleotide phosphate ($NADP^+$), glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PDH)] and $MgCl_2$. Inhibition of metabolic product formation by the test compound for each enzyme was tested in the absence and presence of 0.0457–100 μM of test compound. An enzyme-selective inhibitor was also tested (at eight concentrations) in each assay as a positive control. All determinations were performed in duplicate. Assays for all enzymes were performed in the following manner: the NADPH regenerating system, appropriate buffer solution and vehicle, inhibitor (positive control) solution or test compound solution were dispensed into 96-well microtiter plates. Eight inhibitor and test compound concentrations were tested using 3-fold serial dilutions. The microtiter plates containing 0.1 mL/well of the latter mixture was prewarmed to 37 °C in an incubator. A solution of buffer, microsomes, and substrate were separately prepared and vortex mixed to disperse the protein. The reactions were initiated by the addition of the microsome/substrate solution (0.1 mL) to the wells of the microtiter plates containing the pre-warmed NADPH regenerating system, buffer, and inhibitor or test compound solutions. The final concentration of acetonitrile, the vehicle used for the known inhibitors, was 2%. Following specified incubation times, the reactions were stopped by the addition of 0.075 mL of a STOP solution (see below). Blank (background noise) samples were also assayed by adding the STOP solution prior to the addition of the microsome/substrate mix to the NADPH regenerating system. The amount of metabolic product formed was quantified by fluorescence detection in a fluorescence plate reader utilizing excitation and emission filters that have been optimized for the detection of each metabolite.

CYP1A2	Microsomes: CYP1A2 (0.5 pmol) and supplemental cDNA-expressed human reductase NADPH regenerating system: 1.3 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$ Buffer: 100 mM potassium phosphate buffer, pH 7.4 (PPB) Inhibitor: furafylline (0.0457–100 μM) Substrate: 3-cyano-7-ethoxycoumarin (5 μM CEC) Metabolic product: 3-cyano-7-hydroxycoumarin (CHC) Incubation time: 15 min STOP buffer: 80% acetonitrile/20% 0.5 M Tris base Excitation filter: 400 nm Emission filter: 460 nm
CYP2A6	Microsomes: CYP2A6 (1.0 pmol) and supplemental cDNA-expressed human reductase and human cytochrome b5 NADPH regenerating system: 0.065 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$ Buffer: 100 mM Tris buffer, pH 7.5 Inhibitor: tranylcypromine (0.0457–100 μM) Substrate: coumarin (3 μM) Metabolic product: 7-hydroxycoumarin (7-HC, umbelliferone) Incubation time: 15 min STOP buffer: 80% acetonitrile/20% 0.5 M Tris base Excitation filter: 400 nm Emission filter: 460 nm
CYP2B6	Microsomes: CYP2B6 (1.0 pmol) and supplemental cDNA-expressed human reductase and human cytochrome b5 NADPH regenerating system: 1.3 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$ Buffer: 100 mM PPB Inhibitor: tranylcypromine (0.057–125 μM) Substrate: 7-ethoxy-4-trifluoromethylcoumarin (2.5 μM EFC) Metabolic product: 7-hydroxy-4-trifluoromethylcoumarin (HFC) Incubation time: 30 min STOP buffer: 80% acetonitrile/20% 0.5 M Tris base Excitation filter: 400 nm Emission filter: 530 nm
CYP2C8	Microsomes: CYP2C8 (4.0 pmol) and supplemental cDNA-expressed human reductase and human cytochrome b5 NADPH regenerating system: 1.3 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$ Buffer: 50 mM PPB Inhibitor: quercetin (0.009–20 μM) Substrate: dibenzylfluorescein (1 μM DBF) Metabolic product: fluorescein Incubation time: 30 min STOP Buffer: 2 mM NaOH Excitation filter: 485 nm Emission filter: 530 nm
CYP2C9	Microsomes: CYP2C9*1 (1.0 pmol) and supplemental cDNA-expressed human reductase and human cytochrome b5 (CYP2C9*1 is the most common allele in human populations which has been studied to date) NADPH regenerating system: 1.3 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$ Buffer: 25 mM PPB Inhibitor: sulfaphenazole (0.00457–10 μM) Substrate: 7-methoxy-4-trifluoromethylcoumarin (75 μM MFC) Metabolic product: 7-hydroxy-4-trifluoromethylcoumarin Incubation time: 45 min STOP buffer: 80% acetonitrile/20% 0.5 M Tris base

Excitation filter: 400 nm
Emission filter: 530 nm

CYP2C19 Microsomes: CYP2C19 (0.5 pmol) and supplemental cDNA-expressed human reductase and human cytochrome b5
NADPH regenerating system: 1.3 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$
Buffer: 50 mM PPB
Inhibitor: tranylcypromine (0.229–500 μM)
Substrate: 3-cyano-7-ethoxycoumarin (25 μM)
Metabolic product: 3-cyano-7-hydroxycoumarin
Incubation time: 30 min
STOP buffer: 80% acetonitrile/20% 0.5 M Tris base
Excitation filter: 400 nm
Emission filter: 460 nm

CYP2D6 Microsomes: CYP2D6*1 (1.5 pmol) and supplemental cDNA-expressed human reductase (CYP2D6*1 is the most common human CYP2D6 allele)
NADPH regenerating system: 8.2 μM $NADP^+$, 0.41 mM G6P, 0.4 U/mL G6PDH, 0.41 mM $MgCl_2$
Buffer: 100 mM PPB
Inhibitor: quinidine (0.00023–0.5 μM)
Substrate: 3-[2-(*N*,*N*-diethyl-*N*-methylamino)ethyl]-7-methoxy-4-methylcoumarin (1.5 μM AMMC)
Metabolic product: 3-[2-(*N*,*N*-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin (AHMC)
Incubation time: 30 min
STOP buffer: 80% acetonitrile/20% 0.5 M Tris base
Excitation filter: 400 nm
Emission filter: 460 nm

CYP2E1 Microsomes: CYP2E1 (2.0 pmol) and supplemental cDNA-expressed human reductase and cytochrome b5
NADPH regenerating system: 1.3 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$
Buffer: 100 mM PPB
Inhibitor: diethyldithiocarbamic acid (0.0457–100 μM DDTC)
Substrate: 7-methoxy-4-trifluoromethylcoumarin (100 μM MFC)
Metabolic product: HFC
Incubation time: 45 min
STOP buffer: 80% acetonitrile/20% 0.5 M Tris base
Excitation filter: 400 nm
Emission filter: 530 nm

CYP3A4/BFC Microsomes: CYP3A4 (1.0 pmol) and supplemental cDNA-expressed human reductase and cytochrome b5
NADPH regenerating system: 1.3 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$
Buffer: 200 mM PPB
Inhibitor: ketoconazole (0.00229–5 μM)
Substrate: 7-benzyloxy-4-trifluoromethylcoumarin (50 μM BFC)
Metabolic product: 7-hydroxy-4-trifluoromethylcoumarin
Incubation time: 30 min
STOP buffer: 80% acetonitrile/20% 0.5 M Tris base
Excitation filter: 400 nm
Emission filter: 530 nm

CYP3A4/BQ Microsomes: CYP3A4 (3.0 pmol) and supplemental cDNA-expressed human reductase and cytochrome b5
NADPH regenerating system: 1.3 mM $NADP^+$, 3.3 mM G6P, 0.4 U/mL G6PDH, 3.3 mM $MgCl_2$
Buffer: 200 mM PPB
Inhibitor: ketoconazole (0.00229–5 μM)
Substrate: 7-benzyloxyquinoline (40 μM BQ)
Metabolic product: quinolinol
Incubation time: 30 min

STOP buffer: 80% acetonitrile/20% 0.5 M Tris base
Excitation filter: 400 nm
Emission filter: 530 nm

7.4. Chemistry

Melting points were determined on an electrothermal melting point apparatus. All nominal and accurate mass electronic impact (EI) measurements were made using a JEOL HX110 double focusing mass spectrometer. All nominal and accurate mass electrospray ionization (ESI) measurements were made with a Waters/Micromass QTOF Ultima Global instrument of QQTof geometry. IR spectra were recorded on a Bomem MB-100 spectrometer. ^{1}H NMR spectra were determined at 200 MHz using a Varian XL-200 or at 300 MHz using a Bruker AC-300 spectrometer; ^{13}C NMR spectra were determined at 50.3 MHz using a Varian XL-200 or at 75.5 MHz using a Bruker AC-300 spectrometer; chemical shifts (δ) given using chloroform-*d* as a reference. The silical gel used for chromatography is Kieselgel 60 70–230 mesh Merck. Unless otherwise noted all reagents and solvents obtained from commercial suppliers were used without further purification.

