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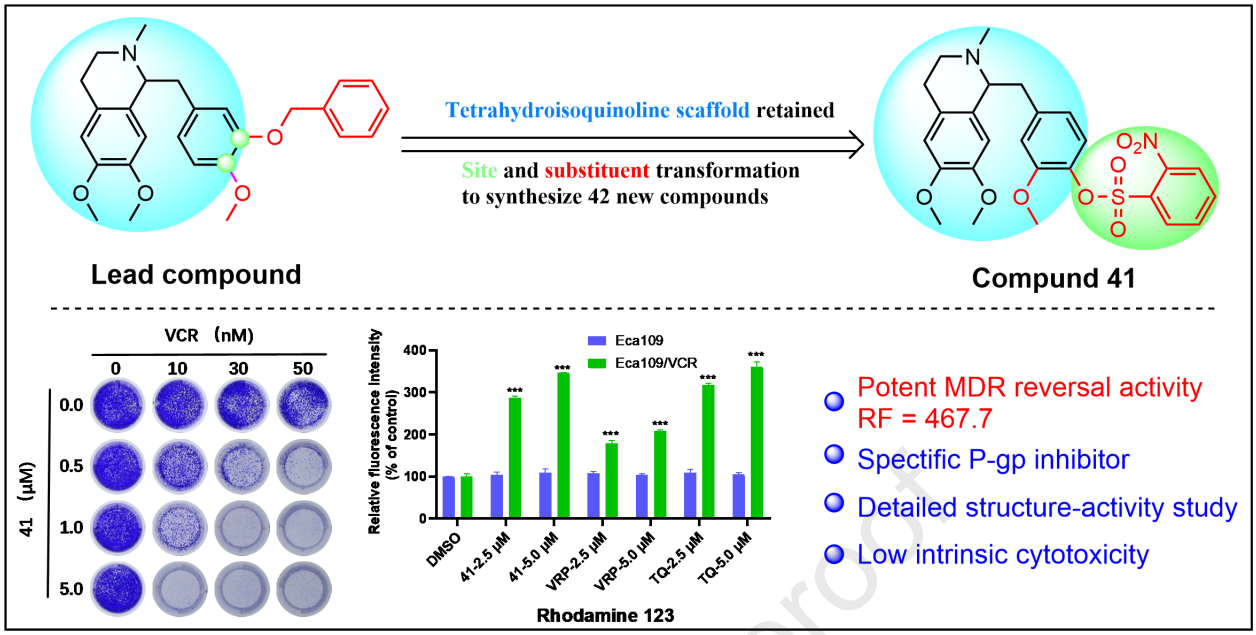

Derivatives Against Multidrug Resistance in Eca109/VCR Cells

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Abstract: The advent of multidrug resistance (MDR) in tumors markedly diminishes the effectiveness of anticancer therapies. P-glycoprotein (P-gp) plays a crucial role in tumor MDR by mediating the efflux of drugs and cytotoxic agents. Presently, small molecule agents targeting P-gp are among the promising therapeutic approaches to counteract MDR. In previous research, our team identified a novel class of P-gp inhibitors featuring a 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline scaffold. To further delineate the structure-activity relationship, this study conducted an extensive structural optimization, synthesizing 42 novel compounds. Evaluation on the drug-resistant cell line Eca109/VCR indicated that the majority of these compounds exhibited remarkable MDR-reversing activity. Notably, the optimized compound **41** demonstrated an outstanding ability to reverse MDR, with a reversal fold of up to 467.7, surpassing the efficacy of the standard third-generation P-gp inhibitor **TQ**, as evidenced by plate cloning assay and flow cytometry analysis. Subsequent mechanism validation experiments—including western blotting, chemosensitization tests, and fluorescent substrate accumulation assays—complemented by molecular docking studies, confirmed that compound **41** exerts its MDR-reversing effects through P-gp inhibition. This research offers new perspectives for the development of drug sensitizers targeting resistant tumors based on the tetrahydroisoquinoline scaffold.

Keywords: tetrahydroisoquinoline derivatives; multidrug resistance; P-glycoprotein; Eca109/VCR.

1. Introduction

The onset of multidrug resistance (MDR)[1] is frequently associated with prolonged chemotherapy treatments. This phenomenon is characterized by the cross-resistance of malignant tumors to a variety of chemotherapeutic agents that differ in both structure and mechanism of action, ultimately diminishing treatment efficacy and heightening the risk of disease recurrence. The emergence of MDR is a complex process, commonly attributed to factors such as enhanced drug efflux[2, 3], altered drug metabolism[4], cellular autophagy[5], and improved DNA damage repair[6]. Among these, the increased drug efflux mediated by P-glycoprotein (P-gp)[7-9] has garnered significant attention as a target for overcoming multidrug resistance due to its well-defined pharmacological properties and structural characteristics.

P-gp (also known as ABCB1 or MDR1) is a 170 kDa[10] plasma membrane glycoprotein encoded by the human mdr1 gene and belongs to the ATP-binding cassette (ABC) transporter family[11-14]. This family comprises nucleotide binding domains (NBDs) that bind and hydrolyze ATP[15, 16], inducing conformational changes in the transmembrane domains (TMDs) to facilitate substrate efflux[17]. In tumor cells, P-gp is often overexpressed[18, 19], leading to the extrusion of drugs from the intracellular environment, thereby reducing the intracellular drug concentration[20] and leading to MDR[21]. Developing effective P-gp inhibitors for use in combination with conventional chemotherapeutic agents has long been considered a promising strategy[22-34] for reversing clinical MDR.

Over recent decades, numerous P-gp inhibitors have been developed[35-61], which can be broadly categorized into three generations. First- and second-generation P-gp inhibitors, such as Verapamil (**VRP**)[50, 58] and Biricodar[56, 57], exhibit several limitations, including systemic toxicity and significant interactions with cytochrome CYP3A4[62], resulting in problematic pharmacokinetic characteristics. In contrast, third-generation P-gp inhibitors, such as Tariquidar (**TQ**)[51, 55], Elacridar[52, 54], and Zosuquidar[53], have demonstrated enhanced selectivity, stronger affinity for P-gp, and greater reversal activity. These characteristics deem them promising candidates in preclinical and clinical studies for overcoming drug resistance and sensitizing chemotherapy agents. However, recent clinical trials of **TQ** have been paused due to significant side effects reported in Phase III trials for lung cancer[63]. Consequently, the development of P-gp inhibitors that increase efficacy and reduce toxicity is of great significance for truly solving clinical tumor MDR.

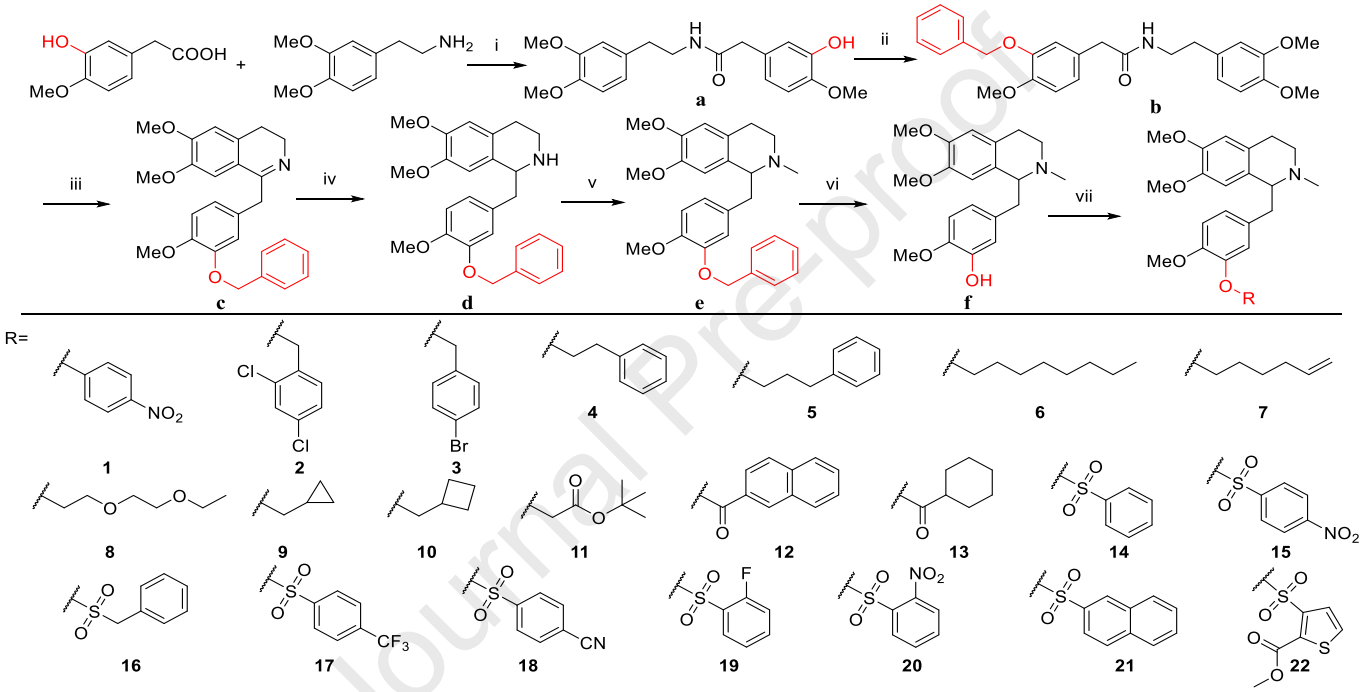
In previous studies, a novel class of P-gp inhibitors with a 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline scaffold was

identi ses on targeted optimization and modification to develop more effective and less toxic novel P-gp inhibitors. Specifically, intermediates **f** (Scheme 1) and **g** (Scheme 2) were utilized as the reaction substrates, performing a series of substitutions on the exposed hydroxyl groups, ultimately yielding 42 compounds. Subsequently, structure-activity relationship was analyzed to further elucidated the impact of different steric hindrances and substituents on pharmacological efficacy of the synthesized compounds. Finally, the optimal compound **41** was identified as a potent P-gp inhibitor through a series of biological characterizations, outperforming the typical third-generation P-gp inhibitor **TQ** in both cytotoxicity and reversal activity.

2. Results and discussion

2.1 Chemistry

The general synthetic route for compounds **1-42** is depicted in **Scheme 1** and **Scheme 2**. The target products were synthesized through a series of reactions[39], including condensation, substitution, the Bischler-Napieralski reaction, reductive amination, debenzylation, and further substitution reactions. All compounds have been reported for the first time, and their final structures were thoroughly characterized using ^{1}H NMR, ^{13}C NMR, and HRMS.

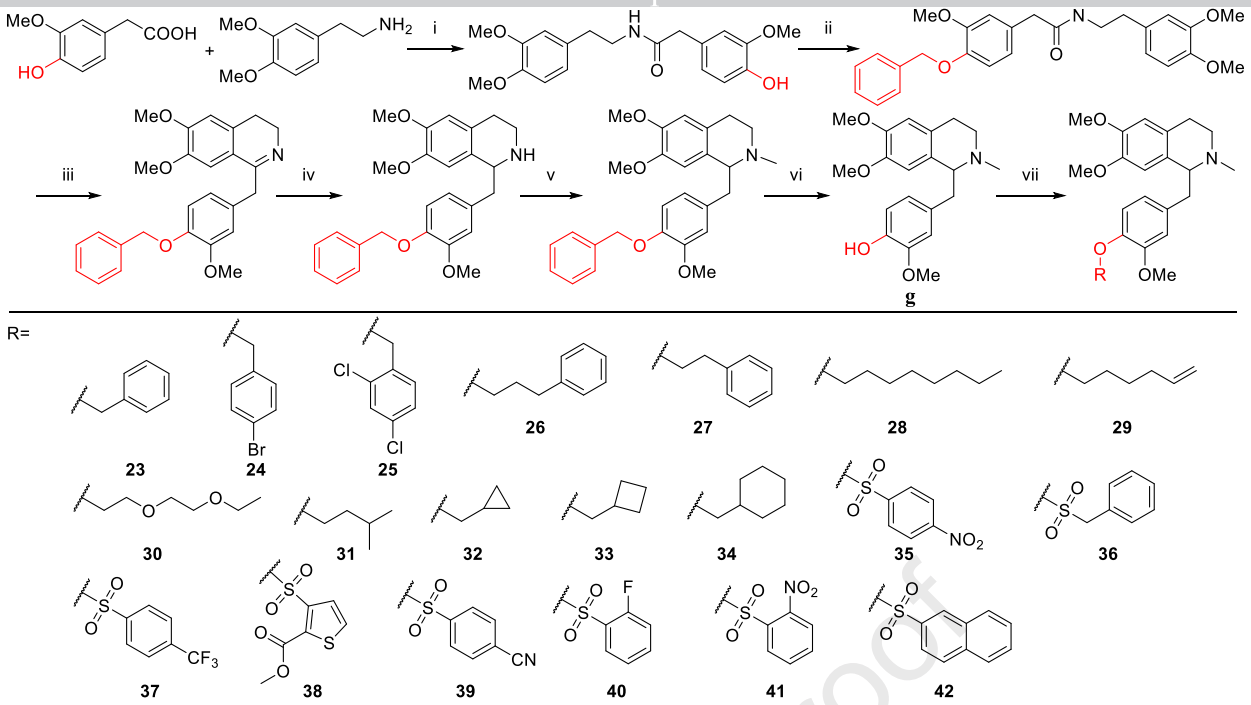


Scheme 1. Synthesis of compounds **1-22**. Reagents and conditions: (i) Tol, H_3BO_3, reflux, 150 °C, 24 h; (ii) K_2CO_3, MeCN, BnBr, reflux, 80 °C, 20 h; (iii) $POCl_3$, DCM, reflux, 60 °C, 8 h; (iv) $NaBH_4$, MeOH, 0 °C, 5 h; (v) HCHO, $NaBH_4$, MeOH, 0 °C, 5 h; (vi) $NiCl_2 \cdot 6\,H_2O$, $NaBH_4$, MeOH, 0 °C, 8 h; (vii) MeCN, K_2CO_3, R-X, reflux, 70 °C, 8 h.

2.2 Biological Screening and Structure-Activity Relationship

The cytotoxicity of all synthesized compounds against Eca109/VCR[39, 64, 65] cells and their effectiveness in reversing vincristine (**VCR**) resistance were evaluated using CCK-8 assays, with results detailed in **Table 1**. Overall, the compounds exhibited relatively low cytotoxicity, with the exception of compound **2**, which resulted in a cell viability of only 55.4%. This suggests that most of these compounds are well-suited for co-administration with antitumor agents in practical applications.

