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Synthesis, Preliminary Pharmacological Evaluation and Receptor Docking Studies Of 10-Amino-3-Methoxy-6,8,12,12a-Tetrahydro-5H-Thiazolo[4',5':4,5]Pyrido[2,1-A]Isoquinolin-2-Ols As Novel Dopamine D_1 Receptor Inhibitors

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ABSTRACT

A series of 10-amino-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2,1-a] isoquinolines with substituent hydroxyl or methoxyl groups at C2 and C3 positions of the benzene ring were designed and synthesized. These compounds are 2-aminothiazole bioisosteres of the natural product stepholidine (SPD). Radioligand binding assays showed that compounds **10a**, **10b**, **10c** and **12b** had moderate binding affinity and activity for the D_1 receptor, as well as high selectivity over the D_2 receptor. Among them, **10c** exhibited moderate D_3 receptor binding affinity. **10a** with an amino group in position 10 showed higher affinities toward D_3 receptor compared to those without an amino group. *In silico* docking analysis of active ligands was performed to explain the result of binding assays and analyze structure-activity relationship for further structure optimization. Our analysis suggested that besides the salt bridge interaction formed by the conserved D3.32 with the protonated nitrogen of ligands and the hydrogen bonds formed by S5.42 with 2-hydroxyl group and S5.46 with 3-methoxyl group, a hydrogen bond formed between S188 located on the second extracellular loop (ECL2) and the nitrogen in the thiazole ring was critical to the affinity of this series of compounds for the D_1 receptor, which was in agreement with the interaction mode between SPD and the D_1 receptor. Our results suggested that the introduction of thiazole ring changes the pharmacological profile of SPD and the optimized compounds have high selective activity to D_1 receptor over D_2 receptor. These thiazole-SPD-derivative compounds have potential clinical use in Parkinson's disease and other conditions related to dopamine receptors.

Keywords: *Dopamine D_1 receptor; Bioisosteres of stepholidine; Inhibitory design; Synthesis; Molecular Modeling*

Introduction

Dopamine (DA), the endogenous ligand of dopaminergic neurotransmission systems, has been associated with many physiological functions such as fine movement coordination, cognition and emotion. DA exerts its effects by activating five distinct dopamine receptors (DRs) which belong to the GPCR superfamily and is classified into two subfamilies, D1-like (D1R and D5R) and D2-like (D2R, D3R and D4R), based on their pharmacological and functional characteristics. It has been established that DRs are primary targets of antipsychotic drugs used to treat psychomotor diseases such as schizophrenia, a

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debilitating mental illness which affects 0.5-1.5% of the worldwide population [1].

Since the discovery of the first D_1 receptor full agonist dihydrexidine (DHX, **1**) (Figure 1) by David *et al* [2], people started to realize that D_1 agonists have potential effect for the treatment of Parkinson's disease (PD), schizophrenia and memory disorders. Stepholidine (SPD, **2**) (Figure 1), a tetrahydroprotoberberine (THPB) alkaloid extracted from the Chinese herb *Stephania intermedia Lo*, has been reported to be a D_1 agonist and D_2/D_3 antagonist with binding affinities of 13 nM, 85 nM and 15 nM respectively [3]. Nevertheless, its clinical use is limited by the low oral bioavailability caused by its poor solubility in water and unwanted pre-systemic metabolism [4]. Pramipexole (**3**) (Figure 1), a D_2/D_3 agonist with binding affinities of 3 nM and 0.5 nM respectively [5], is an orally available anti-Parkinsonian drug bearing the 2-aminothiazole moiety which has proven its value in medicinal chemistry as a stable bioisosteric replacement of a phenol group with improved oral bioavailability and antioxidant function for neuroprotection [6]. In this work, we integrated the aminothiazole in Pramipexole into the scaffold of SPD in light of bioisosterism and designed a series of aminothiazole-containing SPD derivatives. The new designed compounds show highly selective D_1 or D_3 activities over D_2 receptor.

Materials and Methods

1. Chemistry

The solvents were dried according to standard procedures. Reactions involving moisture sensitive compounds were performed under an anhydrous atmosphere of dry argon, unless indicated otherwise. Commercially available chemicals were used without further purification. Reactions were monitored by using thin-layer chromatography (TLC) on silica-coated plastic sheets (silica gel 60 F_{254}) with the indicated eluent. Nuclear magnetic resonance spectra were recorded on a Brucker-DPX 400 MHz spectrometer, Mass spectral data was collected on a HP5973 N analytical mass spectrometer.

2. Syntheses

Syntheses of the target molecules are shown in Scheme 1-3. Scheme 1 describes the syntheses of key intermediates 9a–9f with common scaffold. We applied a classical procedure for the syntheses of protoberberine alkaloids. 4-(Benzyloxy)-3-methoxy -phenylethanamine (4a) was prepared from vanillin [7]. Fusion of 4 and unprotected 2-amino-4-thiazoleacetic acid (5) gave the amides 6 [8]. The amino group of 5 didn't affect the condensation. When 6 was directly subjected the Bischler-Napieralski cyclization with phosphorus oxychloride in dry toluene, the 2-aminothiazole ring was destroyed to form byproducts without expected cyclization product. Therefore, 6 was protected by Cbz or Fmoc before cyclization [9,10]. Thus, the cyclization proceeded smoothly to give the 3,4-dihydroisoquinolines, which was at once reduced with sodium tetrahydroborate to the unstable tetrahydroisoquinolines 8. Pictet-Spengler reaction of 8 with aqueous formaldehyde in acetic acid resulted in the diversely substituted thiazolopyridotetrahydroisoquinolines 9 [11].

Scheme 2 shows synthesis of target molecules with 2-hydroxy-3-methoxy or 2-acyloxy-3-methoxy substituted. Debenzylation of 9b–9d was first tried with Pd/C catalytic hydrogenation, however, the catalyst was poisoned. Therefore, the debenzylation was carried out by refluxing 9b–9d in MeOH with 37% hydrochloric acid, which gave the target compounds 10b and 10c, and 10d whose fluorenylmethyloxycarbonyl group wasn't affected[12]. As for O-benzyl and N-benzyloxycarbonyl protected 9a, thorough removal of the two groups needed stronger condition and longer time. Therefore, 9a was heated to 85°C in 37% hydrochloric acid without other solvent to give the target molecule 10a. Acylation of 10d with different acyl chlorides formed esters 11a–11d. Finally, Fmoc group of these esters were removed by piperidine in DMF to give target compounds 12a–12d[13], which could be considered as prodrugs of 9a.