7.4.1. General procedure for the synthesis of compound 7a–11a and 7b–11b. To a solution of *tert*-butanol, DMF or acetonitrile (20–40 mL) containing the amine (1 equiv) were added sodium iodide (0.5 equiv), sodium carbonate or potassium carbonate (2 equiv) and the corresponding chloronitrile (1.2 equiv). The mixture was stirred under reflux for 24 h (TLC monitoring, dichloromethane/methanol 9:1). The mixture was poured into 20 mL of water and extracted with ethyl acetate. The organic layers were combined and evaporated. The crude product was dissolved in 1 N HCl (10–20 mL) and extracted with ethyl acetate. The aqueous layers were combined and NaOH pellets were added to alkalinize the mixture. The white solution was extracted with ethyl acetate. The organic layer was washed with brine, dried with anhydrous sodium sulfate, filtered, and evaporated under vacuum. Pure product was obtained after column chromatography on silica gel using a 0%–5% methanol/dichloromethane as elution solvent.

7.4.1.1. Preparation of [[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]acetonitrile (7a). Yield: 84%, yellow oil; IR (NaCl, cm^{-1}); 2948, 2835, 2803 (C–H); 2229 (CN); 1591, 1516, 1464, 1418 (C=C Ar); 1264 (tertiary amine); ^{1}H NMR (300 MHz, $CDCl_3$); 6.70 (m, 3H, ArH), 3.74 (s, 3H, OMe), 3.70 (s, 3H, OMe), 3.41 (s, 2H, CH_2), 2.58 (s, 4H, CH_2), 2.26 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$); 148.9, 147.5, 131.9, 120.5, 114.8, 111.9, 111.4, 57.4, 55.8, 55.7, 45.1, 41.9, 33.5.

7.4.1.2. Preparation of (6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)acetonitrile (7b). Yield: 76%, yellow oil; IR (NaCl, cm^{-1}): 3051, 2929, 2850 (C–H); 1589, 1515, 1464 (C=C Ar); 1264 (amine tertiaire); ^{1}H NMR (200 MHz, $CDCl_3$): 6.60 (s, 1H, ArH), 6.53 (s, 1H, ArH), 3.85 (s, 6H, OMe), 3.71 (s, 4H, CH_2), 2.86 (s, 4H, CH_2); ^{13}C NMR (50.3 MHz, $CDCl_3$): 147.8, 147.4, 125.0, 124.9, 114.7, 111.3, 109.3, 55.9, 55.8, 53.8, 49.8, 45.9, 28.5.

7.4.1.3. Preparation of 3-[[2-(3,4-dimethoxyphenyl)-ethyl](methyl)amino]propanenitrile (8a). Yield: 94%, yellow oil; IR (NaCl, cm^{-1}): 3060, 2939, 2801 (C–H), 2248 (CN); 1590, 1516, 1418 (C=C Ar); 1262 (tertiary amine); ^{1}H NMR (300 MHz, $CDCl_3$); 6.54 (m, 3H, ArH), 3.62 (s, 3H, OMe), 3.58 (s, 3H, OMe), 2.47 (m, 6H, CH_2), 2.20 (t, 2H, CH_2, J = 6.7 Hz), 2.09 (s, 3H, CH_3); ^{13}C NMR (50.3 MHz, $CDCl_3$); 148.8, 147.4, 132.6, 120.5, 119.0, 112.1, 111.3, 59.1, 55.9, 55.8, 52.7, 41.6, 33.4, 16.1.

7.4.1.4. Preparation of 3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propanenitrile (8b). Yield: 29%, yellow oil; IR (NaCl, cm^{-1}); 3055, 2930, 2832 (C–H); 2249 (CN); 1604, 1517, 1464 (C=C Ar); 1264 (tertiary amine); ^{1}H NMR (300 MHz, $CDCl_3$); 6.50 (s, 1H, ArH), 6.43 (s, 1H, ArH), 3.73 (s, 6H, OMe), 3.50 (s, 2H, CH_2), 2.66 (m, 6H, CH_2), 2.48 (t, 2H, CH_2, 6.9 Hz); ^{13}C NMR (50.3 MHz, $CDCl_3$); 147.6, 147.3, 125.8, 125.7, 118.8, 111.4, 109.4, 55.9, 55.8, 54.9, 53.0, 50.5, 28.4, 16.2.

7.4.1.5. Preparation of 4-[[2-(3,4-dimethoxyphenyl)-ethyl](methyl)amino]butanenitrile (9a). Yield: 45%, yellow oil; IR (NaCl, cm^{-1}); 2924, 2850 (C–H); 1591, 1516, 1460 (C=C Ar); 1264 (tertiary amine); ^{1}H NMR (200 MHz, $CDCl_3$); 6.70 (m, 3H, ArH), 3.83 (s, 3H, OMe), 3.80 (s, 3H, OMe), 2.65 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 2.48 (m, 2H, CH_2), 2.29 (m, 2H, CH_2), 2.23 (s, 3H, CH_3), 1.72 (m, 2H, CH_2); ^{13}C NMR (50.3 MHz, $CDCl_3$); 148.8, 147.3, 132.9, 120.5, 119.8, 112.1, 111.3, 59.5, 55.9, 55.8, 55.4, 41.9, 33.4, 23.4, 14.6.

7.4.1.6. Preparation of 4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl) butanenitrile (9b). Yield: 65%, yellow solid; IR (NaCl, cm^{-1}); 3046, 2929, 2846 (C–H); 1612, 1517, 1465 (C=C Ar); 1265 (tertiary amine); ^{1}H NMR (200 MHz, $CDCl_3$); 6.58 (s, 1H, ArH), 6.50 (s, 1H, ArH), 3.82 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.52 (s, 2H, CH_2), 2.82 (m, 2H, CH_2), 2.68 (m, 4H, CH_2), 2.45 (t, 2H, CH_2, J = 6.9 Hz), 1.89 (m, 2H, CH_2); ^{13}C NMR (50.3 MHz, $CDCl_3$); 147.6, 147.3, 126.3, 126.1, 119.8, 111.4, 109.4, 55.9, 55.6, 50.9, 28.6, 23.1, 14.8.

7.4.1.7. Preparation of 5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]pentanenitrile (10a). Yield: 89%, sticky yellow oil. IR (NaCl, cm^{-1}); 3046, 2954, 2838, 2797 (C–H), 2247 (CN); 1591, 1516, 1465, 1421 (C=C Ar); 1262 (tertiary amine); ^{1}H NMR (400 MHz, $CDCl_3$); 6.75 (m, 3H, ArH), 3.87 (s, 3H, OMe), 3.85 (s, 3H, OMe), 2.70 (m, 2H, CH_2), 2.57 (m, 2H, CH_2), 2.41 (m, 2H, CH_2), 2.33 (m, 2H, CH_2), 2.28 (s, 3H, CH_3), 1.63 (m, 4H, CH_2); ^{13}C NMR (50.3 MHz, $CDCl_3$); 148.8,

147.3, 133.0, 120.5, 119.7, 112.0, 111.3, 59.7, 56.4, 55.9, 55.8, 41.9, 33.4, 26.1, 23.3, 16.9.

7.4.1.8. Preparation of 5-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)pentanenitrile (10b). Yield: 46%, as sticky yellow oil; IR (NaCl, cm^{-1}); 3056, 2925, 2851 (C–H); 1611, 1517, 1465 (C=C Ar); 1265 (tertiary amine); ^1H NMR (300 MHz, $CDCl_3$); 6.58 (s, 1H, ArH), 6.50 (s, 1H, ArH), 3.82 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.54 (s, 2H, CH_2), 2.79 (m, 2H, CH_2), 2.68 (m, 2H, CH_2), 2.52 (m, 2H, CH_2), 2.39 (m, 2H, CH_2), 1.72 (m, 4H, CH_2); ^{13}C NMR (50.3 MHz, $CDCl_3$); 147.5, 147.1, 126.5, 126.1, 119.7, 111.4, 109.4, 56.9, 55.9, 55.8, 55.7, 50.9, 28.6, 25.9, 23.3, 16.9.