The reversal fold (RF) is a metric used to evaluate the reversing effect of the test compounds, defined as the ratio of the IC_{50} value of **VCR** alone to the IC_{50} value when **VCR** is co-administered with the test compounds. Based on the RF values of the compounds, a SAR analysis was conducted. Using a previously studied compound as a lead, a series of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives (42 compounds) were designed and synthesized by modifying the 3-site and 4-site of its benzyl phenyl ring. Encouragingly, most of the compounds demonstrated good reversal activity against VCR-resistant Eca109/VCR cells, with compound **41** showing the best reversal activity (RF=467.7).



Scheme 2. Synthesis of compounds **23-42**. Reagents and conditions: (i) Tol, H_3BO_3, reflux, 150 °C, 24 h; (ii) K_2CO_3, MeCN, BnBr, reflux, 80 °C, 20 h; (iii) $POCl_3$, DCM, reflux, 60 °C, 8 h; (iv) $NaBH_4$, MeOH, 0 °C, 5 h; (v) HCHO, $NaBH_4$, MeOH, 0 °C, 5 h; (vi) $NiCl_2 \cdot 6 H_2O$, $NaBH_4$, MeOH, 0 °C, 8 h; (vii) MeCN, K_2CO_3, R-X, reflux, 70 °C, 8 h.

2.2.1 Fragment Extension Study on the 3-site of Benzene Ring of Isoquinoline Derivatives

Initially, we conducted a fragment extension study focusing on the 3-site of benzene ring of isoquinoline derivatives. The result showed that substituted benzyl group functions as a suitable moiety, with compounds **1**, **2**, and **3** achieving moderate to excellent RF values. However, the high cytotoxicity associated with compound **2** diminishes confidence in its RF value, suggesting that it might be more applicable as a cytotoxic agent rather than as a sensitizer for drug-resistant tumors.

Further elongation of the alkyl chain led to the synthesis of compounds **4** and **5**, indicating that an inappropriately long carbon chain at this position notably reduces reversal activity. Exploring the reverse activity of alkyl substituents revealed that pure linear alkyl chains (**6**) exhibited significant effects; however, terminal alkenes (**7**) led to a marked reduction in RF. This may be attributed to alkenes' susceptibility to nucleophilic amino acid attacks, resulting in increased off-target effects.

When an oxygen atom was incorporated into the linear alkyl chain, as seen in compound **8**, reversal activity diminished significantly, likely due to the strong influence of the oxygen atom as a hydrogen bond donor. Investigations into cycloalkane substituents showed that ring size greatly impacts activity, with cyclobutane substituents (**10**) outperforming cyclopropane substituents (**9**) in terms of reversal activity.

We also explored introducing various carbonyl compounds. Among these, -CH_2CO_2tBu (**11**) and -cyclohexanecarbonyl (**13**) demonstrated a near-complete loss of reversal activity, while -2-naphthalenecarbonyl (**12**) showed improved reversal activity. This pattern aligns with findings from compounds **1-5**, indicating that aryl groups with appropriate spacing can enhance reverse activity. And this also suggests that, to some extent, aryl groups are more beneficial than alkyl groups in enhancing activity.

Given the unique benefits of sulfonyl groups in drug design, such as enhancing hydrogen bond donation, improving water solubility, and optimizing pharmacokinetics, sulfonyl groups were introduced to synthesize compounds **14-22** in an effort to enhance reverse activity. Encouragingly, most sulfonyl-containing compounds demonstrated excellent reversal activity, particularly compounds **17** and **21**, whose activities were comparable to the positive control, **TQ**. Although the introduction of sulfonyl groups significantly enhances activity, as easily demonstrated by the comparisons between compounds **1** and **15**, as well as **12** and **21**, the negative impact of unsuitable substituted aryl groups on activity must still be considered during structural design (compounds **14**, **19**, and **22**).

Table 1. Cytotoxicity and reversal activity of compounds 1-42 against Eca109 / VCR cells[a]

Compound[b]	Cell viability (%)[c]	IC_{50} (nM)[d]	LogP/ LogD[e]	RF[f]	Compound[b]	Cell viability (%)[c]	IC_{50} (nM)[d]	LogP/ LogD[e]	RF[f]
VCR	-	4152±699.5	-	1.0	**23**	101.4±2.3	51.7±10.6	4.2/4.0	80.3

2	55.4±3.2	26.3±2.5	5.3/4.3	412.3	**25**	92.4±0.8	13.5±4.6	5.3/4.3	307.6
3	97.8±2.0	26.3±2.4	5.0/4.1	158.0	**26**	103.3±0.5	25.6±3.3	4.8/4.2	162.4
4	102.4±2.8	336.5±125.6	4.5/4.1	12.3	**27**	104.2±1.3	2347.0±279.0	4.5/4.1	1.8
5	96.9±3.4	33.5±7.8	4.9/4.2	124.1	**28**	94.7±2.8	50.3±3.8	6.0/4.6	82.6
6	95.3±5.2	26.1±5.0	6.0/4.6	159.0	**29**	103.4±1.2	2141.0±124.8	4.7/4.0	1.9
7	98.2±5.6	272.3±68.7	4.7/4.0	15.2	**30**	103.6±1.6	489.4±135.9	2.5/3.0	8.5
8	103.1±3.3	1864.0±336.6	2.5/3.0	2.2	**31**	105.5±1.3	18.3±1.9	4.4/4.2	227.1
9	105.6±5.8	654.9±99.4	3.9/3.6	6.3	**32**	104.2±1.2	2750.7±526.7	3.9/3.6	1.5
10	102.7±2.3	33.9±7.5	4.3/3.9	122.6	**33**	107.9±5.1	22.0±4.1	4.3/3.9	188.7
11	104.3±4.7	1137.8±240.2	3.7/3.4	3.6	**34**	87.7±2.6	14.8±1.3	5.2/4.8	280.0
12	101.1±1.0	38.5±5.7	5.3/4.4	107.9	**35**	105.6±0.7	24.1±3.6	3.6/3.5	172.2
13	103.6±0.8	926.2±243.7	4.7/3.9	4.5	**36**	102.6±0.7	24.2±3.4	3.6/3.4	171.6
14	102.6±0.5	172.9±69.6	3.6/3.5	24.0	**37**	90.4±1.4	13.5±4.2	4.4/4.0	307.4
15	101.5±1.8	29.3±5.9	3.6/3.5	141.5	**38**	101.9±1.1	22.8±2.5	3.4/3.3	182.2
16	101.5±2.9	23.1±1.8	3.6/3.4	180.0	**39**	99.9±1.2	24.6±1.3	3.4/3.2	168.5
17	100.5±1.3	11.2±1.0	4.5/4.0	371.9	**40**	98.2±1.2	15.1±4.9	3.7/3.5	274.3
18	96.0±1.2	17.6±0.1	3.4/3.1	235.4	**41**	99.1±2.0	8.9±2.3	3.4/3.3	467.7
19	98.9±0.7	252.0±126.4	3.7/3.5	16.5	**42**	84.7±1.4	11.5±3.0	4.8/4.8	362.1
20	102.0±4.2	16.4±1.5	3.4/3.3	253.3	**VRP**	98.9±1.3	38.5±6.3	3.3/3.4	107.9
21	94.7±2.7	12.2±0.2	4.8/4.0	340.5	**TQ**	84.8±4.7	11.4±2.8	5.2/3.7	364.3
22	100.7±1.7	336.5±125.6	3.5/3.2	12.3					

[a] Cell viability and IC_{50} values were obtained from three independent experiments and presented as the mean±SD. The P-gp inhibitors **VRP** and **TQ** were used as positive controls and DMSO was used as negative control. [b] All tested compounds and positive controls were using in 5.0 μM. [c] Cell viability of Eca109/VCR compared to the negative control (DMSO, 100%) after treatment with the tested compound for 48 h. [d] IC_{50} value of **VCR** with or without combination of the tested compounds against drug resistant cells Eca109/VCR. [e] logP and $logD_{7.4}$ were calculated by the webtool ADMETlab 2.0. [f] Reversal fold (RF) was defined as the ratio of (IC_{50} value of **VCR** alone) /(IC_{50} value of **VCR** combined with the tested compounds).

2.2.2 Fragment Extension Study on the 4-site of Benzene Ring of Isoquinoline Derivatives

To explore the impact of different substitution sites on reversal activity and identify the optimal active site, we focused on fragment growth at the 4-site of benzene ring of isoquinoline derivatives, synthesizing compounds **23**-**42**. Interestingly, compounds substituted at the 3-site and 4-site exhibited similar trends regarding single-drug cytotoxicity and reversal activity: 1) Substituted benzyl groups (**23**-**25**) are beneficial to activity, particularly when halogen substituents are present; 2) The introduction of phenyl groups requires an appropriate carbon chain linker, otherwise, the activity may diminish significantly, as observed in compounds **26**, **27**; 3) Long-chain alkyl substituents (**28**, **31**) are beneficial; however, the further introduction of double bonds (**29**) or ether linkages (**30**) has a detrimental effect on reverse activity; 4) Cycloalkyl groups with at least four members are beneficial, with larger rings yielding better results (**32**-**34**); 5) The introduction of sulfonyl groups can significantly enhance reverse activity (**35**-**42**).

In addition to these trends, 4-site substituted compounds generally show advantages over 3-site substituted ones in terms of single-drug cytotoxicity and reversal activity, as exemplified by (**2**) and (**25**), (**20**) and (**41**). Notably, even groups considered unsuitable at the 3-site, like 2-fluorophenyl (**19**) and thienyl (**22**), demonstrated substantial reversal activity when substituted at the 4-site (**38**, **40**).

In summary, our detailed study of the structure-activity relationships of tetrahydroisoquinoline derivatives, summarized in **Fig.1**, led to the synthesis of 42 compounds, identifying compound **41** as the optimal candidate with a RF of up to 467.7. Further exploration of the mechanisms is planned in the next.

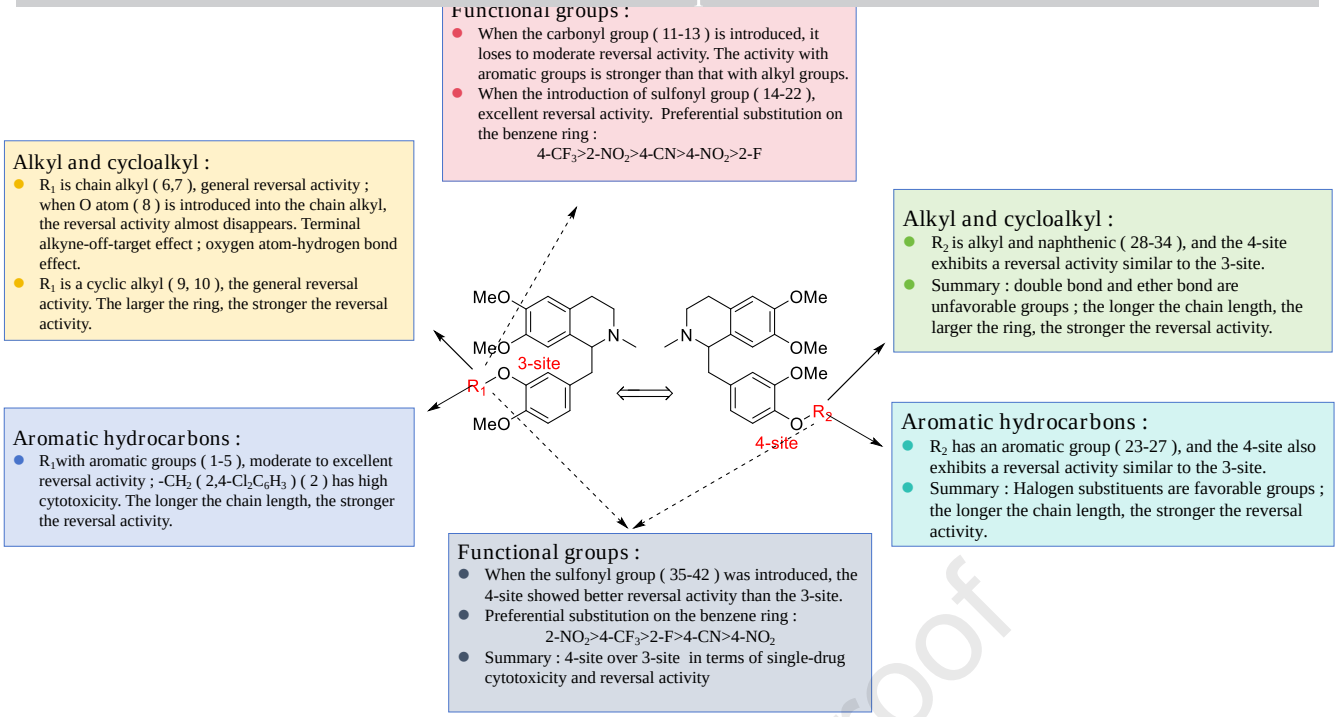


Fig.1. SAR of novel isoquinoline alkaloid derivatives

2.2.3 Impact of Lipophilicity on Reversal Activity

During SAR studies, we assessed the lipophilicity of all synthesized compounds using the ADMETlab 2.0 web tool[66], predicting both logP and logD values and summarizing in **Table 1**. The logP values for these compounds ranged from 2.5 to 6.0, while the logD values ranged from 3.0 to 4.8. Although no clear linear relationship was observed between lipophilicity (LogP/LogD) and RF, a preliminary correlation was identified through grouped statistical analysis. As shown in Support Information **Fig.S1**, compounds in high-RF group (RF > 100) demonstrated a significantly higher average LogP/LogD (Δ LogP = +0.46, Δ LogD = +0.23) compared to the low-RF group (RF < 50), suggesting that relatively higher lipophilicity is beneficial for improving RF From a statistical perspective. Additionally, the majority of sulfonyl-containing compounds (green triangles) are concentrated in the high-RF group (13/17) and predominantly located below the median of the statistical box (15/17 for LogP group and 16/17 for LogD group), with the exception of the compound **42**. This phenomenon may be attributed to the introduction of sulfonyl groups, which enhances water solubility, increases hydrogen-bond acceptors, and elevates topological polar surface area (TPSA), thereby contributing to improved pharmacological profiles, so that exhibiting comparatively lower LogP/LogD values while maintaining relatively high RF values.

2.3 Apoptosis and Flow Cytometry Analysis Experiments

Following the SAR studies and confirming that compound **41** exhibits optimal reversal activity, we further validated its ability to reverse drug-resistant phenotypes using flow cytometry to assess apoptosis in resistant cells. **Fig.2A** and **2B** illustrate that treatment with **VCR** alone resulted in a very low apoptosis rate in Eca109/VCR cells, highlighting their substantial resistance to **VCR**. Additionally, treatment with sensitizers **41**, **VRP**, or **TQ** alone had minimal impact on the apoptosis of the resistant strains. In contrast, combining compound **41** or the positive control drugs (**VRP**, **TQ**) with 100 nM **VCR** significantly enhanced **VCR**'s cytotoxicity against drug-resistant Eca109/VCR cells, as evidenced by a marked increase in apoptotic cells. These flow cytometry analyses confirm that compound **41** exhibits exceptional reversal activity against Eca109/VCR cell drug resistance, with this activity being enhanced at increasing doses of compound **41**.