Scheme 3 describes synthesis of target molecules with 2,3-dimethoxy or 2,3-dihydroxy substituted. 9e was N-defluorenylmethyloxycarbonylated by piperidine to give 13. O-dedimethylations of 9f and 13 were first tried with trimethylsilyl iodide in chloroform[14] and boron tribromide in dichloromethane[15]. However, both of the two methods failed

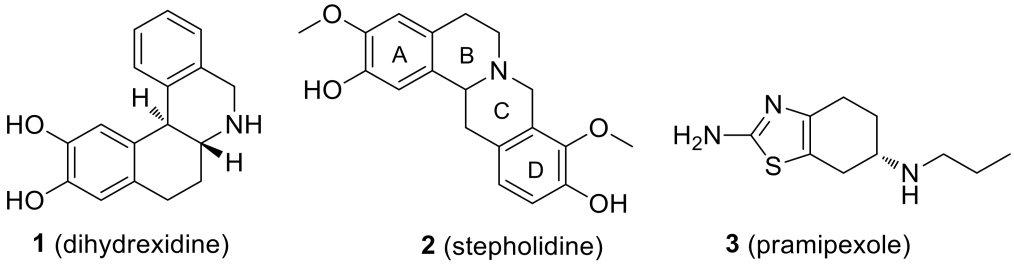


Figure 1: Molecular structures of dihydrexidine, stepholidine and pramipexole.

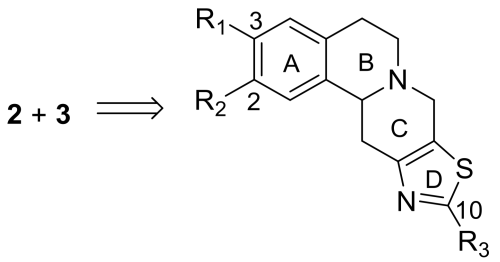


Figure 2: Proposed skeleton by combination of stepholidine and pramipexole.

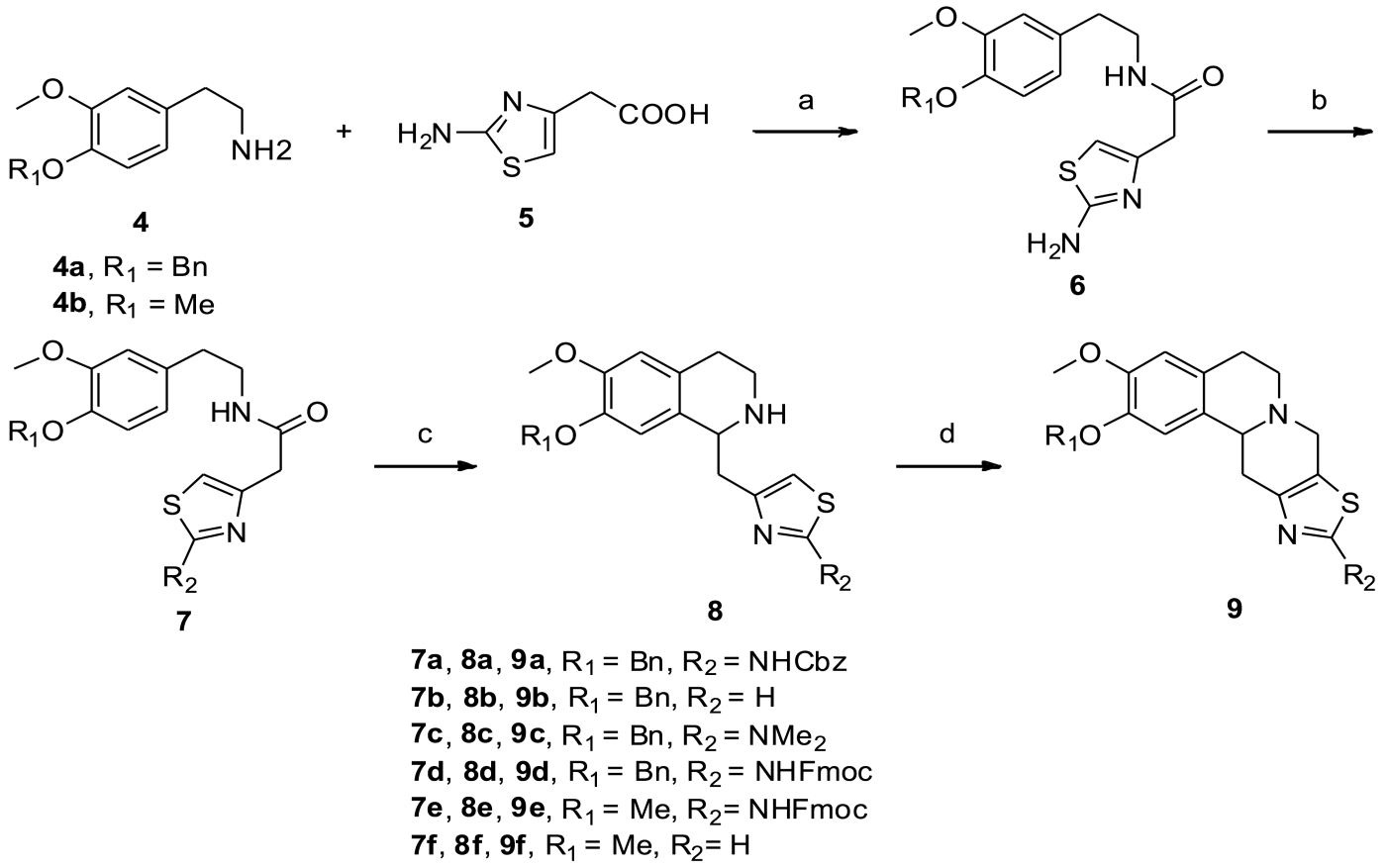
probably due to limited solubility of the substrates in the above-mentioned solvents. Thus, we treated them with 48% hydrobromic acid at 120–130°C[16], yielding the dedimethylated products 14a and 14b successfully.