7.4.1.9. Preparation of 6-[[2-(3,4-dimethoxyphenyl)-ethyl](methyl)amino]hexanenitrile, (11a). Yield: 46%, yellow oil; IR (NaCl, cm^{-1}); 2938, 2861, (C–H); 2243 (CN); 1592, 1517, 1465, 1422 (C=C Ar); 1264 (tertiary amine); ^1H NMR (300 MHz, $CDCl_3$); 6.69 (m, 3H, ArH), 3.82 (s, 3H, OMe), 3.79 (s, 3H, OMe), 2.67 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 2.37 (t, 2H, CH_2, $J = 6.7$ Hz), 2.30 (m, 5H, CH_2 and CH_3), 1,61 (m, 2H, CH_2), 1.43 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$); 148.8, 147.3, 132.9, 120.5, 119.7, 112.1, 111.3, 59.7, 57.1, 55.9, 55.8, 42.0, 33.3, 26.5, 26.4, 25.3, 17.0.

7.4.1.10. Preparation of 6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)hexanenitrile (11b). Yield: 38%, yellow oil; IR (NaCl, cm^{-1}); 2921, 2861 (C–H); 1517, 1464, (C=C Ar); 1265 (tertiary amine); 737 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$); 6.50 (s, 1H, ArH), 6.44 (s, 1H, ArH), 3.72 (s, 6H, OMe), 3.48 (s, 2H, CH_2), 2.73 (t, 2H, CH_2, $J = 4.9$ Hz), 2.63 (t, 2H, CH_2, $J = 5.7$ Hz), 2.43 (t, 2H, CH_2, $J = 7.1$ Hz), 2.25 (t, 2H, CH_2, $J = 7.1$ Hz), 1.57 (m, 4H, CH_2), 1.41 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$); 147.6, 147.2, 126.1, 125.9, 119.7, 111.4, 109.5, 57.6, 55.9, 55.8, 55.5, 50.9, 28.4, 26.5, 26.2, 25.2, 17.0.

7.4.2. General procedure for the synthesis of compounds 12a–16a and 12b–16b. Compounds (**7a**–**11a** and **7b**–**11b**) (1 equiv) were dissolved in 20–100 mL of a solution of freshly prepared 1.4 M NaOH in ethanol. To this solution was added wet Raney nickel (150–200 mg). The bottle was placed in a Parr shaker apparatus and the system was purged three times with hydrogen. The system pressure was adjusted to 40 psi and agitated for 12 h. The reaction mixture was filtered through Celite™ to remove the Raney nickel. The Celite™ pad was washed three times with ethanol and the filtrate was evaporated to a small volume and diluted with 40 mL of distilled water, alkalized with NaOH pellets and extracted with chloroform (3× 30 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum.

7.4.2.1. Preparation of *N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylethane-1,2-diamine (12a). To a 100 mL solution of 1.4 M NaOH in ethanol containing **7a** (6.447 mmol) was added Raney nickel wet (500 mg) to give compound **12a** as yellow oil after hydrogenation (1.455 g, 95%). IR (NaCl, cm^{-1}): 3381 (NH_2); 3052, 2927, 2836 (C–H); 1659 (NH_2 deformation); 1517, 1464, 1420 (C=C Ar); 1264 (tertiary amine); ^1H NMR (300 MHz, $CDCl_3$) δ: 6.71 (m, 3H, ArH), 3.86 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.37 (s, 2H, NH_2), 2.80 (t, 2H, CH_2, $J = 5.9$ Hz), 2.73 (m, 2H, CH_2), 2.70 (m, 2H, CH_2), 2.52 (m, 2H, CH_2), 2.29 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 148.9, 147.3, 132.9, 120.6, 120.1, 112.1, 111.3, 59.7, 58.3, 55.9, 41.9, 38.7, 33.4.

7.4.2.2. Preparation of 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanamine (12b). To a 50 mL solution of 1.4 M NaOH/EtOH containing **7b** (1.19 mmol) was added Raney nickel (150 mg, wet) to give 0.243 g of compound **12b** as yellow oil after hydrogenation (86%). IR (NaCl, cm^{-1}); 3367 (NH_2); 3053, 2926 (C–H); 1612 (NH_2 deformation) 1517, 1465, (C=C Ar); 1264 (tertiary amine); 738 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$) δ: 6.47 (s, 1H, ArH), 6.40 (s, 1H, ArH), 3.71 (s, 3H, OMe), 3.70 (s, 3H, OMe), 3.43 (s, 2H, CH_2), 2.73 (m, 4H, CH_2), 2.59 (m, 2H, CH_2), 2.43 (m, 2H, CH_2), 1.56 (s, 2H, NH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 147.5, 147.2, 126.6, 126.2, 111.4, 109.5, 60.8, 55.9, 55.8, 51.0, 39.1, 29.6, 28.6.

7.4.2.3. Preparation of *N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylpropane-1,3-diamine (13a). To a 75 mL solution of 1.4 M NaOH/EtOH containing **8a** (1.95 mmol) was added 150 mg of Raney nickel wet to give 0.489 g of compound **13a** as yellow oil after hydrogenation (99%). IR (NaCl, cm^{-1}) δ: 3425 (NH_2); 2928, 2851 (C–H); 1649 (NH_2 deformation); 1517, 1464, 1422 (C=C Ar); 1264 (tertiary amine); ^1H NMR (300 MHz, $CDCl_3$) δ: 6.46 (m, 3H, ArH), 3.57 (s, 3H, OMe), 3.54 (s, 3H, OMe), 2.44 (m, 4H, CH_2), 2.32 (m, 2H, CH_2), 2.17 (t, 2H, CH_2, $J = 6.9$ Hz), 2.01 (s, 3H, CH_3), 1.95 (s, 2H, NH_2), 1.35 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 148.6, 147.1, 132.9, 120.3, 111.9, 111.2, 59.7, 55.6, 55.5, 55.3, 41.9, 40.4, 33.2, 30.6.

7.4.2.4. Preparation of 3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propan-1-amine (13b). To a 50 mL solution of 1.4 M NaOH/EtOH containing **8b** (1.04 mmol) was added 150 mg of Raney nickel wet to give 0.227 g of compound **13b** as yellow oil after hydrogenation (87%). IR (NaCl, cm^{-1}); 3396 (NH_2); 2928, 2856 (C–H); 1638 (NH_2 deformation); 1517, 1463, (C=C Ar); 1264 (tertiary amine); 737 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$) δ: 6.56 (s, 1H, ArH), 6.49 (s, 1H, ArH), 3.80 (s, 6H, OMe), 3.52 (s, 2H, CH_2), 2.77 (m, 4H, CH_2), 2.68 (t, 2H, CH_2, $J = 5.5$ Hz), 2.54 (t, 2H, CH_2, $J = 7.2$ Hz), 1.72 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 147.5, 147.2, 126.7, 126.3, 111.4, 109.5, 56.2, 55.9, 51.0, 40.7, 30.7, 29.6, 28.7.

7.4.2.5. Preparation of *N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylbutane-1,4-diamine (14a). To a 50 mL solution of 1.4 M NaOH in ethanol containing **9a** (1.10 mmol) was added Raney nickel (150 mg, wet) to give 0.268 g of compound **14a** as yellow oil after hydrogenation (91%). IR (NaCl, cm^{-1}): 3357 (NH_2); 3055, 2932,

2852, 2798 (C–H); 1590 (NH_2 deformation); 1515, 1465, 1418 (C=C Ar); 1265 (tertiary amine); 737 (CH deformation Ar); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.65 (m, 3H, ArH), 3.75 (s, 3H, OMe), 3.72 (s, 3H, OMe), 2.57 (m, 6H, CH_2), 2.29 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 1.96 (bs, 2H, NH_2), 1.36 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 148.7, 147.2, 133.0, 120.5, 112.0, 111.2, 59.7, 57.4, 55.8, 55.7, 42.1, 41.9, 33.3, 31.5, 24.6.