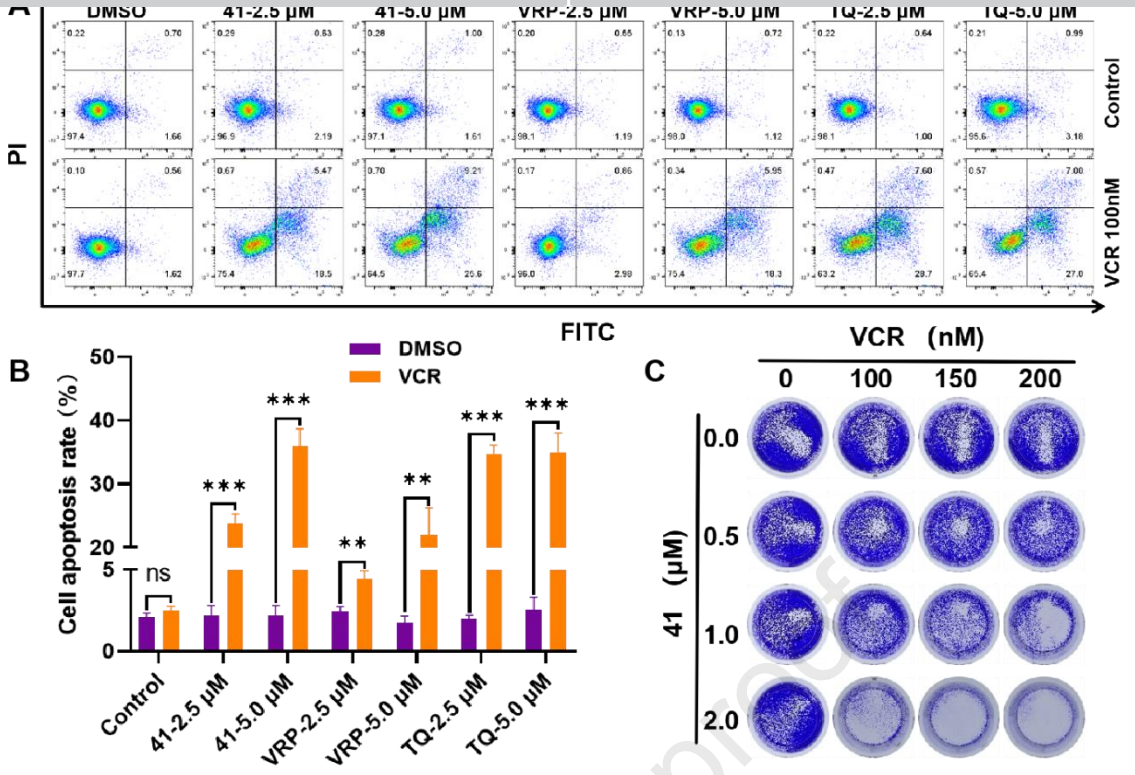


Fig.2. (A) Apoptosis of Eca109/VCR cells treated with **41**, **VRP** and **TQ** (2.5 and 5.0 μM) with or without 100 nM **VCR** for 60 h. (B) Statistical diagram of apoptosis of Eca109/VCR.(n.s, not significant;****,P < 0.0001). (C) Effect of compound **41** combined with **VCR** on Eca109/VCR Cells.

2.4 Plate Cloning Assay

In addition to flow cytometry, we continued to use the plate cloning formation assay to validate the reversal phenotype against MDR by compound **41**. As depicted in **Fig.2C**, treatment with either compound **41** or **VCR** alone did not reduce the plate cloning formation of drug-resistant Eca109/VCR cells. However, when combined, compound **41** and **VCR** significantly enhanced the cytotoxic effects on these resistant cells compared to single-drug treatments. Additionally, the sensitization effect of compound **41** on the resistant strains became more pronounced with increasing doses. It is worth noting that under identical conditions within the same experimental batch, compound **41** demonstrated superior reversal activity compared to **OY-101** (the previously reported optimal compound) in the colony formation assay, as detailed in **Fig.S2**.

2.5 Effect of Compound 41 on P-gp Expression

Having confirmed the ability of compound **41** to reverse the MDR phenotype, we investigated its underlying mechanisms. Initially, Western blot analysis was performed to assess the expression levels of key drug resistance proteins in both parental and resistant strains. As shown in **Fig.3A** and **Fig.3B**, the resistant strains displayed significant overexpression of P-gp compared to the parental strains. Subsequently, Eca109/VCR cells were treated with varying concentrations of compound **41**, alongside blank solvent and positive drug controls. The results showed that neither compound **41** nor the control treatments led to significant changes in the expression levels of P-gp. Therefore, we infer that the reversal activity of compound **41** may primarily occur through direct intervention in P-gp function rather than through regulation of its expression.

2.6 Sensitization Effect of Compound 41 on Other Chemotherapeutic Agents.

To further explore the potential of compound **41** in reversing drug resistance, an assessment was conducted on its capacity to counteract resistance to other chemotherapeutic agents that are recognized substrates of ABC transporters. Specifically, a concentration of 5.0 μM of compound **41** was administered to both parental Eca109 cells and drug-resistant Eca109/VCR cells, and the resulting changes in their IC_{50} values were recorded. As illustrated in **Fig.3C**, the drug-resistant Eca109/VCR cells displayed significant multidrug resistance to P-gp substrates such as mitoxantrone, colchicine, docetaxel, and doxorubicin, whereas no resistance was seen against the non-P-gp substrate lapatinib. Additionally, it was observed that compound **41** exhibited reversing activity against non-P-gp specific substrates, including mitoxantrone (co-transported by P-gp and ABCG2) and doxorubicin (co-transported by P-gp and MRP1), while showing even stronger reversing activity against P-gp specific substrates like colchicine and docetaxel. Consequently, it can be concluded that compound **41** potently inhibits the function of P-gp in drug-resistant Eca109/VCR cells, thereby effectively reversing their MDR.

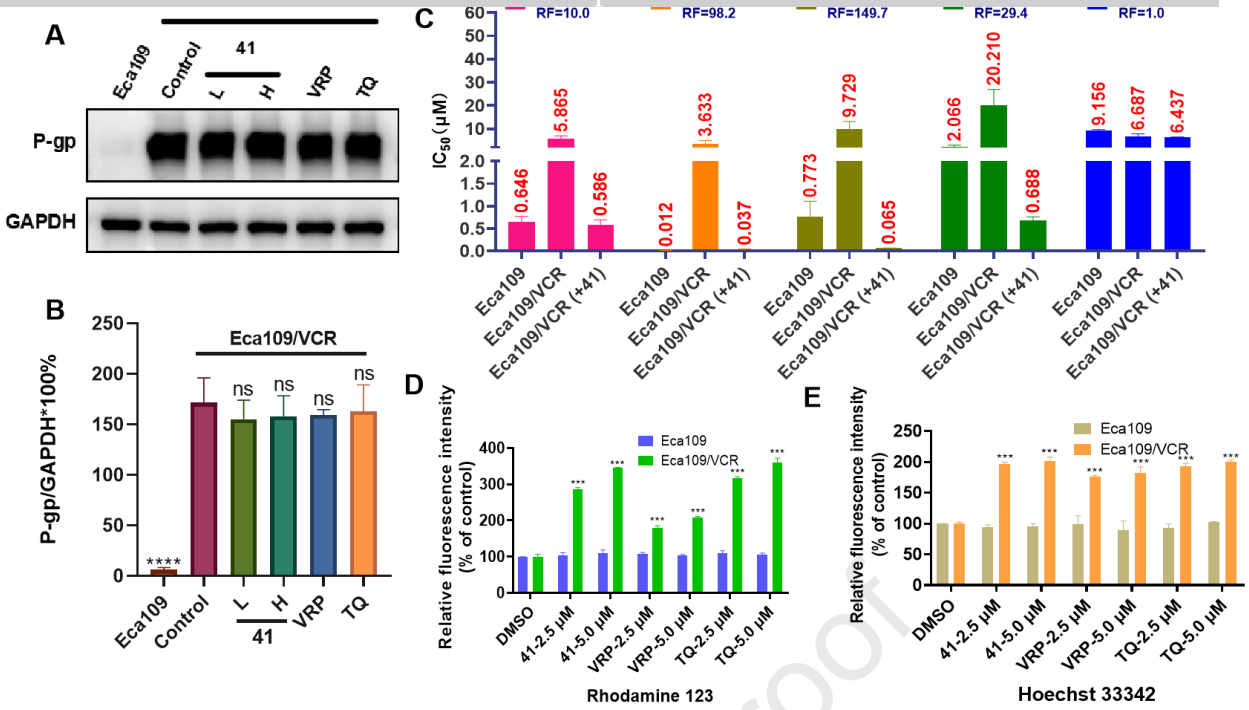


Fig.3. (A,B) Western blotting. The expression of P-gp in parental cells Eca109 and drug-resistant cells Eca109/VCR, and the effects of 41(L: 5.0 μM; H: 10.0 μM), VRP (5.0 μM) or TQ (5.0 μM) on the expression for 24 h were measured by Western blot. Comparisons of the densitometric quantification were statistically estimated and represented as mean ± SD for three independent experiments (n.s, not significant; ****,$P < 0.0001$). (C) Effect of compound **41** on MDR of Eca109/VCR cells. (D,E) Effects of compounds on the accumulation of fluorescent substrates **RH123** and **H33342** in parental Eca109 cells and drug-resistant Eca109/VCR cells (***,$P < 0.001$).

2.7 The Effect of Compound 41 on the Accumulation of Fluorescent Substrates in Eca109/VCR Cells

To verify the specificity of compound **41** as a P-gp inhibitor, its effect on the efflux of the P-gp-specific fluorescent substrate Rhodamine 123 (**RH123**) was assessed. As shown in **Fig.3D**, treatment with compound **41**, along with positive controls **VRP** and **TQ**, did not significantly increase the intracellular accumulation of **RH123** in parental Eca109 cells. In contrast, the same treatment led to a significant increase in the relative content of the fluorescent substrate within drug-resistant Eca109/VCR cells. This suggests that P-gp expression is the primary factor differentiating the parental and resistant cell lines concerning drug resistance-associated proteins. Moreover, similar outcomes were observed when replacing **RH123** with Hoechst 33342 (**H33342**) (**Fig.3E**), a common substrate co-transported by both P-gp and ABCG2. However, the ability to inhibit the efflux of the fluorescent substrate was slightly weaker, likely due to **H33342** being extruded by both P-gp and ABCG2. These findings indicate that, when used in combination with drugs, compound **41** demonstrates a capacity to inhibit P-gp activity comparable to the positive controls **VRP** and **TQ**, thereby influencing the accumulation of fluorescent substrates. Although compound **41**'s activity profile aligns with selective P-gp inhibition, its potential off-target effects on other ABC transporters cannot be ruled out without comprehensive selectivity screening. Such evaluations will be prioritized in future studies.

2.8 Molecular Docking Analysis of Compound 41 with P-gp

The interaction between compound **41** and P-gp (PDB entry: 6QEX)[67] was analyzed using SYBYL-X 2.0[68]. A high-resolution cryo-EM structure of P-gp bound to its native ligand was retrieved from the UniProt database. The protein was preprocessed in PyMol[69] by removing water molecules, cholesterol, and phospholipids. Compound **41** was simulated under physiological ionization states (pH = 7) using SYBYL, and all generated conformers were docked into the P-gp binding pocket. As illustrated in **Fig. 4A**, compound **41** resides within the central cavity of P-gp, forming hydrogen bonds with PHE343, GLN347, PHE983, and GLN990, while engaging in hydrophobic interactions with TRP232, ILE306, PHE336, PHE343, PHE728, and PHE983. Notably, the benzene ring of the tetrahydroisoquinoline scaffold participates in π-π stacking with the aromatic side chain of PHE983, further stabilizing the binding interface.

A similar molecular docking analysis was performed by replacing compound **41** with compound **20** (**Fig.4B**), as they share highly similar structural features, differing only in the position of a substituent—one at the 3-site and the other at the 4-site. Due to the altered substituent position, we observed that the 2-nitrobenzenesulfonyl group of compound **20** cannot effectively approach PHE343 and GLN347 to form hydrogen bonds, nor engage in hydrophobic interactions with TRP232 and ILE306. This positional variation also causes slight deviation of its tetrahydroisoquinoline scaffold from compound **41**, thereby

preve es are reflected in the SYBYL docking scores (energy-based scoring function weighted by intermolecular distance, hydrophobic contributions, electrostatic interactions, and shape complementarity). Compound **41** achieved a score of 9.2051, while **20** scored 8.7826, confirming weaker binding.

In summary, molecular docking analysis demonstrates that compound **41** exhibits strong direct interactions with P-gp, partially explaining the superior impact of 4-position substitution over the 3-position. Additionally, analysis of the results reveals that both compounds **41** and **20** adopt the *S*-configuration in their highest-scoring docking conformations. This observation aligns with prior studies reporting that *S*-configured analogs of this scaffold show enhanced P-gp inhibitory activity compared to their *R*-configured counterparts.

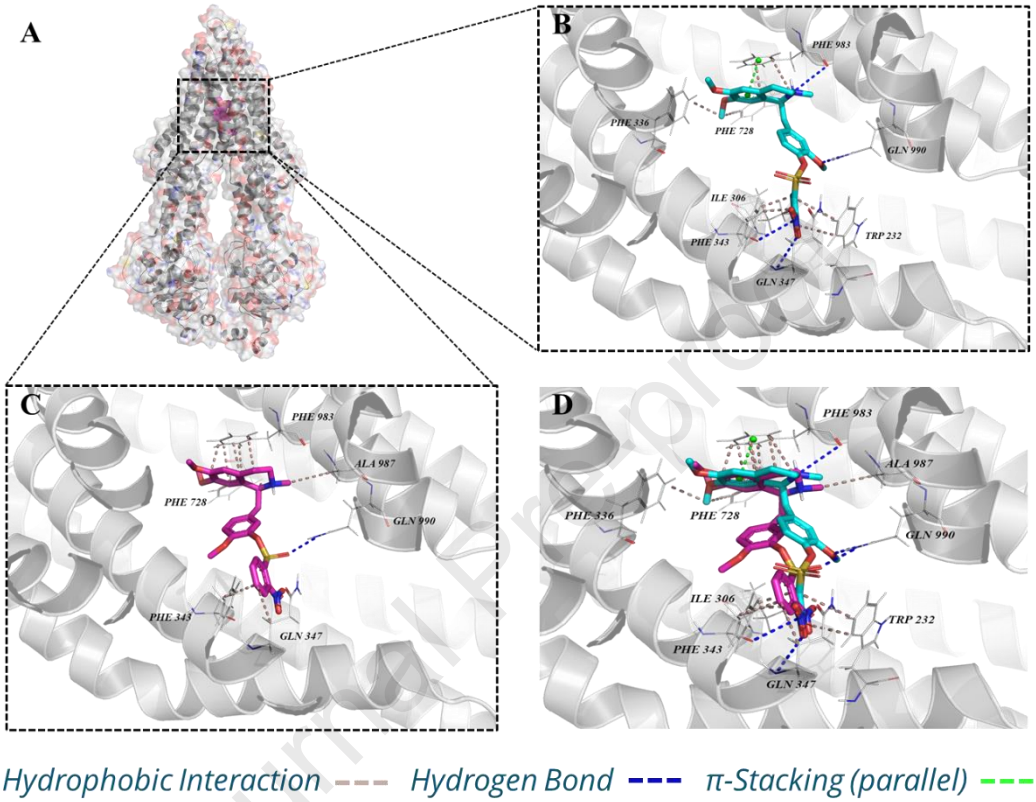


Fig.4 Molecular docking results of P-gp with compounds **41**/**20**. A. Global docking view. B. Interaction details of compound **41** with P-gp. C. Interaction details of compound **20** with P-gp. D. Overlay of compounds **41** and **20**.