Syntheses of the compared molecules are shown in Scheme 4. We adopted a different strategy by which B, C and D rings were constructed in sequence from substituted phenylethylamines. Imines 15 were prepared from 4 in two steps by Pictet-Spengler reaction and oxidation of C-N single bond to double bond with NBS[17], followed by aza-Diels Alder reaction[18] with N,N,N-trimethyl-3-oxobutan-1-aminium iodide[19,20] (16) to give tetrahydropyridoisoquinolinones 17. The next key step was regio-selective bromination at α-carbon (C1) of the carbonyl group. In consideration of bilateral α-carbons with similar reactivity, we attempted to activate C1 by producing an electron withdrawing group next to it. Therefore, NBS was employed as the bromination reagent and oxidant of the benzyl bond between β-C and γ-N. Thus, the specific C1 brominated ketones 18 were obtained. Condensation of 18 with thiourea gave rise to the aminothiazoles 19, followed by reduction of the C-N double bond to give 20, or Sandmeyer reaction as well as reduction to give 23. Subsequent debenzylation or dedimethylation of them produced the target molecules 20b, 21a, 21b and 23.

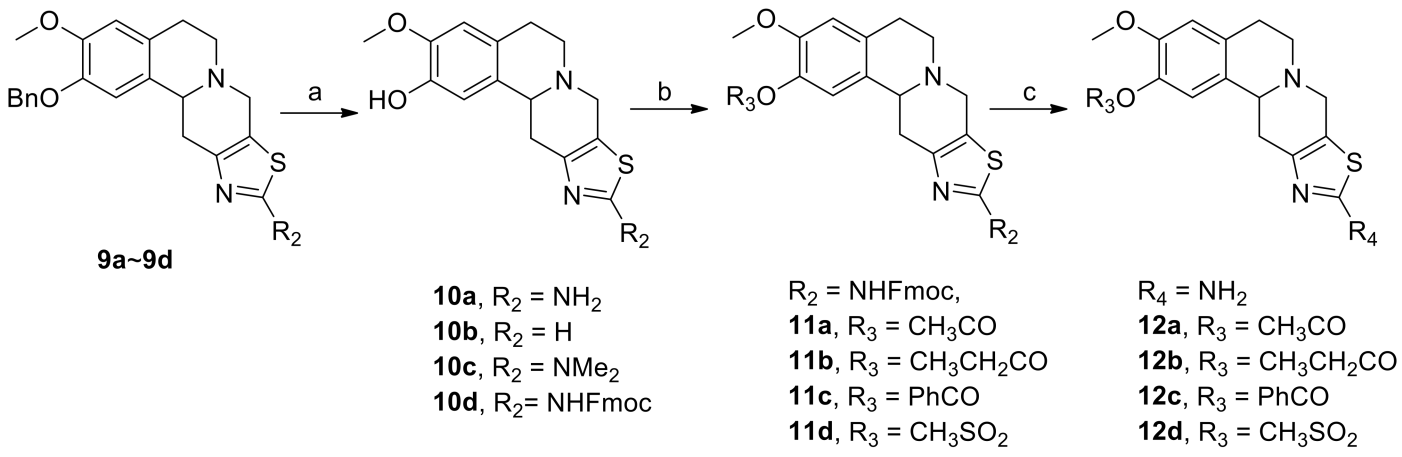
2.1. General procedure for the syntheses of amides 6a and 6b. To a suspension of **5** in $CHCl_3$ was added EDC·HCl (1.5 equivalent) and HOBt (1.5equivalent), stirred at room temperature for 30min, then TEA (3 equivalent)and **4** (1.0 equivalent) were added. The reaction mixture was stirred for overnight at room temperature. The organic layer was washed twice with water and once with brine, then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by washing with EA.

2.1.1. 2-(2-Aminothiazol-4-yl)-N-(4-(benzyloxy)-3-methoxyphenethyl)acetamide(6a). White solid; yield: 60.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.29 (m, 5H), 6.87 (s, 1H), 6.80 (d, *J* = 8.1 Hz, 1H), 6.74 (d, *J* = 1.7 Hz, 1H), 6.60 (dd, *J* = 8.2 Hz, 2.6Hz, 1H), 6.21 (s, 1H), 5.15 (s, 2H), 4.67 (s, 2H), 3.89 (s, 3H), 3.49 (dd, *J* = 12.7, 6.4 Hz, 2H), 3.43 (s, 2H), 2.72 (t, *J* = 6.5 Hz, 2H); MS(ESI)m/z: 398.2$(M+H)^+$.

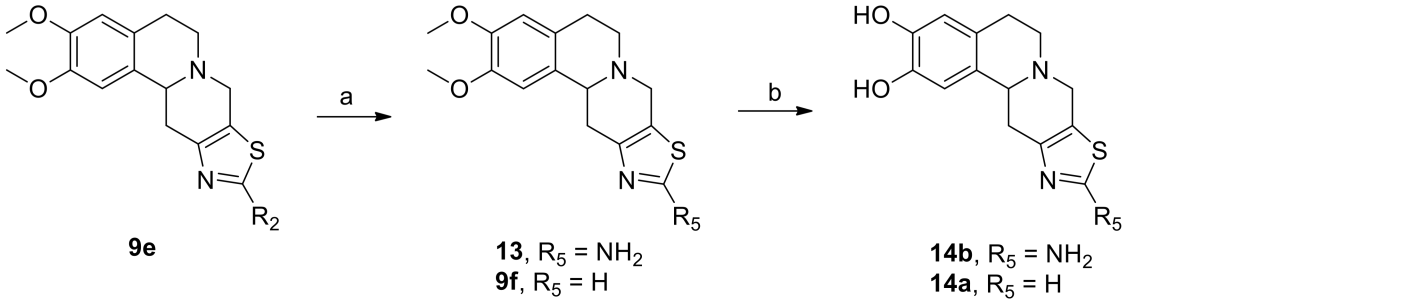
2.2.1.2. 2-(2-Aminothiazol-4-yl)-N-(3,4-dimethoxyphenethyl)acetamide (6b). White solid; yield: 62%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (s, 1H), 6.80 (d, *J* = 7.8 Hz, 1H), 6.70 (d, *J* = 8.3 Hz, 2H), 6.23 (s, 1H), 4.90 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.49 (dd, *J* = 12.7, 6.6 Hz, 2H), 3.44 (s, 2H), 2.74 (t, *J* = 6.8 Hz, 2H); MS(ESI)m/z: 322.1 $(M+H)^+$.