7.4.2.6. Preparation of 4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butan-1-amine (14b). To a 50 mL solution of 1.4 M NaOH in ethanol containing **9b** (0.865 mmol) was added Raney nickel (150 mg, wet) to give 0.229 g of compound **14b** as yellow oil after hydrogenation (99%). IR (NaCl, cm^{-1}); 3430 (NH_2); 2924, 2853 (C–H); 1630 (NH_2 deformation); 1518, 1463, (C=C Ar); 1264 (tertiary amine); 739 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.42 (s, 1H, ArH), 6.35 (s, 1H, ArH), 3.66 (s, 6H, OMe), 3.04 (br s, 4H, CH_2 + NH_2), 2.66 (t, 2H, CH_2, J = 5.5 Hz), 2.56 (m, 4H, CH_2), 2.33 (t, 2H, CH_2, 7.3 Hz), 1.40 (m, 4H, CH_2); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 147.4, 147.0, 126.2, 126.0, 111.2, 109.4, 57.9, 57.1, 55.7, 55.5, 50.8, 41.6, 31.1, 28.2, 24.2.

7.4.2.7. Preparation of *N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylpentane-1,5-diamine (15a). To a 100 mL solution of 1.4 M NaOH in ethanol containing **10a** (3.07 mmol) was added Raney nickel (300 mg, wet) to give 0.689 g of compound **15a** as yellow oil after hydrogenation (80%). IR (NaCl, cm^{-1}); 3347 (NH_2); 2930, 2856 (C–H); 1649 (NH_2 deformation), 1515, 1465, (C=C Ar); 1264 (tertiary amine); 733 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.56 (m, 3H, ArH), 3.68 (s, 3H, OMe), 3.64 (s, 3H, OMe), 2.49 (m, 4H, CH_2), 2.39 (m, 2H, CH_2), 2.21 (t, 2H, CH_2, J = 7.3 Hz), 2.10 (s, 3H, CH_3), 1.75 (s, 2H, NH_2), 1.30 (m, 4H, CH_2), 1.20 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 148.7, 147.2, 133.1, 120.4, 112.0, 111.2, 59.7, 57.5, 55.7, 55.6, 42.0, 41.9, 33.4, 27.1, 24.7.

7.4.2.8. Preparation of 5-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)pentan-1-amine (15b). To a 50 mL solution of 1.4 M NaOH in ethanol containing **10b** (1.02 mmol) was added Raney nickel (150 mg, wet) to give 0.248 g of compound **15b** as yellow oil after hydrogenation (87%). IR (NaCl, cm^{-1}); 3391 (NH_2); 3053, 2930, 2855 (C–H); 1640 (NH_2 deformation), 1517, 1465, (C=C Ar); 1265 (tertiary amine); 737 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.53 (s, 1H, ArH), 6.47 (s, 1H, ArH), 3.78 (s, 6H, OMe), 3.49 (s, 2H, CH_2), 2.76 (m, 2H, CH_2), 2.65 (m, 4H, CH_2), 2.44 (m, 2H, CH_2), 2.20 (2H, NH_2), 1.38 (m, 6H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 147.4, 147.1, 126.7, 126.2, 111.4, 109.5, 58.3, 55.8, 55.7, 51.0, 41.8, 33.1, 28.6, 27.0, 24.8.

7.4.2.9. Preparation of *N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylhexane-1,6-diamine (16a). To a 50 mL solution of 1.4 M NaOH in ethanol containing **11a** (0.262 mmol) was added Raney nickel (150 mg, wet) to give 0.0753 g of compound **16a** as yellow oil after hydrogenation (97%). IR (NaCl, cm^{-1}); 3445 (NH_2); 2923, 2853 (C–H); 1615 (NH_2 deformation), 1517, 1463, (C=C Ar); 1264 (tertiary amine); 739 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.73 (m, 3H, ArH), 3.84 (s, 3H, OMe), 3.82 (s, 3H, OMe), 2.62 (m, 6H, CH_2), 2.36 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 1.32 (m, 10H, NH_2 + CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 148.8, 147.3, 132.9, 120.5, 119.7, 112.1, 111.3, 59.7, 57.1, 55.9, 55.8, 42.0, 33.3, 26.5, 26.4, 25.3, 17.0.

7.4.2.10. Preparation of 6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)hexan-1-amine (16b). To a 50 mL solution of 1.4 M NaOH in ethanol containing **11b** (0.869 mmol) was added Raney nickel (150 mg, wet) to give 0.219 g of compound **16b** as yellow oil after hydrogenation (86%). IR (NaCl, cm^{-1}); 3445 (NH_2); 2929, 2854 (C–H); 1612 (NH_2 deformation), 1517, 1465, (C=C Ar); 1264 (tertiary amine); 740 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.38 (s, 1H, ArH), 6.32 (s, 1H, ArH), 3.61 (s, 6H, OMe), 3.33 (s, 2H, CH_2), 2.60 (t, 2H, CH_2, J = 5.2 Hz), 2.47 (t, 2H, CH_2, J = 5.6 Hz), 2.27 (t, 2H, CH_2, J = 7.5 Hz), 1.83 (s, 2H, NH_2), 1.40 (m, 2H, CH_2), 1.20 (m, 6H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 147.4, 147.1, 126.7, 126.1, 111.3, 109.5, 58.2, 55.7, 50.9, 41.8, 33.3, 28.6, 27.3, 27.1, 26.7.

7.4.3. General procedure for the synthesis of compounds 17a–21a and 17b–21b. To a solution of acetonitrile (15 mL) containing the diamine (**8a**–**16a** or **8b**–**16b**) (1 equiv) was added isantoic anhydride (1 equiv) and the mixture was stirred under reflux for 18 h. The mixture was diluted with 20 mL AcOEt and extracted with HCl 1N. The aqueous layer was combined and alkalized with NaOH pellets. The white solution was extracted with AcOEt. The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Pure product was obtained after column chromatography on silica gel using a 0% to 5% methanol/dichloromethane as elution solvent.

7.4.3.1. Preparation of 2-amino-*N*-{2-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]ethyl} benzamide (17a). Yield: 84%, yellow oil; IR (NaCl, cm^{-1}) 3362 (NHCO + NH_2); 2921, 2851 (C–H); 1642 (CO); 1588 (NH_2 deformation); 1515, 1465, (C=C Ar); 1264 (tertiary amine); 735 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.10 (t, 1H, ArH, J = 6.9 Hz and J = 14.6 Hz), 7.04 (d, 1H, ArH, J = 6.9 Hz), 6.66 (m, 4H, ArH + 1H CONH), 6.55 (m, 2H, ArH), 5.55 (s, 2H, NH_2), 3.77 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.40 (m, 2H, CH_2), 2.66 (m, 6H, CH_2), 2.26 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.3, 148.9, 148.8, 147.4, 132.8, 132.1, 127.3, 120.5, 117.1, 116.5, 115.9, 112.0, 111.3, 58.9, 55.9, 55.7 55.5, 41.6, 36.6, 33.2.

7.4.3.2. Preparation of 2-amino-*N*-[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl] benzamide (17b). Yield 74%, yellow oi;. IR (NaCl, cm^{-1}) 3435 (NHCO + NH_2); 2918, 2849 (C–H); 1711 (CO); 1625 (NH_2 deformation); 1517, 1445, (C=C Ar); 1222 (tertiary amine); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.21 (m, 1H, ArH), 7.08 (m, 1H, ArH), 6.82 (m, 1H, NHCO), 6.50 (m, 4H, ArH), 5.28 (s, 2H, NH_2), 3.74 (s, 3H, OMe),

3.73 (s, 3H, OMe), 3.50 (m, 4H, CH_2), 2.68 (m, 6H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.4, 148.7, 147.7, 147.4, 132.1, 127.5, 126.0, 117.2, 116.5, 116.1, 111.5, 109.5, 56.2, 56.0, 55.9, 55.3, 50.6, 36.4, 28.5.