2.9 Pharmacokinetic study of 41

The pharmacokinetic (PK) parameters of **41** in SD rats after intravenous (1 mg/Kg) and oral administration (10 mg/Kg) were measured (see detail in **SI**, **Table S1**). Compound **41** exhibits an extremely rapid metabolic rate, as evidenced by its half-life ($T_{1/2}$), time to peak concentration (T_{max}), terminal elimination rate constant (λ-z) and apparent clearance rate (Cl/F). Notably, the apparent clearance rate of orally administered compound **41** is as high as 393 L/kg/h, rendering it more suitable for specific clinical scenarios as a P-gp inhibitor: traditional P-gp inhibitors often cause systemic toxicity due to drug-drug interactions[70,71], thereby limiting their clinical application. Clinical reports have documented patient fatalities caused by adverse drug-drug interactions between P-gp inhibitors and co-administered drugs[72]. Compound **41** exhibits a high clearance rate, enabling rapid elimination after achieving therapeutic effects. Theoretically, by administering compound **41** via intravenous injection at the time when the chemotherapy drug reaches its peak plasma concentration, the intracellular accumulation of the chemotherapeutic agent in P-gp-overexpressing resistant tumor cells might be enhanced. Meanwhile, the rapid metabolic clearance of compound **41** reduces the risk of systemic drug-drug interactions, thereby suggesting a promising direction for future research.

3.Conclusions

In this study, a series of tetrahydroisoquinoline derivatives were synthesized and evaluated for their ability to reverse tumor MDR. Compound **41** emerged as the most potent candidate, reducing the IC_{50} of VCR to 8.9 ± 2.3 nM (RF = 467.7) in resistant Eca109/VCR cells, outperforming the third-generation P-gp inhibitor **TQ** with higher specificity and lower toxicity. Mechanistic

studie cking revealed strong binding affinity to P-gp. These findings highlight compound **41** as a promising MDR reversal agent through potent inhibition of P-gp. The PK study demonstrated that the introduction of the sulfonyl group accelerated drug metabolism, which may help address the issue of systemic toxicity caused by drug interactions. Future studies should prioritize in structural optimization to improve pharmacokinetics, and exploration of its broader applicability against other ABC transporters implicated in clinical drug resistance. Collectively, these results affirm a potent P-gp inhibitor and provides new insights into the development of resistance-reversing agents, utilizing isoquinoline as a core framework.

4 Experiments

4.1 Chemistry

Unless otherwise specified, all reagents and chemicals are from commercial suppliers and can be used directly. Unless otherwise stated, all reactions were carried out in a natural environment at room temperature and pressure, monitored by thin layer chromatography (TLC), and purified by column chromatography with a suitable solvent system. The target compounds were characterized by nuclear magnetic resonance spectroscopy and HRMS (ESI). ^{1}H NMR or ^{13}C NMR spectra were recorded on Agilent 600 or 150 MHz NMR spectrometer, respectively (Chemical shifts were reported in ppm from tetramethylsilane with the solvent resonance as the internal standard in $CDCl_3$ solution, unless otherwise noted). ESI-MS data were obtained using quadrupole time-of-flight (QTOF) mass spectrometer (Bruker Impact II, Bremen,Germany). All final compounds were purified to >95% purity as determined by High Performance Liquid Chromatography (HPLC) analysis with following analytical method: Waters e2695 with Shim-pack GIST C18 reversed-phase column (4.6 mm ×250 mm, 1.7 μm, flow rate = 0.5 mL/min or 1.0 mL/min). Melting points were determined using a capillary melting point apparatus.

4.1.1 General procedure for the synthesis of key intermediate f

3,4-dimethoxyphenylethylamine (4.66 mL, 26.8 mmol, 1.0 equiv.), H_3BO_3 (0.13 g, 2.144 mmol, 0.08 equiv.), 3-hydroxy-4-methoxyphenylacetic acid (4.88 g, 26.8 mmol, 1.0 equiv.) were dissolved in 150 mL toluene and stirred at 150 °C for 24 h. After the reaction was completed, the solvent was removed by vacuum distillation, and the residue was washed with EA to obtain a pale yellow solid **a**.

Intermediate **a** (8.48 g, 24.58 mmol, 1.0 equiv.) and K_2CO_3 (6.78 g, 49.16 mmol, 2.0 equiv.) were dissolved in 80 mL MeCN, stirred for 15 min, and then benzyl bromide (3.06 mL, 25.81 mmol, 1.05 equiv.) was added, and refluxed at 80 °C for 20 h. After the reaction is completed, the insoluble impurities are filtered out, and the solvent is removed by pressure distillation to obtain a reddish-brown oily **b**.

The intermediate **b** (9.47 g, 22.95 mmol, 1.0 equiv.) was dissolved in 80 mL DCM, and phosphorus oxychloride (6.41 mL, 68.85 mmol, 3.0 equiv.) was slowly added under ice bath conditions. After stirring for 15 min, it was refluxed and stirred at 60 °C for 8 h. After the reaction was completed, the reaction was quenched with saturated $NaHCO_3$ solution, and then extracted with DCM to obtain the organic phase, dried with anhydrous Na_2SO_4, concentrated and dried to obtain intermediate **c**.

The intermediate **c** (7.37 g, 17.6 mmol, 1.0 equiv.) was dissolved in 80 mL methanol, and the sodium borohydride powder (3.344 g, 88 mmol, 5.0 equiv.) was slowly added under ice bath conditions, stirring for 5 h. After the completion of the reaction, the organic phase was obtained by DCM extraction, dried by anhydrous Na_2SO_4, and concentrated and dried to obtain the intermediate **d**.

The intermediate **d** (6.74 g, 16.07 mmol, 1.0 equiv.) was dissolved in 80 mL of methanol, added with formaldehyde solution (3.64 mL, 48 mmol, 3.0 equiv.), and stirred for 15 min to activate formaldehyde. Then sodium borohydride powder (1.8 g, 48 mmol, 3.0 equiv.) was slowly added under ice bath conditions and stirred for 5 h. After the reaction was completed, the organic phase was extracted with DCM, dried with anhydrous Na_2SO_4, concentrated and dried to obtain intermediate **e**.

The intermediate **e** (1.25 g, 2.87 mmol, 1.0 equiv.) was dissolved in 40 mL of methanol, added with nickel(II) chloride hexahydrate (2.04 g, 8.61 mmol, 3.0 equiv.), and stirred for 20 min. Then, sodium borohydride powder (2.39 g, 63 mmol, 22.0 equiv.) was slowly added under ice bath conditions and stirred for 8 h. After the reaction was completed, it was extracted with DCM, dried with anhydrous Na_2SO_4, concentrated and dried, and purified by silica gel column chromatography (DCM : methanol = 40 : 1) to obtain intermediate **f**.

4.1.2 General procedure for the synthesis of compounds 1-22

The compound **f** (0.5 mmol, 1.0 equiv.) and K_2CO_3 (1.5 mmol, 3.0 equiv.) were dissolved in MeCN (4 mL), stirred for 20 min, added with halogenated compounds (1.0 mmol, 2.0 equiv.), and stirred at 70 °C for 8 h. After the completion of the reaction, the organic phase was extracted with DCM, dried with anhydrous Na_2SO_4, concentrated and dried, and purified by silica gel column chromatography (DCM : methanol = 60 : 1) to obtain products **1-22**.

ng to the general procedure for the synthesis of compounds **1-22**, compound **1** was obtained by using 1-iodo-4-nitrobenzene. Yellow oily, 43 mg, yield: 40 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15 (d, *J* = 9.2 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.85 (d, *J* = 9.2 Hz, 2H), 6.82 (s, 1H), 6.52 (s, 1H), 6.18 (s, 1H), 3.82 (s, 3H), 3.75 (s, 3H), 3.69 (t, *J* = 6.2 Hz, 1H), 3.66 (s, 3H), 3.14 – 3.07 (m, 2H), 2.89 – 2.85 (m, 1H), 2.74 (ddd, *J* = 16.5, 10.0, 4.1 Hz, 2H), 2.54 (t, *J* = 5.1 Hz, 1H), 2.51 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.58, 149.67, 147.44, 146.64, 142.30, 142.02, 133.23, 128.77, 127.86, 126.57, 125.70, 123.82, 115.79, 112.64, 111.28, 110.94, 64.62, 55.93, 55.73, 47.38, 42.75, 39.96, 25.70. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{28}N_2O_6$: 465.2020, found 465.2020.

1-(3-((2,4-dichlorobenzyl)oxy)-4-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(2):

According to the general procedure for the synthesis of compounds **1-22**, compound **2** was obtained by using 2,4-dichlorobenzyl bromide. Brown oily, 83 mg, yield: 72 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.4 Hz, 1H), 7.37 (d, *J* = 2.1 Hz, 1H), 7.23 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.78 (d, *J* = 8.2 Hz, 1H), 6.66 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.63 (d, *J* = 2.0 Hz, 1H), 6.52 (s, 1H), 6.04 (s, 1H), 5.15 – 5.06 (m, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 3.65 – 3.61 (m, 1H), 3.58 (s, 3H), 3.15 – 3.05 (m, 2H), 2.81 – 2.69 (m, 3H), 2.53 (dt, *J* = 16.0, 4.8 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.88, 147.14, 147.06, 146.23, 133.73, 133.58, 132.70, 132.43, 129.43, 128.89, 128.84, 127.18, 125.89, 122.93, 115.77, 111.56, 110.98, 110.71, 67.49, 64.60, 55.97, 55.62, 55.43, 46.82, 42.48, 40.41, 25.29. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{29}Cl_2NO_4$: 502.1546, found 502.1562.

1-(3-((4-bromobenzyl)oxy)-4-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(3): According to the general procedure for the synthesis of compounds **1-22**, compound **3** was obtained by using 4-bromobenzyl bromide. Brown oily, 87 mg, yield: 74 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.2 Hz, 2H), 7.27 – 7.23 (m, 2H), 6.76 (d, *J* = 8.2 Hz, 1H), 6.62 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.56 (d, *J* = 2.0 Hz, 1H), 6.52 (s, 1H), 6.02 (s, 1H), 5.01 – 4.92 (m, 2H), 3.82 (d, *J* = 3.2 Hz, 6H), 3.62 (dd, *J* = 7.5, 5.0 Hz, 1H), 3.56 (s, 3H), 3.13 – 3.03 (m, 2H), 2.73 (tdd, *J* = 18.3, 13.2, 7.4 Hz, 3H), 2.52 (dt, *J* = 16.0, 4.8 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.94, 147.32, 147.20, 146.30, 136.25, 132.17, 131.51, 128.83, 125.95, 122.79, 121.54, 115.85, 111.47, 111.02, 110.80, 70.24, 64.66, 55.95, 55.69, 55.48, 46.93, 42.53, 40.54, 25.38. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{30}BrNO_4$: 512.1431, found 512.1442.

6,7-dimethoxy-1-(4-methoxy-3-phenethoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline(4): According to the general procedure for the synthesis of compounds **1-22**, compound **4** was obtained by using (2-bromoethyl)benzene. Yellow oily, 78 mg, yield: 75 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (t, *J* = 7.5 Hz, 2H), 7.26 (dd, *J* = 9.0, 2.1 Hz, 2H), 7.22 (td, *J* = 7.1, 1.5 Hz, 1H), 6.76 (d, *J* = 7.9 Hz, 1H), 6.61 (d, *J* = 7.8 Hz, 2H), 6.52 (s, 1H), 5.99 (s, 1H), 4.11 (ddd, *J* = 8.5, 7.0, 3.0 Hz, 2H), 3.82 (s, 3H), 3.78 (s, 3H), 3.65 (dd, *J* = 7.9, 5.1 Hz, 1H), 3.53 (s, 3H), 3.16 (ddd, *J* = 12.4, 8.9, 5.1 Hz, 1H), 3.13 – 3.07 (m, 3H), 2.81 (ddd, *J* = 15.4, 9.0, 5.7 Hz, 1H), 2.77 – 2.69 (m, 2H), 2.57 (dt, *J* = 16.0, 4.7 Hz, 1H), 2.51 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.84, 147.74, 147.24, 146.23, 137.98, 132.48, 129.07, 128.48, 126.49, 125.76, 122.26, 114.90, 111.68, 111.06, 69.77, 64.82, 56.16, 55.72, 55.52, 46.76, 42.58, 40.72, 35.76, 25.35. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{28}H_{33}NO_4$: 448.2482, found 448.2490.

6,7-dimethoxy-1-(4-methoxy-3-(3-phenylpropoxy)benzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline(5): According to the general procedure for the synthesis of compounds **1-22**, compound **5** was obtained by using 1-bromo-3-phenylpropane. Brown oily, 72 mg, yield: 68 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26 (t, *J* = 7.6 Hz, 2H), 7.22 – 7.15 (m, 3H), 6.76 (d, *J* = 8.6 Hz, 1H), 6.63 – 6.57 (m, 2H), 6.54 (s, 1H), 5.97 (s, 1H), 3.92 (td, *J* = 6.6, 3.0 Hz, 2H), 3.82 (d, *J* = 7.5 Hz, 6H), 3.74 (dd, *J* = 8.2, 4.7 Hz, 1H), 3.53 (s, 3H), 3.22 – 3.14 (m, 2H), 2.88 – 2.70 (m, 6H), 2.55 (s, 3H), 2.15 – 2.08 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.11, 147.93, 147.41, 146.34, 141.54, 131.96, 128.49, 128.36, 125.87, 125.23, 122.21, 114.99, 111.59, 111.04, 68.07, 64.69, 56.11, 55.72, 55.51, 46.41, 42.15, 40.69, 32.07, 30.63, 24.95. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{29}H_{35}NO_4$: 462.2639, found 462.2640.