Scheme 1. Synthesis of key intermediates. Reagents and condition: (a) EDC·Cl, HOBt, TEA, chloroform, rt; (b) (i) **7a**: CbzCl, 4-DMAP, pyridine, chloroform, 0°C to rt, (ii) **7b**, **7f**: t-BuONO, THF, 60°C, (iii) **7c**: 1) t-BuONO, $CuBr_2$, MeCN, 60°C, 2) Me_2NH, MeCN, sealed tube, 80°C, (iv) **7d-7e**: FmocCl, DIPEA, dioxane, 0°C to rt; (c) 1) $POCl_3$, toluene, 100°C, 2) $NaBH_4$, MeOH, 0°C; (d) HCHO (37% in water), CH_3COOH, 60°C;



Scheme 2. Synthesis of some target compounds. Reagents and condition: (a) **10a**: 37% HCl, 80°C, 6h, **10b**~**10d**: 37% HCl, MeOH, 80°C, 4h; (b) R_3Cl, 4-DMAP, TEA, DCM, rt, **11a**: CH_3COCl, **11b**: CH_3CH_2COCl, **11c**: PhCOCl, **11d**: CH_3SO_2Cl; (c) piperidine, DMF, rt, 15min.



Scheme 3. Synthesis of some target compounds. Reagents and condition: (a) **13**, piperidine, DMF, rt, 15min; (b) 48% HBr, 120°C, 6h.

2.2. Synthesis of Benzyl (4-(2-((4-(benzyloxy)-3-methoxyphenethyl)amino)-2- oxoethyl)thiazol-2-yl)carbamate (Cbz-protection) (7a).

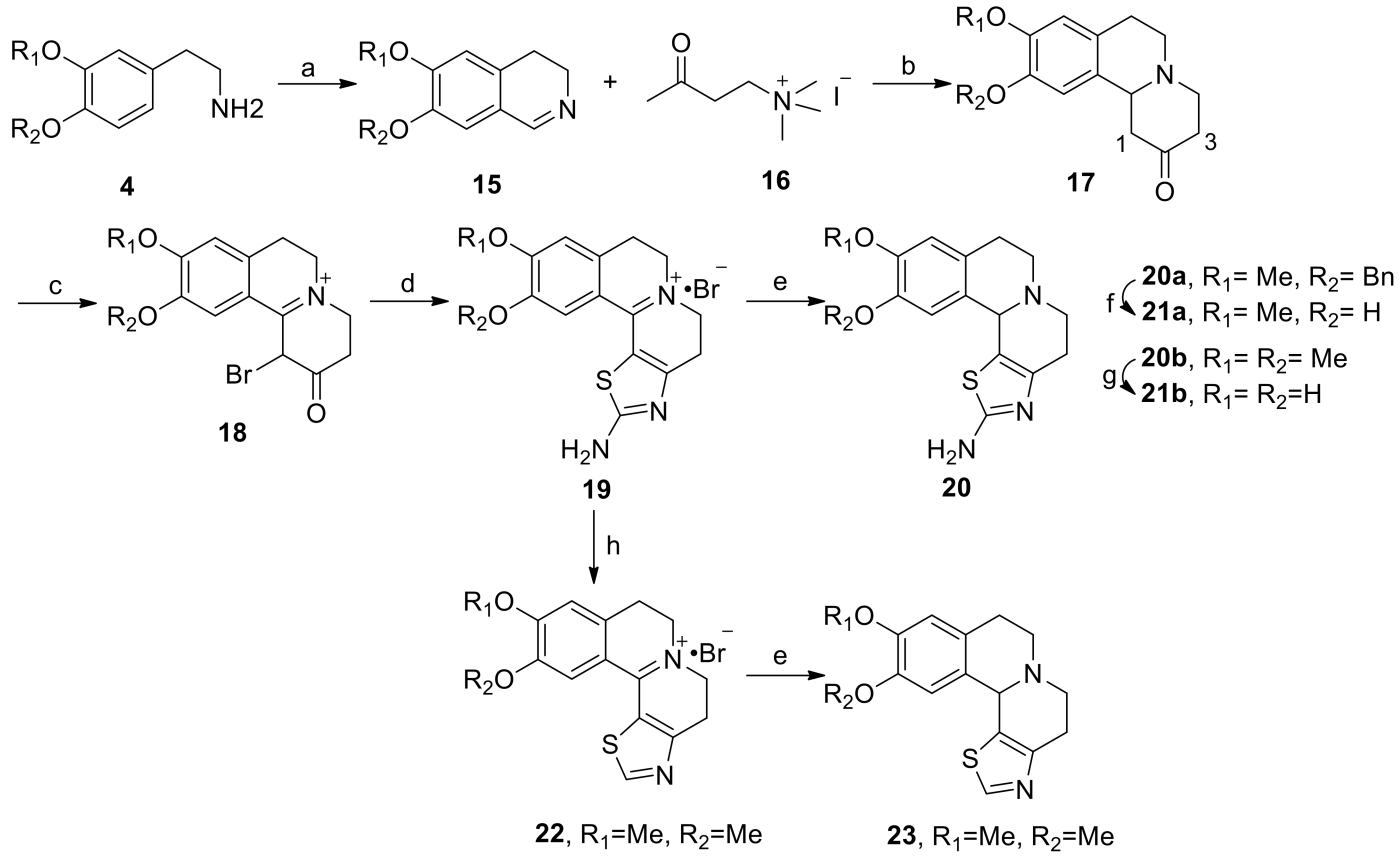
6a (1.5g，3.77mmol) was dissolved in chloroform (30mL) and cooled in an ice bath. Pyridine (0.9mL，11.31mmol) and 4-DMAP (46mg，0.377mmol) were added, and the solution was stirred for 15min. Benzyl chloroformate (1.29g，7.55mmol) was dissolved in chloroform (10mL), and the solution was added dropwise over a period of 15min. The reaction mixture was stirred in the ice bath for 1h and stirred at room temperature overnight. The mixture was washed with water (2×100mL) and brine (50mL), then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by washing with EA (10mL) to give **7a**. Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d6) δ 11.8 (s, 1H), 7.95 (t, *J* = 5.6 Hz, 1H), 7.41 – 7.30 (m, 10H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.78 (s, 2H), 6.63 (dd, *J* = 8.2 Hz, 1.5Hz, 1H), 5.20 (s, 2H), 4.99 (s, 2H), 3.71 (s, 3H), 3.38 (s, 2H), 3.22 (dd, *J* = 13.0 Hz, 6.3 Hz, 2H), 2.60 (t, *J* = 7.4 Hz, 2H); MS(ESI)m/z: 531.62 $(M+H)^+$.