7.4.3.3. Preparation of 2-amino-*N*-{2-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]propyl} benzamide (18a). Yield: 85%, yellow oil; IR (NaCl, cm^{-1}) 3352 (NHCO + NH_2); 3055, 2927, 2853 (C–H); 1635 (CO); 1588 (NH_2 deformation); 1517, 1465, (C=C Ar); 1264 (tertiary amine); 737 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.05 (m, 1H, NHCO), 7.14 (m, 2H, ArH), 6.67 (m, 4H, ArH), 6.56 (t, 1H, ArH, J = 7.6 Hz), 5.64 (br s, 2H, NH_2), 3.82 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.47 (q, 2H, CH_2, J = 5.6 Hz and J = 11.3 Hz), 2.64 (m, 6H, CH_2), 2.29 (s, 3H, CH_3), 1.75 (m, 1H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.2, 149.0, 148.9, 147.4, 132.6, 131.9, 127.2, 120.5, 117.2, 116.3, 116.1, 111.9, 111.4, 60.1, 57.3, 55.9, 55.8, 53.5, 41.9, 40.1, 33.4, 25.3.

7.4.3.4. Preparation of 2-amino-*N*-[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl] benzamide (18b). Yield: 56%, yellow oil; IR (NaCl, cm^{-1}) 3454 (NHCO); 3352 (NH_2); 2924, 2841 (C–H); 1636 (CO); 1584 (NH_2 deformation); 1518, 1464, (C=C Ar); 1264 (tertiary amine); 738 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.59 (s, 1H, NHCO), 6.98 (m, 2H, ArH), 6.59 (s, 1H, ArH), 6.52 (d, 1H, ArH, J = 8.1 Hz), 6.50 (s, 1H, ArH), 5.97 (t, 1H, ArH, J = 7.8 Hz), 5.71 (s, 2H, NH_2), 3.84 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.66 (s, 2H, CH_2), 3.54 (q, 2H, CH_2, J = 5.5 and 10.5 Hz), 2.75 (m, 6H, CH_2), 1.85 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.3, 148.9, 147.8, 147.4, 131.8, 127.4, 125.8, 125.7, 117.1, 116.0, 115.5, 111.4, 109.5, 57.9, 56.0, 55.9, 55.5, 51.2, 40.4, 28.5, 24.4.

7.4.3.5. Preparation of 2-amino-*N*-{2-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]butyl} benzamide (19a). Yield: 80%, yellow oil; IR (NaCl, cm^{-1}) 3454 (NHCO); 3367 (NH_2); 2928, 2853 (C–H); 1640 (CO); 1586 (NH_2 deformation); 1516, 1464, (C=C Ar); 1264 (tertiary amine); 738 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.27 (d, 1H, ArH, J = 6.9 Hz), 7.14 (m, 2H, ArH + NHCO), 6.64 (m, 5H, ArH), 5.49, (s, 2H, NH_2), 3.83 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.36 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 2.55 (m, 2H, CH_2), 2.41 (t, 2H, CH_2, J = 6.4 Hz), 2.24 (s, 3H, CH_3), 1.59 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.5, 148.9, 148.7, 147.4, 133.0, 132.0, 127.3, 120.5, 117.2, 116.6, 116.4, 112.1, 111.4, 59.6, 57.1, 55.9, 55.8, 42.2, 39.6, 33.1, 27.5, 25.1.

7.4.3.6. Preparation of 2-nitro-*N*-[2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl] benzamide (19). To a solution of ethyl acetate (30 mL) containing compound **14b** (0.210 g, 0.796 mmol), were added 2-nitrobenzoylchloride (110 μL, 0.835 mmol) and DOWEX 1X8 (0.261 g, 100–200 mesh) chloride resin. The mixture was stirred for overnight at room temperature, filtered, and extracted with 1 N NaOH (3× 10 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel using a gradient concentration of solvent from 2% to 10% methanol (2 M NH_3) in dichloromethane to give **19** as a yellow oil (0.105 g, 32%). IR (NaCl, cm^{-1}): 3057, 2927, 2855 (C–H); 1661 (CO); 1518, 1465, (C=C Ar); 1531, 1349 (NO_2); 1265 (tertiary amine); 740 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.87 (s, 1H, NHCO), 7.75 (d, 1H, ArH, J = 6.9 Hz), 7.29 (m, 3H, ArH), 6.30 (s, 1H, ArH), 6.19 (s, 1H, ArH), 3.79 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.42 (m, 2H, CH_2), 3.35 (s, 2H, CH_2), 2.54 (m, 6H, CH_2), 1.75 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 166.6, 147.4, 147.1, 133.6, 133.2, 129.5, 128.3, 125.5, 124.1, 111.0, 109.1, 57.8, 55.9, 55.7, 55.6, 50.1, 40.1, 28.0, 27.8, 25.1.

7.4.3.7. Preparation of 2-amino-*N*-[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl] benzamide (19b). To a solution of methanol (20 mL) containing **22** (0.0805 g, 0.195 mmol) were added ammonium chloride (0.0625 g, 1.16 mmol) and dust zinc (0.255 g, 3.89 mol). The mixture was stirred at refluxed for 90 min. The reaction mixture was filtered and the solvent evaporated under vacuum. The crude was diluted with 1 M HCl (15 mL) and extracted with ethyl acetate (3× 15 mL). The aqueous layers were combined, alkalized with NaOH pellets, and extracted with ethyl acetate (3× 15 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated to give **19 b** as yellow oil (0.054 g, 72%). IR (NaCl, cm^{-1}): 3469 (NHCO); 3352 (NH_2); 2928, 2851 (C–H); 1638 (CO); 1584 (NH_2 deformation); 1518, 1464, (C=C Ar); 1264 (tertiary amine); 740 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.12 (m, 3H, ArH + NHCO), 6.58 (d, 1H, ArH, J = 8.1 Hz), 6.55 (s, 1H, ArH), 6.45 (s, 1H, ArH), 6.30 (t, 1H, ArH, J = 7.5 Hz), 5.50 (s, 2H, NH_2), 3.82 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.50 (s, 2H, CH_2), 3.39 (m, 2H, CH_2), 2.78 (t, 2H, CH_2, J = 5.7 Hz), 2.68 (t, 2H, CH_2, J = 5.5 Hz), 2.52 (t, 2H, CH_2, J = 6.5 Hz), 1.70 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.4, 148.6, 147.6, 147.3, 131.9, 127.3, 126.3, 126.1, 117.1, 116.4, 116.3, 111.4, 109.6, 57.3, 55.9, 50.5, 39.5, 28.4, 27.2, 24.8.

7.4.3.8. Preparation of 2-amino-*N*-{2-[[2-(3,4-methoxyphenyl)ethyl](methyl)amino]pentyl} benzamide (20a). Yield: 82%, yellow oil. IR (NaCl, cm^{-1}); 3454 (NHCO); 3347 (NH_2); 2926, 2851 (C–H); 1640 (CO); 1516, 1448, (C=C Ar); 1264 (tertiary amine); 738 (CH deformation Ar); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.20 (m, 1H, ArH), 7.10 (t, 1H, ArH, J = 6.8 Hz), 6.60 (m, 5H, ArH), 6.16 (m, 1H, NHCO), 5.42, (s, 2H, NH_2), 3.78 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.32 (m, 2H, CH_2NHCO), 2.65 (m, 2H, CH_2), 2.50 (m, 2H, CH_2), 2.34 (t, 2H, CH_2, 7.2 Hz), 2.23 (s, 3H, CH_3), 1.53 (m, 4H, CH_2), 1.33 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.4, 148.9, 148.7, 147.4, 133.1, 132.1, 127.1, 120.6, 117.3, 116.5, 116.4, 112.1, 111.3, 59.7, 57.4, 55.9, 55.8, 42.1, 39.6, 33.3, 29.6, 26.8, 24.8.