6,7-dimethoxy-1-(4-methoxy-3-(octyloxy)benzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline(6): According to the general procedure for the synthesis of compounds **1-22**, compound **6** was obtained by using 1-bromooctane. Yellow oily, 57.9 mg, yield: 55 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.74 (d, *J* = 8.1 Hz, 1H), 6.62 (d, *J* = 2.1 Hz, 1H), 6.59 (d, *J* = 8.2 Hz, 1H), 6.54 (s, 1H), 6.01 (s, 1H), 3.89 (td, *J* = 7.2, 3.1 Hz, 2H), 3.81 (d, *J* = 5.3 Hz, 6H), 3.69 (dd, *J* = 8.0, 4.8 Hz, 1H), 3.54 (d, *J* = 2.0 Hz, 3H), 3.16 (ddt, *J* = 13.9, 8.8, 4.2 Hz, 2H), 2.82 (ddd, *J* = 15.1, 8.8, 5.7 Hz, 1H), 2.75 (td, *J* = 13.8, 12.7, 7.1 Hz, 2H), 2.60 (dd, *J* = 12.7, 8.2 Hz, 1H), 2.54 (s, 3H), 1.77 (q, *J* = 7.3 Hz, 2H), 1.41 (q, *J* = 7.5 Hz, 2H), 1.33 – 1.23 (m, 8H), 0.89 – 0.84 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.39, 147.98, 147.47, 146.52, 132.47, 129.12, 125.87, 122.04, 115.07, 111.83, 111.33, 111.26, 69.19, 64.98, 56.21, 55.84, 55.63, 47.05, 42.66, 40.88, 31.87, 29.43, 29.29, 26.03, 25.54, 22.71, 14.14. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{28}H_{41}NO_4$: 456.3108, found 456.3132.

1-(3-(hex-5-en-1-yloxy)-4-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(7): According to

the ge… …v oily, 43.7 mg, yield: 45 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.75 (d, *J* = 8.5 Hz, 1H), 6.62 – 6.59 (m, 2H), 6.55 (s, 1H), 6.02 (s, 1H), 5.81 (ddt, *J* = 16.9, 10.0, 6.7 Hz, 1H), 5.01 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.95 (dt, *J* = 10.3, 1.8 Hz, 1H), 3.91 (td, *J* = 6.9, 3.1 Hz, 2H), 3.82 (d, *J* = 5.1 Hz, 6H), 3.68 (dd, *J* = 7.9, 4.8 Hz, 1H), 3.55 (s, 3H), 3.20 – 3.11 (m, 2H), 2.83 (ddd, *J* = 15.2, 8.8, 5.7 Hz, 1H), 2.75 (ddd, *J* = 13.7, 10.7, 6.1 Hz, 2H), 2.59 (dt, *J* = 15.9, 4.7 Hz, 1H), 2.54 (s, 3H), 2.10 (q, *J* = 7.2 Hz, 2H), 1.81 (p, *J* = 7.0 Hz, 2H), 1.54 (p, *J* = 7.6 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.15, 147.82, 147.28, 146.33, 138.50, 132.40, 129.09, 125.83, 121.95, 114.96, 114.61, 111.67, 111.16, 111.09, 68.80, 64.83, 56.07, 55.70, 55.49, 46.94, 42.59, 40.73, 33.36, 28.60, 25.46, 25.20. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{35}NO_4$: 426.2639, found 426.2632.

1-(3-(2-(2-ethoxyethoxy)ethoxy)-4-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(8): According to the general procedure for the synthesis of compounds **1-22**, compound **8** was obtained by using 1-(2-bromoethoxy)-2-ethoxyethane. Brown oily, 60.7 mg, yield: 57 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.74 (d, *J* = 8.1 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 6.60 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.54 (s, 1H), 6.00 (s, 1H), 4.09 (hept, *J* = 5.2 Hz, 2H), 3.84 (td, *J* = 5.4, 2.6 Hz, 2H), 3.81 (d, *J* = 10.4 Hz, 6H), 3.69 (td, *J* = 7.2, 5.8, 2.8 Hz, 3H), 3.61 – 3.56 (m, 2H), 3.54 (s, 3H), 3.51 (q, *J* = 7.0 Hz, 2H), 3.20 – 3.13 (m, 2H), 2.90 – 2.67 (m, 4H), 2.59 (dt, *J* = 15.9, 4.6 Hz, 1H), 2.53 (s, 3H), 1.19 (t, *J* = 7.0 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.01, 147.95, 147.35, 146.37, 132.29, 128.76, 125.62, 122.57, 115.66, 111.78, 111.19, 111.06, 70.77, 69.75, 69.55, 68.49, 66.54, 64.80, 56.00, 55.69, 55.50, 46.75, 42.44, 40.68, 25.23, 15.06. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{37}NO_6$: 460.2694, found 460.2698.

1-(3-(cyclopropylmethoxy)-4-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(9): According to the general procedure for the synthesis of compounds **1-22**, compound **9** was obtained by using (Bromomethyl)cyclopropane. Yellow solid, 65.2 mg, m.p.: 141-143 °C. Yield: 71 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.76 (d, *J* = 8.0 Hz, 1H), 6.62 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 6.55 (s, 1H), 6.01 (s, 1H), 3.83 (d, *J* = 3.2 Hz, 6H), 3.74 (dd, *J* = 7.0, 2.0 Hz, 2H), 3.69 (dd, *J* = 8.1, 4.9 Hz, 1H), 3.55 (s, 3H), 3.17 (ddt, *J* = 14.3, 9.7, 4.7 Hz, 2H), 2.83 (ddd, *J* = 15.1, 8.8, 5.8 Hz, 1H), 2.76 (td, *J* = 13.6, 6.8 Hz, 2H), 2.60 (dt, *J* = 15.8, 4.6 Hz, 1H), 2.55 (s, 3H), 1.26 (ddd, *J* = 11.9, 8.5, 5.6 Hz, 1H), 0.61 (dd, *J* = 8.0, 4.6 Hz, 2H), 0.32 (dd, *J* = 4.8, 1.6 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.16, 147.98, 147.37, 146.39, 132.23, 125.75, 122.19, 115.50, 111.61, 111.18, 111.13, 74.03, 64.88, 56.06, 55.74, 55.54, 46.93, 42.56, 40.71, 25.43, 10.29, 3.35, 3.30. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{24}H_{31}NO_4$: 398.2326, found 398.2333.

1-(3-(cyclobutylmethoxy)-4-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(10): According to the general procedure for the synthesis of compounds **1-22**, compound **10** was obtained by using (bromomethyl)cyclobutane. Yellow solid, 67.5 mg, m.p.: 145-147 °C, yield: 71 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.75 (d, *J* = 8.1 Hz, 1H), 6.61 (d, *J* = 7.4 Hz, 2H), 6.56 (s, 1H), 6.00 (s, 1H), 3.89 (dd, *J* = 7.0, 4.0 Hz, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.72 (dd, *J* = 8.0, 4.7 Hz, 1H), 3.55 (s, 3H), 3.20 (dd, *J* = 12.6, 4.8 Hz, 2H), 2.89 – 2.82 (m, 2H), 2.79 – 2.73 (m, 2H), 2.65 – 2.61 (m, 1H), 2.57 (s, 3H), 2.13 (dq, *J* = 7.5, 3.7, 3.3 Hz, 2H), 1.98 – 1.90 (m, 2H), 1.86 – 1.80 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.54, 148.11, 147.47, 146.47, 129.04, 122.16, 115.60, 112.08, 111.21, 111.17, 73.61, 64.90, 56.24, 55.75, 55.53, 46.85, 42.45, 40.73, 34.60, 25.10, 25.08, 24.86, 18.56. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{25}H_{33}NO_4$: 412.2482, found 412.2476.

tert-butyl2-(5-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenoxy)acetate(11): According to the general procedure for the synthesis of compounds **1-22**, compound **11** was obtained by using tert-butyl bromoacetate. Yellow oily, 62.6 mg, yield: 60 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.77 – 6.70 (m, 1H), 6.63 – 6.56 (m, 2H), 6.53 (s, 1H), 5.96 (s, 1H), 4.51 (dd, *J* = 2.6, 1.2 Hz, 2H), 3.82 (d, *J* = 1.3 Hz, 3H), 3.81 (d, *J* = 1.3 Hz, 3H), 3.65 (dd, *J* = 8.0, 5.0 Hz, 1H), 3.54 (d, *J* = 1.3 Hz, 3H), 3.20 – 3.07 (m, 2H), 2.88 – 2.77 (m, 2H), 2.70 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.59 (td, *J* = 13.0, 11.5, 4.1 Hz, 1H), 2.53 – 2.46 (m, 3H), 1.46 – 1.41 (m, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.92, 147.87, 147.32, 147.11, 146.39, 132.28, 128.92, 125.68, 123.32, 115.29, 111.82, 111.17, 111.04, 81.99, 66.53, 64.76, 56.02, 55.72, 55.55, 46.75, 42.54, 40.76, 28.01, 25.27. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{35}NO_6$: 458.2537, found 458.2554.

5-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl2-naphthoate(12): According to the general procedure for the synthesis of compounds **1-22**, compound **12** was obtained by using 2-naphthoyl chloride. Yellow oily, 47.6 mg, yield: 43 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.20 (dd, *J* = 8.7, 1.7 Hz, 1H), 8.00 (d, *J* = 8.1 Hz, 1H), 7.93 (dd, *J* = 16.1, 8.4 Hz, 2H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.06 (s, 1H), 6.90 (s, 2H), 6.57 (s, 1H), 6.09 (s, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.77 (t, *J* = 6.5 Hz, 1H), 3.65 (s, 3H), 3.24 (dt, *J* = 14.7, 5.8 Hz, 2H), 2.87 (dd, *J* = 15.8, 6.2 Hz, 1H), 2.82 (dt, *J* = 13.5, 6.4 Hz, 2H), 2.67 – 2.61 (m, 1H), 2.56 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.89, 149.78, 147.46, 146.58, 139.86, 135.78, 132.53, 131.93, 129.46, 128.46, 128.26, 127.78, 126.71, 125.63, 124.04, 112.26, 111.24, 111.07, 64.80, 56.06, 55.75, 55.71, 46.70, 42.47, 40.42, 25.16. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{31}H_{31}NO_5$: 498.2275, found 498.2266.

((e(13): According to the general procedure for the synthesis of compounds **1-22**, compound **13** was obtained by using cyclohexanecarboxylic acid chloride. Pale yellow solid, 63 mg, m.p.: 151-153 °C, yield: 64 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.82 (s, 1H), 6.81 (s, 2H), 6.55 (s, 1H), 6.01 (s, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 3.57 (s, 3H), 3.18 (ddd, *J* = 19.4, 11.3, 4.7 Hz, 2H), 2.80 (dddd, *J* = 46.8, 21.8, 11.6, 7.0 Hz, 4H), 2.60 (ddt, *J* = 15.2, 7.3, 4.4 Hz, 2H), 2.53 (s, 3H), 2.09 – 2.03 (m, 2H), 1.81 (dt, *J* = 12.7, 3.8 Hz, 2H), 1.58 (td, *J* = 12.3, 11.7, 3.5 Hz, 2H), 1.39 – 1.33 (m, 2H), 1.31 – 1.23 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.06, 149.54, 147.37, 146.48, 139.78, 132.37, 127.92, 125.54, 123.84, 112.13, 111.18, 111.05, 64.70, 56.00, 55.73, 55.63, 46.66, 42.97, 42.41, 40.36, 29.04, 29.01, 25.76, 25.35, 25.19. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{35}NO_5$: 454.2588, found 454.2612.

((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenylbenzenesulfonate(14): According to the general procedure for the synthesis of compounds **1-22**, compound **14** was obtained by using benzenesulfonyl chloride. Yellow solid, 67 mg, m.p.: 141-143 °C, yield: 60 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 – 7.80 (m, 2H), 7.67 – 7.60 (m, 1H), 7.52 – 7.46 (m, 2H), 7.01 (d, *J* = 2.1 Hz, 1H), 6.84 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.67 (d, *J* = 8.3 Hz, 1H), 6.55 (s, 1H), 6.09 (s, 1H), 3.92 (d, *J* = 29.4 Hz, 1H), 3.83 (s, 3H), 3.63 (s, 3H), 3.50 (s, 3H), 3.15 (ddd, *J* = 13.4, 8.3, 4.7 Hz, 1H), 3.04 (dd, *J* = 14.3, 5.6 Hz, 1H), 2.81 – 2.76 (m, 2H), 2.74 (dt, *J* = 10.2, 4.8 Hz, 1H), 2.57 (dt, *J* = 15.7, 4.8 Hz, 1H), 2.48 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.96, 147.41, 146.64, 137.99, 136.50, 133.71, 129.30, 129.02, 128.62, 128.53, 127.19, 125.01, 122.44, 112.12, 111.34, 110.86, 64.57, 55.77, 55.73, 55.58, 47.10, 42.65, 40.04, 25.43. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{29}NO_6S$: 484.1788, found 484.1794.

5-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl4-nitrobenzenesulfonate(15): According to the general procedure for the synthesis of compounds **1-22**, compound **15** was obtained by using 4 -nitrobenzenesulfonyl chloride. Brown solid, 30.1 mg, m.p.: 145-147 °C, yield: 25 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (d, *J* = 8.8 Hz, 2H), 8.02 (d, *J* = 8.6 Hz, 2H), 7.01 – 6.91 (m, 2H), 6.73 (d, *J* = 8.4 Hz, 1H), 6.57 (s, 1 H), 6.11 (s, 1H), 3.86 (s, 3H), 3.76 (d, *J* = 8.3 Hz, 1H), 3.65 (s, 3H), 3.52 (s, 3H), 3.20 (d, *J* = 17.4 Hz, 2H), 2. 84 (ddd, *J* = 27.1, 12.5, 6.1 Hz, 3H), 2.64 (d, *J* = 16.3 Hz, 1H), 2.56 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 15 0.76, 149.73, 146.92, 142.12, 137.55, 129.83, 125.07, 123.74, 112.40, 111.37, 110.82, 64.59, 56.50, 55.81, 55.79, 55. 59, 46.97, 42.28, 39.86. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{28}N_2O_8S$: 529.1639, found 529.1643.