2.3. General method for Fmoc-protection

6 was dissolved in dioxane and cooled in an ice bath. DIPEA (3 equivalent) was added, and the solution was stirred for 15min. Fluoranthenylmethoxycarbonyl chloride (1.2 equivalent) was dissolved in dioxane, and the solution was added dropwise over a period of 15min. The reaction mixture was stirred in the ice bath for 1h and stirred at room temperature overnight. The mixture was washed twice with water and once with brine, then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by washing with EA.

2.3.1. (9H-Fluoren-9-yl)methyl(4-(2-((4-(benzyloxy)-3-methoxyphenethyl)amino)-2- oxoethyl)thiazol-2-yl)carbamate (7d) White solid; yield: 55.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.57 (d, *J* = 7.5 Hz, 2H), 7.46 – 7.33 (m, 4H), 7.33 – 7.20 (m, 5H), 6.77 (d, 2H), 6.63 (s, 1H), 6.58 (dd, *J* = 1.8 Hz , 1H), 5.06 (s, 2H), 4.52 (d, *J* = 5.5 Hz, 2H), 4.22 (t, *J* = 6.6 Hz, 1H), 3.86 (s, 3H), 3.54 (s, 2H), 3.45 (dd, *J* = 12.8, 6.6 Hz, 2H), 2.69 (t, *J* = 6.7 Hz, 2H).; MS(ESI)m/z: 620.3 $(M+H)^+$.

2.3.2. (9H-Fluoren-9-yl)methyl(4-(2-((3,4-dimethoxyphenethyl)amino)-2-oxoethyl) thiazol-2-yl)carbamate (7e). White solid; yield: 57.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.31 (td, *J* = 7.5, 1.0 Hz, 2H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.72 (d, *J* = 1.7 Hz, 1H), 6.66 (s, 1H), 6.63 (dd, *J* = 8.1, 1.9 Hz, 1H), 4.60 (d, *J* = 5.2 Hz, 2H), 4.31 (t, *J* = 6.6 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.54 (s, 2H), 3.46 (dd, *J* = 12.8, 6.6 Hz, 2H), 2.70 (t, *J* = 6.8 Hz, 2H); MS(ESI)m/z: 544.2 $(M+H)^+$.



Scheme 4. Syntheses of the compared compounds. Reagents and condition: (a) (i) HCHO, HCOOH, 55°C, (ii) NBS, DCM, rt; (b) N,N,N-trimethyl-3-oxobutan-1-aminium iodide, MeOH, reflux; (c) NBS, NH_4OAc (cat.), DCM; (d) thiourea, EtOH, reflux; (e) $NaBH_4$, MeOH, 0°C; (f) 37% HCl, MeOH, 80°C, 4h; (g) 48% HBr, 120°C, 6h; (h) t-BuONO, DMF, 60°C.

2.4. General method for cleavage of the amino group

To a solution of **6** (6.8mmol) in THF (50mL) at 50 °C was added *t*-Butyl nitrite (13.6mmol), the mixture was stirred at 50 °C for 5h and concentrated, the residue was purified by flash chromatography (DCM:MeOH=30:1).

2.4.1. N-(4-(benzyloxy)-3-methoxyphenethyl)-2-(thiazol-4-yl)acetamide (7b). Light yellow solid; yield: 31.3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 1.9 Hz, 1H), 7.49 – 7.28 (m, 5H), 7.12 (d, *J* = 1.9 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.68 (d, *J* = 1.9 Hz, 1H), 6.51 (dd, *J* = 8.1, 1.9 Hz, 1H), 5.15 (s, 2H), 3.86 (s, 3H), 3.73 (s, 2H), 3.48 (dd, *J* = 12.7 Hz, 6.8 Hz, 2H), 2.70 (t, *J* = 6.9 Hz, 2H); MS(ESI)m/z: 383.0 $(M+H)^+$.

2.4.2. N-(3,4-dimethoxyphenethyl)-2-(thiazol-4-yl)acetamide (7f). Light yellow solid; yield: 36.2%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 1.9 Hz, 1H), 7.12 (d, *J* = 1.9 Hz, 1H), 6.79 (d, *J* = 8.1 Hz, 1H), 6.70 (d, *J* = 1.8 Hz, 1H), 6.62 (dd, *J* = 8.1, 1.8 Hz, 1H), 3.84 (s, 6H), 3.72 (s, 2H), 3.49 (dd, *J* = 12.8 Hz, 6.8 Hz, 2H), 2.72 (t, *J* = 6.9 Hz, 2H); MS(ESI)m/z: 306.9 $(M+H)^+$.