7.4.3.9. Preparation of 2-amino-*N*-[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)pentyl] benzamide (20b). Yield: 74%, yellow oil. IR (NaCl, cm^{-1}): 3450 (NHCO);

3347 (NH_2); 2929, 2855 (C–H); 1644 (CO); 1585 (NH_2 deformation); 1516, 1464, (C=C Ar); 1264 (tertiary amine); 739 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25 (m, 1H, ArH), 7.14 (m, 1H, ArH), 6.55 (m, 3H, ArH), 6.49 (s, 1H, ArH), 6.31 (s, 1H, NHCO), 5.46 (s, 2H, NH_2), 3.80 (s, 6H, OMe), 3.52 (s, 2H, CH_2), 3.35 (q, 2H, CH_2, J = 6.7 Hz and 12.9 Hz), 2.78 (t, 2H, CH_2, J = 5.5 Hz), 2.67 (t, 2H, CH_2, J = 5.3 Hz), 2.47 (t, 2H, CH_2, J = 7.2 Hz), 1.60 (m, 4H, CH_2), 1.40 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.4, 148.6, 147.5, 147.2, 132.1, 127.2, 126.5, 126.1, 117.2, 116.5, 116.4, 111.4, 109.5, 58.1, 55.9, 55.8, 55.7, 51.0, 39.5, 29.6, 28.5, 26.8, 24.8.

7.4.3.10. Preparation of 2-amino-*N*-{2-[[2-(3,4-methoxyphenyl)ethyl](methyl)amino]hexyl} benzamide (21a). Yield: 70%, yellow oil. IR (NaCl, cm^{-1}); 3479 (NHCO); 3357 (NH_2); 3051, 2928, 2853 (C–H); 1641 (CO); 1587 (NH_2 deformation); 1516, 1465, (C=C Ar); 1264 (tertiary amine); 737 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.27 (m, 1H, ArH), 7.15 (m, 1H, ArH), 6.71 (m, 3H, ArH), 6.59 (m, 2H, ArH), 6.20 (m, 1H, NHCO), 5.49 (br s, 2H, NH_2), 3.83 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.35 (q, 2H, CH_2, 6.3 and 6.7 Hz), 2.70 (m, 2H, CH_2), 2.57 (m, 2H, CH_2), 2.39 (t, 2H, CH_2, J = 7.2 Hz), 2.29 (s, 3H, CH_3), 1.51 (m, 4H, CH_2), 1.32 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.4, 148.9, 148.6, 147.4, 132.9, 132.1, 127.1, 120.6, 117.2, 116.5, 116.4, 112.1, 111.4, 59.7, 57.5, 55.9, 55.8, 42.1, 39.6, 33.2, 29.6, 27.1, 27.0, 26.9.

7.4.3.11. Preparation of 2-amino-*N*-[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)hexyl] benzamide (21b). Yield: 76%, gum yellow solid. IR (NaCl, cm^{-1}): 3450 (NHCO); 3352 (NH_2); 3052, 2927, 2853 (C–H); 1644 (CO); 1586 (NH_2 deformation); 1517, 1465, (C=C Ar); 1264 (tertiary amine); 738 (CH deformation Ar). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25 (m, 1H, ArH), 7.10 (m, 1H, ArH), 6.54 (m, 2H, ArH), 6.47 (s, 1H, ArH), 6.34 (t, 1H, ArH, J = 5.4 Hz), 5.48 (s, 2H, NH_2), 3.78 (s, 6H, OMe), 3.50 (s, 2H, CH_2), 3.31 (m, 2H, CH_2), 2.77 (t, 2H, CH_2, J = 5.5 Hz), 2.65 (t, 2H, CH_2, J = 5.5 Hz), 2.44 (t, 2H, CH_2, J = 7.9 Hz), 1.53 (m, 4H, CH_2), 1.34 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.4, 148.6, 147.5, 147.2, 132.0, 127.1, 126.6, 126.2, 117.2, 116.5, 111.5, 109.5, 58.2, 55.9, 55.7, 51.0, 39.6, 29.6, 28.6, 27.2, 27.0, 26.9.

7.4.4. General procedure for the synthesis of compounds 22a–26a and 22b–26b. To a solution of dicloromethane (10–20 mL) containing compounds **17a**–**21a** or **17b**–**21b** (1 equiv) were added 3-quinoline acid chloride (1.5 equiv) and DOWEX 1X8 (100–200 mesh) chloride resin. The mixture was stirred for 30 h at room temperature. The mixture was filtered and extracted with 1 N NaOH (3× 10 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Pure product was obtained by flash chromatography on silica gel using a gradient concentration of solvent from 2% to 10% methanol (2 M NH_3) in dichloromethane.

7.4.4.1. *N*-[2-({[{[2-(3,4- methoxyphenyl)ethyl](methyl)amino}ethyl]amino}carbonyl) phenyl]quinoline-3-carboxamide (22a). To a solution of dichloromethane (10 mL) containing **17a** (0.119 mmol) were added 3-quinoline acid chloride and DOWEX 1X8 (0.112 g, 100–200 mesh) to give a yellow solid after purification (0.0425 g, 70%). Mp 149–150 °C; IR (NaCl, cm^{-1}): 2925, 2851 (C–H); 1672, 1600 (CO); 1515, 1463, 1448 (C=C Ar); 1264 (tertiary amine); 738 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.79 (s, 1H, NHCO), 9.55 (d, 1H, ArH, J = 2.08 Hz), 8.84 (d, 1H, ArH, J = 8.4 Hz), 8.78 (d, 1H, ArH, J = 1.6 Hz), 8.15 (d, 1H, ArH, J = 8.5 Hz), 8.00 (d, 1H, ArH, J = 8.1 Hz), 7.80 (t, 1H, ArH, J = 6.9 Hz and J = 14.4 Hz), 7.53 (m, 2H, ArH), 7.21 (d, 1H, ArH, J = 7.2 Hz), 7.07 (m, 2H, ArH + NHCO), 6.70 (m, 3H, ArH), 3.82 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.49 (q, 2H, CH_2 J = 4.9 Hz and J = 5.4 Hz), 2.69 (m, 6H, CH_2), 2.35 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 168.9, 163.8, 149.4, 149.0, 148.9, 147.5, 140.0, 135.7, 132.7, 132.4, 131.3, 129.4, 129.1, 127.6, 127.3, 127.0, 126.7, 123.2, 121.4, 120.4, 119.8, 111.9, 111.3, 58.5, 55.8, 55.3, 41.6, 36.8, 32.9; MS: 513.22 $[M+H]^+$. HRMS $[M]^+$: calcd for $C_{30}H_{32}N_4O_4$ 512.2423; Found: 512.2431.

7.4.4.2. Preparation of *N*-[2-({[{[2-(3,4-methoxyphenyl)ethyl](methyl)amino}propyl]amino}carbonyl)phenyl]-quinoline-3-carboxamide (23a). To a solution of dichloromethane (10 mL) containing **18a** (0.184 mmol) were added 3-quinoline acid chloride and DOWEX 1X8 (0.172 g, 100–200 mesh) to give a yellow solid after purification (0.0765 g, 79%). Mp 92–94 °C; IR (NaCl, cm^{-1}): 3054, 2925, 2851 (C–H); 1673, 1598 (CO); 1517, 1464, 1446 (C=C Ar); 1265 (tertiary amine); 738 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 13.09 (s, 1H, NHCO), 9.55 (d, 1H, ArH, J = 2.04 Hz), 8.92 (m, 1H, NHCO), 8.85 (m, 1H, ArH, J = 8.3Hz), 8.79 (m, 2H, ArH), 8.13 (d, 1H, ArH, J = 8.5 Hz), 7.97 (d, 1H, ArH, J = 8.2 Hz), 7.77 (m, 1H, ArH), 7.53 (m, 2H, ArH), 7.30 (d, 1H, ArH, J = 7.9 Hz), 7.03 (m, 1H, ArH), 6.70 (m, 3H, ArH), 3.84 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.57 (m, 2H, CH_2), 2.66 (m, 6H, CH_2), 2.33 (s, 3H, CH_3), 1.79 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 168.8, 163.8, 149.3, 149.1, 148.9, 147.5, 140.3, 135.6, 132.5, 132.2, 131.2, 129.3, 129.2, 127.6, 127.3, 126.9, 126.6, 122.9, 121.3, 120.4, 119.9, 111.8, 111.3, 60.1, 57.5, 55.8, 41.9, 40.9, 33.3, 24.6; MS: 526.29 $[M^+]$. HRMS $[M^+]$: calcd for $C_{31}H_{34}N_4O_4$ 526.2580; Found: 526.2584.