((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenylphenylmethanesulfonate(16): According to the general procedure for the synthesis of compounds **1-22**, compound **16** was obtained by using a lpha-toluenesulfonyl chloride. Yellow oily, 57.8 mg, yield: 51 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 6.6, 3.0 Hz, 2H), 7.40 – 7.37 (m, 3H), 6.92 – 6.85 (m, 2H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.54 (s, 1H), 6.08 (s, 1H), 4.55 (d, *J* = 5.3 Hz, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.61 (s, 3H), 3.14 (ddd, *J* = 13.3, 8.7, 5.1 Hz, 1H), 3.04 (dd, *J* = 13.9, 5.7 Hz, 1H), 2.84 – 2.68 (m, 4H), 2.55 (dt, *J* = 15.7, 4.7 Hz, 1H), 2.49 (s, 3H). ^{13}C NMR (150 MHz, CD Cl_3) δ 149.85, 147.38, 146.62, 138.17, 132.96, 130.95, 129.31, 129.04, 128.83, 127.76, 126.26, 125.11, 112.42, 111.3 4, 110.91, 64.43, 57.47, 56.07, 55.75, 55.73, 47.01, 42.68, 40.02, 25.43. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{31}NO_6S$: 498.1945, found 498.1939.

((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl4-(trifluoromethyl)benzenesulfonate(17): According to the general procedure for the synthesis of compounds **1-22**, compound **17** was obtained by using 4-(trifluoromethyl)benzene-1-sulfonyl chloride. Yellow oily, 64 mg, yield: 55 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.1 Hz, 2H), 7.77 (t, *J* = 10.2 Hz, 2H), 6.97 (d, *J* = 22.9 Hz, 2H), 6.70 (d, *J* = 8.3 Hz, 1H), 6.5 7 (s, 1H), 6.08 (s, 1H), 3.85 (s, 3H), 3.76 (s, 1H), 3.63 (s, 3H), 3.47 (s, 3H), 3.21 (d, *J* = 29.4 Hz, 2H), 2.85 (dd, *J* = 13.7, 7.4 Hz, 3H), 2.67 (s, 1H), 2.56 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.86, 146.95, 140.02, 137.65, 135.52, 135.30, 129.71, 129.06, 123.04 (q, J_{C-F} = 271.5 Hz), 125.71 (q, J_{C-F} = 3.0 Hz), 125.21, 112.70, 112.25, 11 1.38, 110.88, 109.24, 64.62, 56.49, 55.93, 55.77, 55.43, 46.81, 42.16, 39.93. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{28}F_3NO_6S$: 552.1662, found 552.1657.

((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl4-cyanobenzenesulfonate(18): According to the general procedure for the synthesis of compounds **1-22**, compound **18** was obtained by using 4 -cyanobenzenesulfonyl chloride. Yellow solid, 84 mg, m.p.: 141-143 °C, yield: 72 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.2 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 2.1 Hz, 1H), 6.93 (dd, *J* = 8.4, 2.1 Hz, 1H), 6. 68 (d, *J* = 8.4 Hz, 1H), 6.55 (s, 1H), 6.17 (s, 1H), 3.84 (s, 3H), 3.66 (s, 4H), 3.48 (s, 3H), 3.14 (ddd, *J* = 12.9, 7.8, 5.0 Hz, 1H), 3.05 (dd, *J* = 14.1, 5.8 Hz, 1H), 2.85 (dd, *J* = 14.0, 6.6 Hz, 1H), 2.80 – 2.70 (m, 2H), 2.56 (dt, *J* = 15.1, 4.9 Hz, 1H), 2.49 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.49, 147.46, 146.76, 140.62, 137.49, 132.

6, 42.70, 39.85, 25.50. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{28}N_2O_6S$: 509.1741, found 509.1752.

((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl2-fluorobenzenesulfonate(19): According to the general procedure for the synthesis of compounds **1-22**, compound **19** was obtained by using 2-fluorobenzenesulfonyl chloride. Yellow solid, 79.7 mg, m.p.: 151-153 °C, yield: 70 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74 (ddd, *J* = 8.2, 6.9, 1.8 Hz, 1H), 7.63 (qd, *J* = 7.2, 1.8 Hz, 1H), 7.29 – 7.24 (m, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.02 (d, *J* = 2.1 Hz, 1H), 6.82 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.53 (s, 1H), 6.07 (s, 1H), 3.85 (d, *J* = 8.8 Hz, 1H), 3.82 (s, 3H), 3.60 (s, 3H), 3.51 (s, 3H), 3.12 (ddd, *J* = 13.0, 8.6, 5.0 Hz, 1H), 3.03 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.83 – 2.74 (m, 2H), 2.72 (dt, *J* = 13.0, 5.3 Hz, 1H), 2.54 (dt, *J* = 15.8, 4.8 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (150 MHz, CDCl3) δ 160.63, 158.91, 149.88, 147.38, 146.59, 137.98, 136.13 (d, J_{C-F} = 7.5 Hz), 132.67, 131.12, 129.52, 126.22, 125.09, 123.89, 117.09 (d, J_{C-F} = 21.0 Hz), 112.16, 111.30, 110.82, 64.54, 55.76, 55.70, 55.63, 47.01, 42.61, 40.04, 25.39. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{28}FNO_6S$: 502.1694, found 502.1687.

((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl2-nitrobenzenesulfonate(20): According to the general procedure for the synthesis of compounds **1-22**, compound **20** was obtained by using 2-nitrobenzenesulfonyl chloride. Yellow oily, 95.7 mg, yield: 79 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.85 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.80 (td, *J* = 7.8, 1.4 Hz, 1H), 7.68 (td, *J* = 7.7, 1.3 Hz, 1H), 7.02 (d, *J* = 2.1 Hz, 1H), 6.87 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 1H), 6.54 (s, 1H), 6.12 (s, 1H), 3.83 (s, 3H), 3.66 (d, *J* = 6.2 Hz, 1H), 3.64 (s, 3H), 3.54 (s, 3H), 3.13 (ddd, *J* = 13.2, 8.4, 5.1 Hz, 1H), 3.06 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.84 – 2.80 (m, 1H), 2.79 – 2.69 (m, 2H), 2.55 (dt, *J* = 15.5, 4.8 Hz, 1H), 2.48 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.71, 148.40, 147.38, 146.68, 137.98, 134.72, 132.87, 131.74, 131.45, 130.27, 129.77, 128.84, 126.47, 125.01, 124.61, 112.36, 111.39, 110.84, 64.47, 55.78, 55.77, 55.71, 47.17, 42.67, 39.99, 25.49. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{28}N_2O_8S$: 529.1639, found 529.1641.

((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenylnaphthalene-2-sulfonate(21): According to the general procedure for the synthesis of compounds **1-22**, compound **21** was obtained by using 2-naphthalenesulfonyl chloride. Yellow oily, 66 mg, yield: 54 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.96 – 7.85 (m, 4H), 7.66 (ddd, *J* = 8.2, 6.8, 1.2 Hz, 1H), 7.59 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 7.00 (d, *J* = 2.2 Hz, 1H), 6.81 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.63 (d, *J* = 8.4 Hz, 1H), 6.54 (s, 1H), 6.06 (s, 1H), 3.83 (s, 3H), 3.60 (s, 3H), 3.57 – 3.52 (m, 1H), 3.38 (s, 3H), 3.10 (ddd, *J* = 13.3, 8.8, 5.1 Hz, 1H), 3.01 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.76 (td, *J* = 14.7, 14.0, 6.2 Hz, 2H), 2.68 (dt, *J* = 12.5, 5.1 Hz, 1H), 2.57 – 2.51 (m, 1H), 2.40 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.02, 147.41, 146.63, 138.07, 135.32, 133.36, 132.54, 131.73, 130.22, 129.34, 128.85, 128.71, 127.88, 127.62, 126.20, 124.99, 123.34, 112.15, 111.33, 110.84, 64.57, 55.77, 55.72, 55.53, 47.04, 42.54, 40.12, 25.36. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{30}H_{31}NO_6S$: 534.1945, found 534.1961.

Methyl-3-((5-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenoxy)sulfonyl)thiophene-2-carboxylate(22): According to the general procedure for the synthesis of compounds **1-22**, compound **22** was obtained by using methyl 3-chlorosulfonylthiophene-2-carboxylate. Yellow oily, 68 mg, yield: 54 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.43 (dd, *J* = 5.3, 1.3 Hz, 1H), 7.04 (d, *J* = 1.9 Hz, 1H), 6.82 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.73 – 6.68 (m, 1H), 6.53 (s, 1H), 6.07 (s, 1H), 3.95 (d, *J* = 1.3 Hz, 3H), 3.82 (d, *J* = 1.4 Hz, 3H), 3.62 (d, *J* = 6.3 Hz, 1H), 3.60 (d, *J* = 1.4 Hz, 3H), 3.59 (d, *J* = 1.3 Hz, 3H), 3.12 (ddd, *J* = 13.4, 8.7, 5.2 Hz, 1H), 3.03 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.77 (dt, *J* = 17.8, 7.0 Hz, 2H), 2.71 (dt, *J* = 12.2, 5.0 Hz, 1H), 2.53 (dt, *J* = 15.9, 4.8 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.71, 149.84, 147.34, 146.57, 138.64, 138.19, 136.08, 132.70, 131.32, 129.38, 128.92, 126.27, 125.12, 112.20, 111.31, 110.85, 64.52, 55.81, 55.76, 55.71, 53.04, 46.99, 42.64, 40.04, 25.40. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{29}NO_8S_2$: 548.1407, found 548.1396.

4.1.3 General procedure for the synthesis of key intermediate g

According to the general method of synthesizing the key intermediate **f**, the raw material 3-hydroxy-4-methoxyphenylacetic acid in the first step was replaced with 4-hydroxy-3-methoxyphenylacetic acid, and the key intermediate compound **g** could be synthesized without changing the other operations.

4.1.4 General procedure for the synthesis of compounds 23-42

The compound **g** (0.5 mmol, 1.0 equiv.) and K_2CO_3 (1.5 mmol, 3.0 equiv.) were dissolved in MeCN (4 mL), stirred for 20 min, added with halogenated compounds (1.0 mmol, 2.0 equiv.), and stirred at 70 °C for 8 h. After the completion of the reaction, the organic phase was extracted with DCM, dried with anhydrous Na_2SO_4, concentrated and dried, and purified by silica gel column chromatography (DCM : methanol = 60 : 1) to obtain products **23-42**.

to the general procedure for the synthesis of compounds **23-42**, compound **23** was obtained by using benzyl bromide. Yellow solid, 47 mg, m.p.: 145-147 °C, yield: 47 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (d, *J* = 7.3 Hz, 2H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.30 – 7.26 (m, 1H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.63 (d, *J* = 2.0 Hz, 1H), 6.56 (d, *J* = 6.1 Hz, 2H), 5.98 (s, 1H), 5.12 (s, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 3.72 (dd, *J* = 8.3, 4.7 Hz, 1H), 3.49 (s, 3H), 3.19 (ddd, *J* = 14.9, 7.4, 3.5 Hz, 2H), 2.87 – 2.78 (m, 2H), 2.75 (dd, *J* = 13.6, 8.1 Hz, 1H), 2.61 (dt, *J* = 15.3, 4.6 Hz, 1H), 2.56 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.33, 147.36, 146.45, 146.31, 137.32, 132.77, 128.49, 127.76, 127.17, 125.43, 121.87, 113.91, 113.58, 111.12, 111.04, 71.03, 64.82, 55.97, 55.75, 55.47, 46.68, 42.38, 40.87, 25.15. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{31}NO_4$: 434.2326, found 434.2332.

(4-((4-bromobenzyl)oxy)-3-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(24): According to the general procedure for the synthesis of compounds **23-42**, compound **24** was obtained by using 4-bromobenzyl bromide. Yellow solid, 43 mg, m.p.: 141-143 °C, yield: 37 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.62 (d, *J* = 1.9 Hz, 1H), 6.56 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.55 (s, 1H), 6.03 (s, 1H), 5.06 (s, 2H), 3.83 (s, 3H), 3.79 (s, 3H), 3.68 (dd, *J* = 7.8, 4.9 Hz, 1H), 3.52 (s, 3H), 3.21 – 3.10 (m, 2H), 2.82 (ddd, *J* = 15.2, 8.8, 5.6 Hz, 1H), 2.79 – 2.72 (m, 2H), 2.58 (dt, *J* = 16.0, 4.7 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.29, 147.25, 146.28, 146.04, 136.39, 133.44, 131.60, 129.05, 128.86, 125.90, 121.81, 121.63, 114.03, 113.57, 111.14, 110.99, 70.38, 64.78, 55.90, 55.75, 55.49, 46.91, 42.62, 40.89, 25.43. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{30}BrNO_4$: 512.1431, found 512.1428.

1-(4-((2,4-dichlorobenzyl)oxy)-3-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(25): According to the general procedure for the synthesis of compounds **23-42**, compound **25** was obtained by using 2,4-dichlorobenzyl bromide. Brown oily, 44 mg, yield: 38 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.3 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.24 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.63 (d, *J* = 2.0 Hz, 1H), 6.59 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.55 (s, 1H), 6.04 (s, 1H), 5.15 (s, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 3.70 (dd, *J* = 7.8, 5.0 Hz, 1H), 3.55 (s, 3H), 3.20 – 3.12 (m, 2H), 2.82 (ddd, *J* = 15.2, 8.9, 5.7 Hz, 1H), 2.80 – 2.73 (m, 2H), 2.58 (dt, *J* = 15.9, 4.7 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.24, 147.24, 146.26, 145.81, 133.82, 133.68, 132.79, 129.40, 129.03, 128.96, 127.22, 125.88, 121.88, 113.92, 113.64, 111.12, 110.95, 67.67, 64.71, 55.92, 55.72, 55.48, 46.82, 42.55, 40.84, 25.35. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{29}Cl_2NO_4$: 502.1546, found 502.1557.

6,7-dimethoxy-1-(3-methoxy-4-(3-phenylpropoxy)benzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline(26): According to the general procedure for the synthesis of compounds **23-42**, compound **26** was obtained by using 1-bromo-3-phenylpropaneX. Yellow oily, 39 mg, yield: 37 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 – 7.24 (m, 2H), 7.22 – 7.15 (m, 3H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.62 – 6.57 (m, 2H), 6.55 (s, 1H), 6.03 (s, 1H), 3.97 (td, *J* = 6.6, 1.9 Hz, 2H), 3.82 (s, 3H), 3.77 (s, 3H), 3.70 (dd, *J* = 8.0, 4.8 Hz, 1H), 3.54 (s, 3H), 3.16 (ddd, *J* = 13.8, 9.2, 4.4 Hz, 2H), 2.85 – 2.73 (m, 5H), 2.62 – 2.57 (m, 1H), 2.54 (s, 3H), 2.16 – 2.09 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.17, 147.28, 146.77, 146.30, 141.51, 132.64, 129.04, 128.47, 128.37, 125.89, 125.83, 121.96, 113.58, 113.24, 111.16, 111.07, 68.24, 64.85, 55.98, 55.75, 55.53, 46.91, 42.63, 40.85, 32.10, 30.70, 25.45. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{29}H_{35}NO_4$: 462.2639, found 426.2641

6,7-dimethoxy-1-(3-methoxy-4-phenethoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline(27): According to the general procedure for the synthesis of compounds **23-42**, compound **27** was obtained by using (2-bromoethyl)benzene. Yellow oily, 51 mg, yield: 48 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (t, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 1.6 Hz, 1H), 7.25 (d, *J* = 4.8 Hz, 1H), 7.22 (td, *J* = 7.0, 1.5 Hz, 1H), 6.79 – 6.68 (m, 2H), 6.58 (s, 1H), 6.54 (dd, *J* = 8.2, 2.0 Hz, 1H), 5.89 (s, 1H), 4.18 – 4.11 (m, 2H), 4.07 (dd, *J* = 9.1, 3.8 Hz, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 3.65 (d, *J* = 13.3 Hz, 1H), 3.48 (s, 3H), 3.45 – 3.40 (m, 1H), 3.12 (t, *J* = 7.6 Hz, 3H), 2.98 (td, *J* = 10.6, 5.3 Hz, 1H), 2.90 – 2.84 (m, 2H), 2.74 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.37, 148.45, 147.18, 147.00, 137.88, 129.01, 128.50, 126.53, 122.30, 113.70, 113.02, 111.02, 69.90, 65.20, 56.26, 55.82, 55.55, 45.91, 41.30, 40.81, 35.74, 23.15. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{28}H_{33}NO_4$: 448.2482, found 448.2458.