2.5. Synthesis of N-(4-(benzyloxy)-3-methoxyphenethyl)-2-(2-(dimethylamino)thiaz- ol-4-yl)acetamide (7c).

A solution of copper(II) bromide (1.235g, 5.53mmol) in CH_3CN (50mL) was heated to 60 °C. *t*-Butyl nitrite (0.713g, 6.92mmol) was added and stirred for 15min. **6a** (1.833g, 4.61mmol) was added portionwise. The mixture was allowed to stir for 30min at 80 °C, then concentrated under reduced pressure, and the residue was dissolved in DCM and washed with water (2×100mL) and satd $NaHCO_3$ solution (50mL). The precipitate was removed by filtration, and the DCM extract was dried over Na_2SO_4, filtered and concentrated, the residue was purified by flash chromatography (DCM: MeOH=100:1) to give bromide product (1.06g, 50%). The bromide product was dissolved in CH_3CN (20mL) in a sealed tube, dimethylamine (33% in water) was added. The mixture was heated to 80 °C an stirred for 8h. The volatile was removed under reduced pressure. The residue was dissolved in DCM and washed with water (2×100mL) and brine (50mL), then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (DCM: MeOH=80:1) to give **7c**. Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.26 (m, 5H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.67 (d, *J* = 1.9 Hz, 1H), 6.59 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.18 (s, 1H), 5.12 (s, 2H), 3.83 (s, 3H), 3.50 (dd, *J* = 13.2 Hz, 6.6 Hz, 2H), 3.46 (s, 2H), 2.93 (s, 6H), 2.71 (t, *J* = 6.8 Hz, 2H).; MS(ESI)m/z: 426.1 $(M+H)^+$.

2.6. General procedure for the synthesis of 8a–8f

A mixture of amide **7a–7f** (3mmol), toluene, and freshly distilled $POCl_3$ (6mmol) was heated for 1h at 100 °C. The solvent and excess $POCl_3$ were removed *in vacuo*, and the residue taken up

with MeOH (20mL). To the cooled (0 °C) solution was added $NaBH_4$ (9mmol) portionwise and the mixture was stirred at this temperature for 15min. Acetone was added, and stirring was maintained 10min. The mixture was concentrated, and the residue was taken up with DCM. The organic solution was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography (DCM: MeOH=30:1).

2.6.1. 4-((7-(benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-N,N- dimethylthiazol-2-amine (8c). Light yellow solid; yield: 30.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.26 (m, 5H), 6.57 (s, 1H), 6.55 (s, 1H), 5.80 (s, 1H), 5.20 (d, *J* = 12.5 Hz, 1H), 5.07 (d, *J* = 12.5 Hz, 1H), 4.70 (d, *J* = 5.5 Hz, 1H), 3.85 (s, 3H), 3.55 (s, 1H), 3.42 (s, 1H), 3.19 – 3.16 (m, 2H), 3.03 (s, 6H), 2.95 – 2.86 (m, 2H); MS(ESI)m/z: 410.2 $(M+H)^+$.

2.7. General procedure for the synthesis of 9a–9f

The amine **8a–8f** (0.85mmol)was dissolved in acetic acid (10mL), then 37% w/w aqueous methanal (1.7mmol) was added. The solution was stirred at 60 °C for 6h. The solution was basified to pH 10 with aqueous ammonia in ice bath. The resultant cream precipitate was extracted with DCM (3×30mL), the organic layers were combined and washed with brine (20mL), dried and concentrated. The residue was purified by flash chromatography (DCM: MeOH=100:1).

2.7.1. Benzyl(2-(benzyloxy)-3-methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5] pyrido[2,1-a]isoquinolin-10-yl)carbamate (9a). Off-white solid; yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.27 (m, 10H), 6.69 (s, 1H), 6.65 (s, 1H), 5.30 (s, 1H), 5.23 (s, 2H), 5.10 (s, 2H), 3.99 (d, *J* = 14.4 Hz, 1H), 3.88 (s, 3H), 3.73 – 3.61 (m, 2H), 3.53 – 3.44 (m, 1H), 3.24 – 2.99 (m, 2H), 2.83 – 2.50 (m, 3H); MS(ESI)m/z: 527.7 $(M+H)^+$.

2.7.2. 2-(Benzyloxy)-3-methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido [2,1-a]isoquinoline (9b). Off-white solid; yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.46 – 7.29 (m, 5H), 6.75 (s, 1H), 6.65 (s, 1H), 5.15 (q, 2H), 4.15 (d, 1H, *J* =14.3 Hz), 3.89 (s, 3H), 3.76 (d, 1H, *J* =14.6 Hz), 3.69 (dd, 1H, J = 3.8 Hz, 11 Hz), 3.38 (d, 1H, *J* = 16.2 Hz), 3.2 – 3.16 (m, 2H), 2.83 – 2.67 (m, 3H); MS(ESI)m/z: 378.49 $(M+H)^+$.

2.7.3. 2-(Benzyloxy)-3-methoxy-N,N-dimethyl-6,8,12,12a-tetrahydro-5H-thiazolo [4', 5':4,5]pyrido[2,1-a]isoquinolin-10-amine (9c). Off-white solid; yield: 63%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.28 (m, 5H), 6.76 (s, 1H), 6.63 (s, 1H), 5.12 (d, *J* = 12.2 Hz, 1H), 5.06 (d, *J* = 12.1 Hz, 1H), 3.90 (d, *J* = 13.9 Hz, 1H), 3.87 (s, 3H), 3.68 – 3.52 (m, 2H), 3.22 – 3.09 (m, 3H), 3.07 (s, 6H), 2.78 – 2.52 (m, 3H).; MS(ESI)m/z: 422.2 $(M+H)^+$.

2.7.4. (9H-Fluoren-9-yl)methyl(2-(benzyloxy)-3-methoxy-6,8,12,12a-tetrahydro-5H -thiazolo[4',5':4,5]pyrido[2,1-a]isoquinolin-10-yl)carbamate (9d). Off-white solid; yield: 62.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.52 (d, *J* = 7.5 Hz, 2H), 7.43 – 7.32 (m, 6H), 7.32 – 7.26 (m, 3H), 6.63 (s, 1H), 6.57 (s, 1H), 5.03 (s, 2H), 4.51 (d, *J* = 6.9 Hz, 2H), 4.20 (t, *J* = 7.0 Hz, 1H), 3.98 (d, *J* = 14.7 Hz, 1H), 3.81 (s, 3H), 3.72 (d, *J* = 14.6 Hz, 1H), 3.64 (dd, *J* = 10.7, 3.6 Hz, 1H), 3.22 – 3.06 (m, 2H), 3.05 – 2.88 (m, 1H), 2.78 – 2.53 (m, 3H).; MS(ESI)m/z: 616.4 $(M+H)^+$.