7.4.4.3. Preparation of *N*-[2-({[{[2-(3,4-methoxyphenyl)ethyl](methyl)amino} butyl]amino} carbonyl)phenyl]quinoline-3-carboxamide (24a). To a solution of dichloromethane (10 mL) containing **19a** (0.256 mmol) were added 3-quinoline acid chloride and DOWEX 1x8 (0.180 g, 100–200 mesh) to give a sticky yellow oil after purification (0.106 g, 77%); IR (NaCl, cm^{-1}): 3396 (NHCO); 2924, 2849 (C–H); 1672, 1597 (CO); 1516, 1447, (C=C Ar); 1264 (tertiary amine); 737 (CH deformation Ar); ^{1}H NMR(300 MHz, $CDCl_3$) δ: 12.74 (s, 1H, NH), 9.52 (d, 1H, ArH, J = 2.04 Hz), 8.77 (m, 2H, ArH), 8.13 (m, 2H, ArH + NH), 7.97 (d, 1H, ArH, J = 8.0 Hz), 7.76 (t, 1H, ArH, J = 8.4 Hz), 7.53

(m, 3H, ArH), 7.03 (t, 1H, ArH, J = 7.5 Hz), 6.72 (d, 1H, ArH, J = 8.5 Hz), 6.63 (d, 1H, ArH, J = 6.9 Hz), 3.80 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.42 (m, 2H, CH_2), 2.64 (m, 4H, CH_2), 2.42 (t, 2H, CH_2, J = 5.9 Hz), 2.22 (s, 3H, CH_3), 1.62 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.3, 163.8, 149.4, 149.0, 148.9, 147.4, 139.8, 135.7, 132.6, 132.5, 131.3, 129.3, 129.2, 127.5, 127.4, 127.0, 126.9, 122.9, 121.4, 120.6, 120.5, 112.0, 111.4, 59.4, 56.9, 55.9, 55.8, 42.2, 40.1, 32.9, 27.3, 25.1; MS: 541.26 $[M+H]^+$. HRMS $[M+H]^+$: calcd for $C_{32}H_{36}N_4O_4$ 540.2736; Found: 541.2833.

7.4.4.4. Preparation of *N*-[2-({[{[2-(3,4-methoxyphenyl)ethyl](methyl)amino}pentyl]amino} carbonyl)phenyl]-quinoline-3-carboxamide (25a). To a solution of dichloromethane (10 mL) containing **20a** (0.167 mmol) were added 3-quinoline acid chloride and DOWEX 1X8 (0.0742 g, 100–200 mesh) to give a sticky yellow oil after purification (0.0413 g, 45%). IR (NaCl, cm^{-1}); 2926, 2853 (C–H); 1672, 1599 (CO); 1516, 1448, (C=C Ar); 1264 (tertiary amine); 738 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.60 (s, 1H, NH), 9.54 (d, 1H, ArH, J = 2.3 Hz), 8.77 (m, 2H, ArH + NH), 8.15 (d, 1H, ArH, J = 8.3 Hz), 7.98 (d, 1H, ArH, J = 8.1 Hz), 7.77 (m, 1H, ArH), 7.53 (m, 3H, ArH), 7.10 (t, 1H, ArH, J = 7.1 Hz), 6.74 (m, 4H, ArH), 3.85 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.45 (q, 2H, CH_2, J = 6.1 and 7.0 Hz), 2.70 (m, 2H, CH_2), 2.60 (m, 2H, CH_2), 2.41 (t, 2H, CH_2, J = 7.0 Hz), 2.29 (s, 3H, CH_3), 1.59 (m, 4H, CH_2), 1.37 (m, 2H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.2, 163.8, 149.4, 149.0, 148.9, 147.4, 139.8, 135.8, 132.9, 132.7, 131.4, 129.4, 129.1, 127.5, 127.4, 126.9, 126.7, 123.2, 121.6, 120.6, 112.2, 111.4, 59.7, 57.3, 55.9, 55.8, 42.1, 40.0, 33.3, 29.2, 26.8, 24.7; MS: 555.27 $[M+H]^+$. HRMS $[M+H]^+$: calcd for $C_{33}H_{38}N_4O_4$ 554.2893; Found: 555.2981.

7.4.4.5. Preparation of *N*-[2-({[{[2-(3,4-methoxyphenyl)ethyl](methyl)amino}hexyl]amino} carbonyl)phenyl]-quinoline-3-carboxamide (26a). To a solution of dichloromethane (10 mL) containing **21a** (0.167 mmol) were added 3-quinoline acid chloride and DOWEX 1X8 (0.157 g, 100–200 mesh) to give a yellow solid after purification (0.0542 g, 57%). Mp 100–101 °C; IR (NaCl, cm^{-1}): 3420 (NHCO); 3060, 2926, 2853 (C–H); 1673, 1598 (CO); 1517, 1448, (C=C Ar); 1265 (tertiary amine); 739 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.59 (s, 1H, NHCO), 9.51 (d, 1H, ArH, J = 2.0 Hz), 8.75 (m, 2H, ArH), 8.13 (d, 1H, ArH, J = 8.4 Hz), 7.98 (d, 1H, ArH, J = 8.1 Hz), 7.79 (m, 1H, ArH), 7.59 (m, 2H, ArH), 7.49 (m, 1H, ArH), 7.05 (m, 1H, ArH), 6.88 (m, 1H, NHCO), 6.69 (m, 3H, ArH), 3.82 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.43 (m, 2H, CH_2), 2.70 (m, 2H, CH_2), 2.60 (m, 2H, CH_2), 2.41 (t, 2H, CH_2, J = 7.1 Hz), 2.29 (s, 3H, CH_3), 1.62 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 1.32 (m, 4H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.2, 163.8, 149.4, 149.0, 148.9, 147.4, 139.7, 135.8, 132.7, 132.6, 131.4, 129.4, 129.2, 127.5, 127.4, 126.9, 126.8, 123.2, 121.6, 120.6, 112.1, 111.4, 59.5, 57.3, 55.9, 55.8, 41.9, 40.0, 33.1, 29.3, 26.9, 26.8; MS: 569.2873 $[M+H]^+$. HRMS $[M+H]^+$: calcd for $C_{34}H_{40}N_4O_4$ 568.3049; Found: 569.3130.

7.4.4.6. Preparation of N-[2-({[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl]amino}carbonyl) phenyl]quinoline-3-carboxamide (22b). To a solution of dichloromethane (10 mL) containing **17b** (0.279 mol) were added 3-quinoline acid chloride and 0.262 g of DOWEX 1x8 (100–200 mesh) to give a yellow solid after purification (give 0.0556 g, 39%). Mp 192–194 °C; IR (NaCl, cm^{-1}); 3055, 2924, 2853 (C–H); 1674, 1600 (CO); 1518, 1448, (C=C Ar); 1265 (tertiary amine); 739 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.75 (s, 1H, NH), 9.56 (d, 1H, ArH, J = 2.2 Hz), 8.83 (m, 2H, ArH), 8.16 (d, 1H, ArH, J = 8.0 Hz), 8.00 (d, 1H, ArH, J = 8,0 Hz), 7.83 (m, 1H, ArH), 7.63 (t, 3H, ArH, J = 7.0 Hz), 7.54 (t, 1H, ArH, J = 8.9 Hz), 7.33 (m, 1H, NH), 7.11 (t, 1H, ArH, J = 7.2 Hz), 6.60 (s, 1H, ArH), 6.52 (s, 1H, ArH), 3.85 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.67 (m, 4H, CH_2), 2.83 (m, 6H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.2, 163.8, 149.4, 149.0, 147.9, 147.5, 139.9, 135.8, 132.9, 131.3, 129.4, 129.1, 127.6, 127.4, 127,0, 126.9, 125.8, 125.6, 123.3, 121.5, 120.0, 111.4, 109.5, 55.9, 55.8, 55.3, 50.6, 36.6, 28.4; MS: 510.3 $[M^+]$. HRMS $[M^+]$: calcd for $C_{30}H_{30}N_4O_4$ 510.2267; Found: 510.2263.