6,7-dimethoxy-1-(3-methoxy-4-(octyloxy)benzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline(28): According to the general procedure for the synthesis of compounds **23-42**, compound **28** was obtained by using 1-bromooctane. Yellow oily, 46 mg, yield: 44 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.79 (d, *J* = 2.1 Hz, 1H), 6.72 (d, *J* = 8.2 Hz, 1H), 6.62 (s, 1H), 6.54 (dd, *J* = 8.2, 2.0 Hz, 1H), 5.81 (s, 1H), 4.43 – 4.35 (m, 1H), 3.93 (td, *J* = 7.0, 2.9 Hz, 2H), 3.84 (s, 3H), 3.78 (s, 3H), 3.63 (td, *J* = 12.7, 6.4 Hz, 1H), 3.47 (s, 3H), 3.17 – 3.09 (m, 1H), 3.05 (ddd, *J* = 18.3, 6.9, 2.3 Hz, 1H), 2.94 (d, *J* = 6.8 Hz, 1H), 2.91 (s, 3H), 1.78 (q, *J* = 7.3 Hz, 2H), 1.43 – 1.38 (m, 2H), 1.27 (dddd, *J* = 22.3, 11.5, 9.1, 5.5 Hz, 10H), 0.85 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.45, 149.20, 147.79, 146.95, 126.86, 122.90, 121.06, 120.02, 114.28, 112.64, 111.62, 110.61, 70.69, 68.97, 56.93, 55.90, 55.51, 49.43, 37.76, 31.66, 31.43, 29.21, 29.07, 28.86, 25.81, 22.50, 22.40, 13.96. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{28}H_{41}NO_4$: 456.3108, found 456.3101.

1-(4-(hex-5-en-1-yloxy)-3-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(29): According to

the g[illegible] Yellow oily, 64 mg, yield: 65 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.75 (d, *J* = 7.9 Hz, 1H), 6.59 (d, *J* = 8.2 Hz, 2H), 6.55 (s, 1H), 6.00 (s, 1H), 5.83 – 5.75 (m, 1H), 5.01 (dd, *J* = 17.1, 1.8 Hz, 1H), 4.98 – 4.93 (m, 1H), 3.97 (t, *J* = 6.8 Hz, 2H), 3.82 (s, 3H), 3.76 (s, 3H), 3.73 (dd, *J* = 8.1, 4.7 Hz, 1H), 3.54 (s, 3H), 3.23 – 3.16 (m, 2H), 2.87 – 2.82 (m, 1H), 2.78 (ddd, *J* = 22.0, 13.3, 8.5 Hz, 2H), 2.66 – 2.59 (m, 1H), 2.56 (s, 3H), 2.11 (q, *J* = 7.2 Hz, 2H), 1.84 – 1.79 (m, 2H), 1.55 (tt, *J* = 9.9, 6.5 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.11, 147.39, 146.90, 146.36, 138.50, 132.18, 121.96, 114.69, 113.54, 112.96, 111.15, 111.10, 68.94, 64.87, 55.96, 55.75, 55.52, 46.75, 42.43, 40.84, 33.42, 28.66, 25.25, 25.18. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{35}NO_4$: 426.2639, found 426.2632.

(4-(2-(2-ethoxyethoxy)ethoxy)-3-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(30): According to the general procedure for the synthesis of compounds **23-42**, compound **30** was obtained by using 1-(2-bromoethoxy)-2-ethoxyethane. Yellow oily, 87 mg, yield: 82 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.79 (d, *J* = 8.1 Hz, 1H), 6.61 – 6.56 (m, 2H), 6.53 (s, 1H), 6.04 (s, 1H), 4.15 – 4.10 (m, 2H), 3.85 (t, *J* = 5.3 Hz, 2H), 3.81 (s, 3H), 3.74 (d, *J* = 13.3 Hz, 3H), 3.68 (ddd, *J* = 10.5, 6.9, 4.6 Hz, 3H), 3.58 (dd, *J* = 5.8, 3.8 Hz, 2H), 3.55 (s, 3H), 3.51 (q, *J* = 7.0 Hz, 2H), 3.17 – 3.10 (m, 2H), 2.80 (ddd, *J* = 15.0, 8.8, 5.5 Hz, 1H), 2.77 – 2.69 (m, 2H), 2.56 (dd, *J* = 16.1, 4.8 Hz, 1H), 2.52 (d, *J* = 4.2 Hz, 3H), 1.19 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.31, 147.33, 146.66, 146.42, 133.22, 129.20, 125.99, 121.94, 114.01, 113.81, 111.28, 111.15, 70.84, 69.81, 69.69, 68.79, 66.59, 64.82, 55.91, 55.76, 55.59, 47.03, 42.63, 40.86, 25.54, 15.11. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{37}NO_6$: 460.2694, found 460.2690.

1-(4-(isopentyloxy)-3-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(31): According to the general procedure for the synthesis of compounds **23-42**, compound **31** was obtained by using 1-bromo-3-methylbutane. Brown oily, 37 mg, yield: 39 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.77 (d, *J* = 7.8 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 2H), 6.56 (s, 1H), 6.01 (s, 1H), 3.99 (t, *J* = 7.0 Hz, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 3.73 (dd, *J* = 8.1, 4.8 Hz, 1H), 3.55 (s, 3H), 3.19 (ddd, *J* = 13.4, 9.1, 4.5 Hz, 2H), 2.88 – 2.73 (m, 3H), 2.61 (dt, *J* = 14.8, 4.2 Hz, 1H), 2.56 (s, 3H), 1.81 (dp, *J* = 13.3, 6.7 Hz, 1H), 1.71 (q, *J* = 6.9 Hz, 2H), 0.95 (s, 3H), 0.94 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.08, 147.32, 146.93, 146.29, 132.13, 128.65, 125.51, 121.93, 113.45, 112.83, 111.09, 111.04, 67.54, 64.83, 55.96, 55.73, 55.49, 46.71, 42.44, 40.81, 37.87, 25.20, 25.07, 22.57. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{25}H_{35}NO_4$: 414.2639,found 414.2655.

1-(4-(cyclopropylmethoxy)-3-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(32): According to the general procedure for the synthesis of compounds **23-42**, compound **32** was obtained by using (Bromomethyl)cyclopropane. Yellow solid, 83 mg, m.p.: 144-146 °C, yield: 91 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.74 (d, *J* = 8.1 Hz, 1H), 6.61 – 6.56 (m, 2H), 6.54 (s, 1H), 6.01 (s, 1H), 3.81 (s, 3H), 3.78 (d, *J* = 7.0 Hz, 2H), 3.76 (s, 3H), 3.72 (dd, *J* = 8.1, 4.8 Hz, 1H), 3.54 (s, 3H), 3.18 (dt, *J* = 13.8, 5.3 Hz, 2H), 2.82 (dp, *J* = 18.8, 5.9 Hz, 2H), 2.75 (dd, *J* = 13.7, 8.1 Hz, 1H), 2.60 (dt, *J* = 15.4, 4.1 Hz, 1H), 2.55 (s, 3H), 0.84 (dt, *J* = 14.9, 6.7 Hz, 1H), 0.60 (d, *J* = 7.7 Hz, 2H), 0.31 (d, *J* = 5.0 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.20, 147.29, 146.83, 146.32, 132.63, 125.77, 121.86, 113.49, 113.43, 111.13, 111.06, 74.18, 64.85, 55.92, 55.75, 55.54, 46.90, 42.59, 40.89, 25.41, 10.36, 3.38. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{24}H_{31}NO_4$: 398.2326, found 398.2354.

1-(4-(cyclobutylmethoxy)-3-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(33): According to the general procedure for the synthesis of compounds **23-42**, compound **33** was obtained by using (bromomethyl)cyclobutane. Yellow solid, 85 mg, m.p.: 141-143 °C, yield : 84 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.76 (d, *J* = 8.5 Hz, 1H), 6.59 (dd, *J* = 4.3, 2.4 Hz, 2H), 6.54 (s, 1H), 6.02 (s, 1H), 3.94 (d, *J* = 7.0 Hz, 2H), 3.81 (s, 3H), 3.74 (s, 3H), 3.68 (dd, *J* = 7.9, 4.8 Hz, 1H), 3.55 (s, 3H), 3.18 – 3.10 (m, 2H), 2.81 (ddd, *J* = 19.9, 8.3, 5.1 Hz, 2H), 2.75 (dt, *J* = 13.7, 6.4 Hz, 2H), 2.61 – 2.56 (m, 1H), 2.53 (s, 3H), 2.15 – 2.09 (m, 2H), 1.95 – 1.87 (m, 2H), 1.85 – 1.80 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.37, 147.32, 147.18, 146.37, 132.74, 129.24, 125.94, 122.01, 113.99, 113.80, 111.26, 111.20, 73.81, 64.85, 56.08, 55.76, 55.55, 46.98, 42.63, 40.81, 34.67, 25.55, 25.10, 18.58. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{25}H_{33}NO_4$: 412.2482, found 412.2493.

1-(4-(cyclohexylmethoxy)-3-methoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline(34): According to the general procedure for the synthesis of compounds **23-42**, compound **34** was obtained by using cyclohexylmethyl bromide. Yellow solid, 45 mg, m.p.: 151-153 °C, yield: 45 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.74 (d, *J* = 7.9 Hz, 1H), 6.62 – 6.57 (m, 2H), 6.55 (s, 1H), 6.00 (s, 1H), 3.82 (s, 3H), 3.76 (s, 3H), 3.74 (s, 1H), 3.71 (dd, *J* = 8.1, 4.8 Hz, 1H), 3.54 (s, 3H), 3.22 – 3.15 (m, 2H), 2.87 – 2.77 (m, 2H), 2.75 (dd, *J* = 13.7, 8.2 Hz, 1H), 2.61 (dt, *J* = 15.8, 4.6 Hz, 1H), 2.55 (s, 3H), 1.88 (d, *J* = 14.0 Hz, 3H), 1.73 (dt, *J* = 13.0, 3.5 Hz, 2H), 1.68 (dt, *J* = 12.8, 3.4 Hz, 1H), 1.28 (tt, *J* = 11.8, 3.3 Hz, 2H), 1.21 (ddt, *J* = 28.3, 12.6, 2.8 Hz, 2H), 1.00 (qd, *J* = 12.3, 3.5 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.27, 147.40, 147.33, 146.40, 132.20, 128.83, 125.65, 122.01, 113.93, 113.24, 111.23, 111.19, 74.79, 64.88, 56.14, 55.77, 55.54, 46.84, 42.50, 40.80, 37.50, 29.95, 26.54, 25.74, 25.32. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{37}NO_4$: 440.2795, found 440.2820.

4-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenylphenylmethanesulfonate(35): According to the general procedure for the synthesis of compounds **23-42**, compound **35** was obtained by using 4-nitrobenzenesulfonyl chloride. Brown oily, 78.5 mg, yield: 65 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31 (d, *J* = 8.8 Hz, 2H), 8.02 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.68 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.53 – 6.48 (m, 2 H), 6.11 (s, 1H), 3.81 (s, 3H), 3.69 (t, *J* = 6.2 Hz, 1H), 3.62 (s, 3H), 3.37 (s, 3H), 3.12 – 3.06 (m, 2H), 2.83 (dd, *J* = 13.8, 6.9 Hz, 1H), 2.78 – 2.68 (m, 2H), 2.51 (d, *J* = 5.0 Hz, 1H), 2.49 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.73, 150.56, 147.50, 146.69, 142.08, 140.92, 136.13, 129.86, 128.68, 126.55, 123.74, 123.24, 122.21, 114.33, 111.33, 110.99, 64.45, 55.82, 55.79, 55.33, 47.31, 42.71, 40.87, 25.67. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{28}N_2O_8S$: 529.1639, found 529.1642.

4-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenylphenylmethanesulfonate (36): According to the general procedure for the synthesis of compounds **23-42**, compound **36** was obtained by using alpha-toluenesulfonyl chloride. Brown oily, 110 mg, yield: 96 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 6.6, 3.0 Hz, 2H), 7.40 – 7.37 (m, 3H), 6.98 (d, *J* = 7.9 Hz, 1H), 6.69 (d, *J* = 8.3 Hz, 2H), 6.56 (s, 1H), 6.04 (s, 1 H), 4.56 (s, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 3.73 (dd, *J* = 7.7, 5.0 Hz, 1H), 3.58 (s, 3H), 3.17 (td, *J* = 13.0, 12.6, 5.2 Hz, 2H), 2.86 – 2.76 (m, 3H), 2.58 (dt, *J* = 15.9, 4.7 Hz, 1H), 2.54 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.01, 147.51, 146.61, 140.24, 136.78, 130.90, 129.04, 128.83, 128.61, 127.69, 126.01, 123.70, 122.25, 114.54, 111.40, 111.04, 64.65, 57.49, 55.81, 55.75, 46.92, 42.57, 41.05, 25.39. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{31}NO_6S$: 498.1945, found 498.1936.