2.7.5. (9H-Fluoren-9-yl)methyl(2,3-dimethoxy-6,8,12,12a-tetrahydro-5H-thiazolo [4',5':4,5]pyrido[2,1-a]isoquinolin-10-yl)carbamate (9e). Off-white solid; yield: 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.74 (m, 3H), 7.71 (tt, *J* = 7.4 Hz, 1.2 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.44 – 7.35 (m, 2H), 7.30 (tt, *J* = 7.4 Hz, 1.2 Hz, 1H), 6.60 (s, 1H), 6.57 (s, 1H), 4.55 (d, *J* = 6.5 Hz, 2H), 4.26 (s, 1H), 4.01 (d, *J* = 14.8 Hz, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 3.73 – 3.67 (m, 1H), 3.24 (d, *J* = 17.0 Hz, 1H), 3.15 (s, 1H), 3.04 (s, 1H), 2.71 (d, *J* = 11.5 Hz, 3H).; MS(ESI)m/z: 540.3 $(M+H)^+$.

2.7.6. 2,3-Dimethoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2,1-a]iso- quinoline (9f). Off-white solid; yield: 81.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66(s, 1H), 6.74(s, 1H), 6.63(s, 1H), 4.17(d, 1H, *J*=14.9Hz), 3.87(s, 6H), 3.80 – 3.74(m, 2H,), 3.53(d, 1H, *J*=16.0Hz), 3.26 – 3.04(m, 2H), 2.88(t, 1H, *J*=13.3Hz), 2,81 – 2.64(m, 2H); MS(ESI)m/z: 303.0 $(M+H)^+$; ESI-FTMS m/z: calcd. for $C_{16}H_{18}N_2O_2S$ $(M+H)^+$ 303.1123, found 303.1159.

2.2.8. Synthesis of 10-Amino-3-methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4, 5] pyrido[2,1-a]isoquinolin-2-ol (10a)

A mixture of **9a** (100mg) and 37%HCl (5mL) was stirred under argon at 80 °C for 6h. The solution was basified to pH 9 with 15%NaOH and sat. $NaHCO_3$, filtered to give green solid, and purified by flash chromatography (DCM: MeOH=20:1) to give **10a**. Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77(s, 1H), 6.59(s, 1H), 4.81(s, 2H), 3.88(d, 1H, *J*=14.5Hz), 3.87(s, 3H), 3.69(dd, 1H, *J*=3.9Hz, 11Hz), 3.60(d, 1H, *J*=14.5Hz), 3.23 – 3.01 (m, 3H), 2.73 – 2.50 (m, 3H); MS(ESI)m/z: 303.9$(M+H)^+$; ESI-FTMS m/z: calcd. for $C_{15}H_{17}N_3O_2S$ $(M+H)^+$ 304.1075, found 304.1115.

2.9. General procedure for debenzylation

A mixture of **9b** (100mg), MeOH (6mL) and 37%HCl (3mL) was stirred under argon at 80 °C for 4h. MeOH was removed under reduced pressure. The remaining solution was basified to pH 9 with sat. $NaHCO_3$, filtered to give gray solid. The crude product was purified by flash chromatography (DCM: MeOH=50:1).

2.9.1. 3-Methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2,1-a]isoquin- olin-2-ol (10b). White solid; yield: 73.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66(s, 1H), 6.82(s, 1H), 6.61(s, 1H), 5.62(s, 1H), 4.15(d, 1H, *J*=14.9Hz), 3.88(s, 3H), 3.82 – 3.68 (m, 2H), 3.48(d, 1H, *J*=15.7Hz), 3.27 – 3.04(m, 2H), 2.85(t, 1H, *J*=13.1Hz), 2.79 – 2.64(m, 2H); MS(ESI)m/z: 289.1 $(M+H)^+$; ESI-FTMS m/z: calcd. for $C_{15}H_{16}N_2O_2S$ $(M+H)^+$ 290.0890, found 290.0956.

2.9.2. 10-(Dimethylamino)-3-methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5] pyrido[2,1-a]isoquinolin-2-ol (10c). White solid; yield: 64.9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78(s, 1H), 6.59(s, 1H), 5.62(s, 1H), 3.91(d, 1H, *J*=14.3Hz), 3.67(dd, 1H, *J*=3.8Hz,

10.8Hz), 3.61(d, 1H, *J*=14.3Hz), 3.26 – 3.09(m, 3H), 3.07(s, 6H), 2.77 – 2.54(m, 3H); MS(ESI)m/z: 332.1 $(M+H)^+$; ESI-FTMS m/z: calcd. for $C_{17}H_{21}N_3O_2S$ $(M+H)^+$ 332.1388, found 332.1432.

2.10. General procedure for acylation

To a suspension of **10d**·HCl (0.18mmol) in DCM was added TEA (0.54mmol), 4-DMAP (0.44mmol) and acyl chloride (0.54mmol). The mixture was stirred at room temperature for 8h, then washed with water and brine. The organic layer was dried an concentrated. The residue was purified by flash chromatography (DCM: MeOH=100:1).

2.11. General procedure for deprotection of Fmoc

To a solution of **11a–11d** or **9f** in DMF was added piperidine, then stirred at room temperature for 15min. The mixture was concentrated in vacuo. The residue was purified by flash chromatography (DCM: MeOH=30:1).

2.11.1. 10-Amino-3-methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido [2, 1-a]isoquinolin-2-yl acetate (12a). White solid; yield: 64.9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87(s, 1H), 6.71(s, 1H), 4.92(s, 2H), 3.89(d, 1H, *J*=14.0Hz), 3.82(s, 3H), 3.72(dd, 1H, *J*=3.5Hz, 10.9Hz), 3.59(d, 1H, *J*=14.0Hz), 3.26 – 3.02(m, 3H), 2.87 – 2.56(m, 3H), 2.33(s, 3H); MS(ESI)m/z: 346.0 $(M+H)^+$, ESI-FTMS m/z: calcd. for $C_{17}H_{19}N_3O_3S$ $(M+H)^+$ 346.1181, found 346.1222.