7.4.4.7. Preparation of *N*-[2-({[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl]amino} carbonyl)phenyl]-quinoline-3-carboxamide (23b). To a solution of dichloromethane (10 mL) containing **18b** (0.252 mol) were added 3-quinoline acid chloride and 0.157 g of DOWEX 1X8 (100–200 mesh) to give a yellow solid after purification (0.0553 g, 42%); mp 158–159 °C IR (NaCl, cm^{-1}): 3057, 2923, 2853 (C–H); 1672, 1598 (CO); 1518, 1445, (C=C Ar); 1265 (tertiary amine); 736 (CH deformation Ar); NMR ^{1}H (300 MHz, $CDCl_3$) δ: 13.19 (s, 1H, NH), 9.60 (br s, 1H, NH), 9.54 (d, 1H, ArH, J = 2.10 Hz), 8.77 (m, 2H, ArH), 8.15 (d, 1H, ArH, J = 8.5 Hz), 8.00 (d, 1H, ArH, J = 8.1 Hz), 7.76 (t, 1H, ArH, J = 8.3 Hz), 7.59 (t, 1H, ArH, J = 7.7 Hz), 7.31 (t, 1H, ArH, J = 7.9 Hz), 7.08 (d, 1H, ArH, J = 7.8 Hz), 6.63 (s, 1H, ArH), 6.53 (s, 1H, ArH), 6.28 (t, ArH, J = 7.6 Hz), 3.89 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.66 (s, 2H, CH_2), 3.60 (m, 2H, CH_2), 2.80 (m, 6H, CH_2), 1.89 (m, 2H, CH_2); NMR ^{13}C (75.5 MHz, $CDCl_3$) δ: 168.9, 163.9, 149.3, 149.2, 147.9, 147.6, 140.2, 135.6, 132.3, 131.2, 129.4, 129.2, 127.7, 127.3, 127.0, 126.9, 125.8, 125.7, 122.6, 121.1, 119.5, 111.5, 109.5, 58.6, 56.1, 55.9, 55.6, 51.4, 41.5, 28.8, 23.6; MS: 524.3 $[M^+]$. HRMS $[M^+]$: calcd for $C_{31}H_{32}N_4O_4$ 524.2423; Found: 524.2430.

7.4.4.8. Preparation of *N*-[2-({[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl]amino} carbonyl)phenyl]quinoline-3-carboxamide (24b). To a solution of dichloromethane (10 mL) containing **19b** (0.119 mol) were added 3-quinoline acid chloride and DOWEX 1X8 (0.111 g, 100–200 mesh) to give a sticky yellow oil after purification (0.0507 g, 79%). IR (NaCl, cm^{-1}); 2927, 2856 (C–H); 1600 (CO); 1519, 1448, (C=C Ar); 1265 (tertiary amine); 739 (CH deformation Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.80 (s, 1H, NH) 9.54 (d, 1H, ArH, J = 2.1 Hz), 8.75 (m, 2H, ArH), 8.32 (m, 1H, NH), 8.15 (d, 1H, ArH, J = 8.4 Hz), 8.00 (d, 1H,

ArH, J = 8,0 Hz), 7.78 (t, 1H, ArH, J = 7.9 Hz), 7.61 (t, 1H, ArH, J = 7.5Hz) 7.40 (t, 1H, ArH, J = 7.5 Hz), 7.26 (m, 1H, ArH), 6.51 (m, 2H, ArH), 6.40 (s, 1H, ArH), 3.84 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.50 (m, 4H, CH_2), 2.74, (m, 4H, CH_2), 2.56 (m, 2H, CH_2), 1.78 (m, 4H, CH_3); NMR ^{13}C (75.5 MHz, $CDCl_3$) δ: 169.2, 163.8, 149.4, 149.1, 147.8, 147.5, 139.6, 135.7, 132.2, 131.2, 129.4, 129.1, 127.6, 127.3, 127,0, 126.9, 125.8, 122.9, 121.3, 120.4, 111.4, 109.5, 56.9, 56.2, 55.9, 49.9, 39.9, 28.3, 26.8, 24.8; MS: 538.3 [M^+]. HRMS [M^+]: calcd for $C_{32}H_{34}N_4O_4$ 538.2580; Found: 538.2590.

7.4.4.9. Preparation of *N*-[2-({[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)pentyl]amino} carbonyl)phenyl]-quinoline-3-carboxamide (25b). To a solution of dichloromethane (10 mL) containing 20**b** (0.249 mol) were added 3-quinoline acid chloride and 0.155 g of DOWEX 1X8 (100–200 mesh) to give a yellow solid after purification (0.0751 g, 55%). Mp 87–89 °C; IR (NaCl, cm^{-1}); 3459 (NHCO); 3055, 2923, 2854 (C–H); 1672, 1598 (CO); 1519, 1446, (C=C Ar); 1267 (tertiary N); 740 (CH deformation Ar); NMR ^1H (300 MHz, $CDCl_3$) δ: 12.58 (s, 1H, NH), 9.52 (d, 1H, ArH, J = 2.1 Hz), 8.74 (m, 2H, ArH), 8.13 (d, 1H, ArH, J = 8.5 Hz), 7.97 (d, 1H, ArH, J = 8.1 Hz), 7.78 (t, 1H, ArH, J = 7.9 Hz), 7.59 (t, 1H, ArH, J = 7.7 Hz), 7.49 (t, 1H, ArH, J = 8.3 Hz), 7.44 (br s, 1H, NH), 6.98 (t, 1H, ArH, J = 7.1 Hz), 6.53 (s, 1H, ArH), 6.46 (s, 1H, ArH), 3.80 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.50 (s, 2H, CH_2), 3.44 (m, 2H, CH_2), 2.76 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 2.46 (t, 2H, J = 7.2 Hz), 1.60 (m, 4H, CH_2), 1.40 (m, 2H, CH_2); NMR ^{13}C (75.5 MHz, $CDCl_3$) δ: 169.2, 163.9, 149.4, 148.9, 147.6, 147.3, 139.6, 135.7, 132.6, 131.4, 129.4, 129.2, 127.5, 127.4, 126.9, 126.7, 126.3, 126.1, 123.2, 121.5, 120.6, 111.4, 109.5, 57.9, 55.9, 55.8, 55.7, 50.9, 39.9, 29.3, 28.5, 26.7, 24.7; MS: 553.27 [M+H]$^+$. HRMS [M+H]$^+$: calcd for $C_{33}H_{36}N_4O_4$ 552.2736; Found: 553.2815.

7.4.5. Preparation of *N*-[2-({[2-(6,7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)hexyl]amino} carbonyl)phenyl]quinoline-3-carboxamide (26b). To a solution of dichloromethane (10 mL) containing **21b** (0.324 mol) were added 3-quinoline acid chloride and DOWEX 1X8 (0.303 g, 100–200 mesh) to give a yellow solid after purification (0.113 g, 62%). Mp 78–79 °C;IR (NaCl, cm^{-1}); 3055, 2927, 2856 (C–H); 1674, 1599 (CO); 1518, 1447, (C=C Ar); 1265 (tertiary amine); 739 (CH deformation Ar). NMR ^1H (300 MHz, $CDCl_3$) δ: 12.59 (s, 1H, NH), 9.49 (m, 1H, ArH), 8.70 (m, 2H, ArH), 8.10 (d, 1H, ArH, J = 8.4 Hz), 7.93 (d, 1H, ArH, J = 8.1 Hz), 7.75 (t, 1H, ArH, J = 7.0 Hz), 7.53 (m, 1H, ArH), 7.42 (t, 1H, ArH, J = 7.6 Hz), 7.17 (m, 1H, NH), 6.97 (t, 1H, ArH, J = 7.7 Hz), 6.50 (s, 1H, ArH), 6.43 (s, 1H, ArH), 3.76 (s, 3H, OMe), 3.74 (s, 3H, OMe), 3.42 (m, 4H, CH_2), 2.73 (m, 2H, CH_2), 2.60 (m, 2H, CH_2), 2.40 (m, 2H, CH_2), 1.40 (m, 8H, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 169.2, 163.8, 149.3, 148.9, 147.5, 147.2, 139.6, 135.7, 132.5, 131.4, 129.3, 127.4, 126.9, 126.7, 126.2, 123.2, 121.4, 120.6, 111.4, 109.5, 58.2, 55.9, 55.8, 55.7, 50.9, 40.1, 29.4, 28.6, 27.2, 27.1, 26.9; MS: 567.26 [M+H]$^+$. HRMS [M+H]$^+$: calcd for $C_{34}H_{38}N_4O_4$ 566.2893; Found: 567.2978.

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