4-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl4-(trifluoromethyl)benzenesulfonate(37): According to the general procedure for the synthesis of compounds **23-42**, compound **37** was obtained by using 4-(trifluoromethyl)benzene-1-sulfonyl chloride. Brown solid, 77 mg, m.p.: 141-143 °C, yield: 61 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.1 Hz, 2H), 7.76 (d, *J* = 8.2 Hz, 2H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.68 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.53 (s, 1H), 6.49 (d, *J* = 2.0 Hz, 1H), 6.10 (s, 1H), 3.81 (s, 3H), 3.70 (t, *J* = 6.2 Hz, 1 H), 3.62 (s, 3H), 3.34 (s, 3H), 3.10 (dd, *J* = 13.8, 5.9 Hz, 2H), 2.83 (dd, *J* = 13.8, 7.1 Hz, 1H), 2.75 (ddt, *J* = 28.0, 17.7, 5.5 Hz, 2H), 2.53 (t, *J* = 5.1 Hz, 1H), 2.50 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.71, 147.51, 146.69, 140.68, 140.03, 136.25, 135.36 (q, J_{C-F} = 33.0 Hz), 129.08, 128.66, 126.41, 125.72 (q, J_{C-F} = 4.5 Hz), 123.41, 123.10 (q, J_{C-F} = 271.5 Hz), 122.11, 114.18, 111.32, 110.99, 64.51, 55.78, 55.77, 55.21, 47.23, 42.66, 40.97, 25.62. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{28}F_3NO_6S$: 552.1662, found 552.1651.

Methyl-3-((4-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenoxy)sulfonyl)thiophene-2-carboxylate(38): According to the general procedure for the synthesis of compounds **23-42**, compound **38** was obtained by using methyl 3-chlorosulfonylthiophene-2-carboxylate. Brown oily, 80.7 mg, yield: 65 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, *J* = 5.2 Hz, 1H), 7.42 (d, *J* = 5.3 Hz, 1H), 7.06 (d, *J* = 8.2 Hz, 1H), 6.65 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.56 – 6.47 (m, 2H), 6.05 (s, 1H), 3.93 (s, 3H), 3.80 (s, 3H), 3.67 (dd, *J* = 7.3, 5.0 Hz, 1H), 3.58 (s, 3H), 3.49 (s, 3H), 3.10 (ddt, *J* = 13.2, 9.0, 3.8 Hz, 2H), 2.78 (ddd, *J* = 15.9, 7.4, 4.8 Hz, 2H), 2.74 – 2.69 (m, 1H), 2.54 – 2.50 (m, 1H), 2.49 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.72, 147.51, 146.69, 140.69, 140.04, 136.26, 135.48, 135.26, 129.08, 128.68, 126.43, 125.73, 123.41, 122.11, 114.18, 111.32, 110.99, 64.52, 55.79, 55.77, 55.21, 47.24, 42.67, 40.98, 25.63. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{29}NO_8S_2$: 548.1407, found 548.1397.

4-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl4-cyanobenzenesulfonate (39): According to the general procedure for the synthesis of compounds **23-42**, compound **39** was obtained by using 4-cyanobenzenesulfonyl chloride. Yellow oily, 73.6 mg, yield: 63 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 – 7.90 (m, 2H), 7.83 – 7.73 (m, 2H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.68 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.53 (s, 1H), 6.50 (d, *J* = 1.9 Hz, 1H), 6.10 (s, 1H), 3.82 (s, 3H), 3.69 (t, *J* = 6.2 Hz, 1H), 3.62 (s, 3H), 3.38 (s, 3H), 3.10 (td, *J* = 13.8, 13.0, 5.0 Hz, 2H), 2.83 (dd, *J* = 13.8, 7.0 Hz, 1H), 2.80 – 2.69 (m, 2H), 2.52 (t, *J* = 4.9 Hz, 1H), 2.49 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.61, 147.51, 146.68, 140.85, 140.62, 136.15, 132.36, 129.12, 128.66, 126.49, 123.29, 122.17, 117.41, 117.05, 114.29, 111.36, 111.02, 64.47, 55.83, 55.82, 55.34, 47.25, 42.69, 40.90, 25.62. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{28}N_2O_6S$: 509.1741, found 509.1746.

4-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl2-fluorobenzenesulfonate (40): According to the general procedure for the synthesis of compounds **23-42**, compound **40** was obtained by using 2-fluorobenzenesulfonyl chloride. Brown oily, 150 mg, yield: 95 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 – 7.76 (m, 1H), 7.68 – 7.61 (m, 1H), 7.28 (d, *J* = 9.1 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 6.66

(dd, 3H), 3.46 (s, 3H), 3.19 – 3.11 (m, 2H), 2.84 – 2.75 (m, 3H), 2.56 (dd, *J* = 16.0, 5.6 Hz, 1H), 2.53 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.64, 158.91, 151.12, 147.56, 146.66, 136.72, 136.12, 136.06, 131.07, 125.16, 125.06, 123.92 (d, J_{C-F} = 3.0 Hz) 123.55, 122.06, 117.09 (d, J_{C-F} = 21.0 Hz), 114.32, 111.32, 110.94, 64.67, 55.80, 55.54, 46.99, 42.50, 41.15, 29.66, 25.37. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{28}FNO_6S$: 502.1694, found 502.1683.

4-((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenyl2-nitrobenzenesulfonate(41): According to the general procedure for the synthesis of compounds **23-42**, compound **41** was obtained by using 2-nitrobenzenesulfonyl chloride. Yellow oily, 120 mg, yield: 98 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.82 – 7.75 (m, 2H), 7.67 (td, *J* = 7.4, 1.8 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.65 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.58 – 6.49 (m, 2H), 6.07 (s, 1H), 3.79 (s, 3H), 3.68 (dd, *J* = 7.3, 5.2 Hz, 1H), 3.59 (s, 3H), 3.41 (s, 3H), 3.14 – 3.05 (m, 2H), 2.82 – 2.73 (m, 2H), 2.70 (dt, *J* = 12.4, 5.0 Hz, 1H), 2.54 – 2.49 (m, 1H), 2.48 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.85, 148.34, 147.45, 146.63, 140.89, 136.59, 134.87, 131.85, 131.38, 130.15, 128.77, 126.35, 124.59, 123.32, 122.12, 114.52, 111.44, 111.03, 64.54, 55.80, 55.54, 47.12, 42.64, 40.92, 25.58. HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{26}H_{28}N_2O_8S$: 529.1639, found 529.1641.

((6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-2-methoxyphenylnaphthalene-2-sulfonate(42): According to the general procedure for the synthesis of compounds **23-42**, compound **42** was obtained by using 2-naphthalenesulfonyl chloride. Yellow oily, 51.6 mg, yield: 43 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.40 (d, *J* = 1.9 Hz, 1H), 7.95 (d, *J* = 8.7 Hz, 1H), 7.92 (dd, *J* = 8.2, 3.5 Hz, 2H), 7.88 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.67 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 7.60 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.64 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.49 (s, 1H), 6.45 (d, *J* = 1.9 Hz, 1H), 6.06 (s, 1H), 3.80 (s, 3H), 3.70 – 3.66 (m, 1H), 3.58 (s, 3H), 3.30 (s, 3H), 3.12 – 3.07 (m, 2H), 2.80 – 2.74 (m, 2H), 2.74 – 2.69 (m, 1H), 2.49 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.10, 147.44, 146.61, 140.26, 136.68, 135.32, 133.44, 131.74, 130.18, 129.37, 129.33, 128.84, 128.79, 127.88, 127.60, 126.28, 123.36, 123.32, 121.97, 114.24, 111.31, 110.96, 64.59, 55.78, 55.74, 55.37, 47.15, 42.66, 41.14, 25.62. **HRMS** (ESI+): m/z $[M+H]^+$ calcd for $C_{30}H_{31}NO_6S$: 534.1945, found 534.1958.

4.2 Biological experiments

4.2.1 Cell culture and collection

The vincristine -resistant Eca109/VCR subline was established by Gene Pharmaceutical Co., Ltd. (China) and cultured in a medium containing 100 nM vincristine (HYN0488, MCE, China) to sustain its resistance to chemotherapeutic agents. The esophageal cancer cell line Eca109, sourced from the American Type Culture Collection (ATCC), was maintained in RPMI-1640 medium (R8758, Sigma, USA) supplemented with 10 % fetal bovine serum (S711-001S, Lonsera, China), 100 μg/mL penicillin, and 100 μg/mL streptomycin (15140-122, Gibco, USA). Cultures were incubated at 37 °C in a 5 % CO_2 atmosphere for approximately 48 hours, and passages were performed when cell confluence reached 70-80 %.

4.2.2 Cytotoxicity and MDR reversal assays.

Eca109 and Eca109/VCR cells were seeded at a density of 5,000 to 6,500 cells per well into a white transparent bottom 96-well plate. To maintain moisture and provide buffering, phosphate-buffered saline (PBS) was added to the outer three rows of wells, and the cells were incubated for 24 hours. Each compound was dissolved in dimethyl sulfoxide (DMSO) to prepare a 10 mM stock solution. This stock solution was then further diluted in complete culture medium, and the diluted solutions were added to the cell culture plates to achieve the desired concentrations.In the multidrug resistance (MDR) reversal assay, cells were treated at 37 °C for 48 hours with varying concentrations of anticancer drugs in conjunction with 5 μM of different compounds or DMSO (as a negative control), while **TQ** and **VRP** served as positive controls. Following a 1-hour incubation with CCK-8 reagent, the absorbance at 450 nm was measured using a multifunctional microplate reader to calculate cell viability.

The cell viability rates of proliferation were calculated with the following equation:

Viability ratio (%) = $[(OD_{compd} - OD_{blank})/(OD_{DMSO} - OD_{blank})] \times 100$

4.2.3 Plate clone formation experiment

Cells were seeded in a 24-well plate at approximately 10,000 cells per well and incubated for 24 hours. Following this, they were treated according to specific experimental conditions for 48 hours. The drug-free medium was replaced every two days. After 8 days, the cells were fixed with 4 % paraformaldehyde and subsequently stained with 0.1 % crystal violet.

4.2.4 Cell apoptosis and flow cytometry analysis

Cells treated with various drugs for 60 hours were digested using 0.25 % trypsin and collected by centrifugation, followed by two washes with ice-cold phosphate-buffered saline (PBS). The cells were then resuspended in binding buffer at a concentration of 5 × 10^5 cells per mL. To quantify apoptotic cells, annexin V-FITC/PI apoptosis kit (K2003,APExBIO,USA) was used according to the manufacturer's protocol. A total of 5 μL of Annexin V-FITC and PI staining solution was added to each cell suspension. After gentle mixing, the samples were incubated in the dark at room temperature for 20 minutes. Finally, flow cytometry analysis was performed using a LongCyteTM (Challenbio,China).

4.2.5 Western blot analysis experiment

Protein expression was evaluated using Western blot analysis. Cells were lysed with RIPA buffer (P0013B, Beyotime, China) containing 1 % protease inhibitor. The resulting protein extracts were centrifuged at 4 °C and 12,000 rpm for 15 minutes, after which the total protein concentration in the supernatant was measured using a BCA assay kit. Samples containing 20 μg of total protein were then prepared and subjected to 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer of the proteins onto a PVDF membrane. Bands corresponding to the molecular weights of the target proteins were identified and excised, and the membrane was incubated with the appropriate primary antibodies (P-gp, 22336-1-AP Proteintech, China; Gapdh, CPA9165,Cohesion Biosciences,England) overnight at 4 °C. Subsequently, species-specific secondary antibodies conjugated with horseradish peroxidase were employed to detect the primary antibodies. Finally, the protein bands were visualized using an enhanced chemiluminescence kit (P0018, Beyotime, China).

4.2.6 Determination of intracellular accumulation of fluorescent dyes

Eca109 or Eca109/VCR cell lines (15,000-20,000 cells/well) were plated in black transparent bottom 96-well plates containing 80 μL of culture medium and allowed to incubate overnight. Test compounds (10 μL) dissolved in culture medium were then added to the 96-well plates to achieve a final concentration range of 5 μM, followed by incubation at 37 °C for 30 minutes. Subsequently, 10 μL of **RH123** or **H33342** were added to each well to reach a final concentration of 5 μM, and the cells were cultured for 120 minutes. Afterward, the wells were washed three times with cold phosphate-buffered saline (PBS). The total fluorescence intensity was measured using a multi-mode detection platform (SpectraMax Paradigm, Molecular Devices, USA); the excitation wavelength for **RH123** was 488 nm and the emission wavelength was 530 nm, while the excitation and emission wavelengths for **H33342** were 360 nm and 461 nm, respectively. Under these experimental conditions, the experiments were independently repeated three times, and the data were aggregated for analysis to assess the effect of the compounds on the accumulation of intracellular fluorescent substrates.

The relative total fluorescence intensity was calculated with the following equation:

Relative total fluorescence intensity (%)= $[(FL_{compd} - FL_{blank})/(FL_{DMSO} - FL_{blank})] \times 100$

4.2.7 Molecular docking experiment

The molecular docking study of P-gp (PDB ID: 6QEX; https://www.rcsb.org/)[67] was conducted using SYBYL-X 2.0[68]. The cryo-EM structure of P-gp was preprocessed in PyMOL[69] by removing water molecules, cholesterol, and phospholipids. The ligand preparation module of SYBYL-X 2.0 simulated the ionization states of small molecules at pH 7.0, generating all possible conformers. The protein structure was processed using the Structure Prepare Tool: the original paclitaxel ligand was extracted from the cryo-EM structure, followed by hydrogen atom addition (AddHydrogens: All; Hydrogens Orientation in Water: Random) and charge assignment (Biopolymers: Amber7FF99; Ligands: Gasteiger-Huckel), as per the SYBYL user manual.The Surflex-Dock GeomX (SFXC) protocol was employed for docking. The binding pocket was defined using the ligand-based method with parameters set to threshold = 0.50 and bloat = 0. Molecular docking was performed in Surflex-Dock GeomX mode, and the top-scoring conformation was selected for interaction analysis via the PLIP web server[73]. All structural visualizations were generated using PyMOL v3.0.3.

4.2.8 Pharmacokinetic Study

8-week-old male SD rats (300-400g were placed in standard feeding conditions (clean, ventilated, constant temperature and humidity SPF environment). Plasma samples were collected at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after intravenous administration of **41** (1 mg/kg) and at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after intragastric administration of **41** (10 mg/kg). The supernatant plasma was stored at -20 °C until analysis. The rat plasma was thawed at room temperature. An aliquot of 20 μL sample was mixed with 180 μL of the IS (tolbutamide, dissolved in ACN with 0.1% FA, 200 ng/mL). The mixture was vortexed for 40 minutes and then centrifuged at 3500 rpm for 20 minutes at 4 °C. A volume of 100 μL of the supernatant was transferred to a 96-well plate, followed by the addition of 100 μL of water with 0.1% FA. After mixing for 30 minutes, the sample

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Author contribution

Bo Xu, Tao Yu and Hong-Yuan Liu contributed equally to this work.

Declaration of interests

The authors state that they have no known competing economic interests or personal relationships, which may affect the work of this report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://xxxxx

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Highlights

• 42 new chemical entities were designed, synthesized, and subjected to comprehensive SAR analysis

• Compound **41** emerged as a highly potent P-gp inhibitor

• Compound **41** effectively reversed MDR in vitro, with reversal fold as high as 467.7

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