2.11.2. 10-Amino-3-methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido [2, 1-a]isoquinolin-2-yl propionate (12b). White solid; yield: 71.2%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86(s, 1H), 6.69(s, 1H), 4.87(s, 2H), 3.88(d, 1H, *J*=14.1Hz), 3.80(s, 3H), 3.70(dd, 1H, *J*=3.5Hz, 10.6Hz), 3.59(d, 1H, *J*=14.1Hz), 3.24 – 3.05(m, 3H), 2.85 – 2.65(m, 3H), 2.62(q, 2H, *J*=7.83Hz), 1.28(t, 3H, *J*=7.83Hz); MS(ESI)m/z: 360.0 $(M+H)^+$; ESI-FTMS m/z: calcd. for $C_{18}H_{21}N_3O_3S$ $(M+H)^+$ 360.1337, found 360.1377.

2.11.3. 10-Amino-3-methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2, 1-a]isoquinolin-2-yl benzoate (12c). White solid; yield: 70.3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23(d, 2H, *J*=7.4Hz), 7.64(t, 1H, *J*=7.4Hz), 7.51(t, 2H, *J*=7.4Hz), 6.99(s, 1H), 6.75(s, 1H), 4.97(s, 2H), 3.90(d, 1H, *J*=14.5Hz), 3.80(s, 3H), 3.74(dd, 1H, *J*=3.8Hz, 10.8Hz), 3.61(d, 1H, *J*=14.5Hz), 3.28 – 3.06(m, 3H), 2.88 – 2.57(m, 3H); MS(ESI)m/z: 408.1 $(M+H)^+$, ESI-FTMS m/z: calcd. for $C_{22}H_{21}N_3O_3S$ $(M+H)^+$ 408.1337, found 408.1377.

2.11.4. 10-Amino-3-methoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2, 1-a]isoquinolin-2-yl methanesulfonate (12d). White solid; yield: 64.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13(s, 1H), 6.74(s, 1H), 4.84(s, 2H), 3.89(d, 1H, *J*=14.1Hz), 3.88(s, 3H), 3.73(dd, 1H, *J*=3.9Hz, 11.0Hz), 3.62(d, 1H, *J*=14.1Hz), 3.18(s, 3H), 3.24 – 3.08(m, 3H), 2..85 – 2.55(m, 3H); MS(ESI)m/z: 382.0 $(M+H)^+$, ESI-FTMS m/z: calcd. for $C_{16}H_{19}N_3O_4S_2$ $(M+H)^+$ 382.0851, found 382.0889.

2.11.5. 2,3-Dimethoxy-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2,1-a]is- oquinolin-10-amine (13). White solid; yield: 89.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70(s, 1H), 6.61(s, 1H), 4.79(s, 2H), 3.90(d, 1H, *J*=14.5Hz), 3.87(s, 3H), 3.85(s, 3H), 3.72(dd, 1H, *J*=3.9Hz, 11Hz), 3.62(d, 1H, *J*=14.5Hz), 3.30 – 3.05(m, 3H), 2.81 – 2.57(m, 3H); MS(ESI)m/z: 318.3 $(M+H)^+$, ESI-FTMS m/z: calcd. for $C_{16}H_{19}N_3O_2S$ $(M+H)^+$ 318.1232, found 318.1266.

2.12. General procedure for dedimethylation

A mixture of 9f or 13 and 48%HBr was stirred at 120 °C for 6h. The solution was basified to pH 9 with 15%NaOH and sat. $NaHCO_3$, filtered to give brown solid, then purified by flash chromatography (DCM: MeOH=10:1).

2.12.1. 6,8,12,12a-Tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2,1-a]isoquinoline-2,3- diol (14a). White solid; yield: 75.8%; ^{1}H-NMR(400MHz, CD_3OD) δ 8.89(s, 1H), 6.72(s, 1H), 6.54 (s, 1H), 4.18(d, 1H, *J*=15.3Hz), 3.81 – 3.65(m, 2H), 3.48(dd, 1H, *J*=2.5Hz, 16.2Hz), 3.24 – 3.09(m, 1H), 3.09 – 2.88(m, 1H), 2.84 – 2.56(m, 3H); MS(ESI)m/z: 275.0$(M+H)^+$, ESI-FTMS m/z: calcd. for $C_{14}H_{14}N_2O_2S$ $(M+H)^+$ 275.0810, found 275.0841.

2.12.2. 10-Amino-6,8,12,12a-tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2,1-a]isoqui- noline-2,3-diol (14b). White solid; yield: 76.9%; ^{1}H-NMR (400MHz, CD_3OD) δ 6.67(s, 1H), 6.52(s, 1H), 3.83(d, 1H, *J*=14.5Hz), 3.65(dd, 1H, *J*=3.8Hz, 10.8Hz), 3.51(d, 1H, *J*=14.5Hz), 3.18 – 3.06(m, 2H), 3.06 – 2.88(m, 1H), 2.74 – 2.56(m, 2H), 2.56 – 2.38(m, 1H); MS(ESI)m/z: 290.0$(M+H)^+$, ESI-FTMS m/z: calcd. for $C_{14}H_{15}N_3O_2S$ $(M+H)^+$ 290.0919, found 290.0956.

2.13. General procedure for the synthesis of 3,4-dihydroisoquinolines 15

To a solution of **4** (59.4mmol) in formic acid (100mL) was added paraformaldehyde (59.4mmol), the mixture was stirred at 60 °C for 3h. Much of the formic acid was removed under reduced pressure, the remaining solution was basified to pH 9 with 15%NaOH, then extracted with DCM (3×100mL). The organic layers were combined, dried, concentrated and the residue was taken up with DCM (100mL). To this solution was added NBS (65.3mmol) portionwise. The mixture was stirred at room temperature for 3h, then basified to pH 9 with 15%NaOH. The organic layer was washed with brine (50mL), dried and concentrated. The residue was purified by flash chromatography (DCM: MeOH=40:1).

2.14. Preparation of N,N,N-trimethyl-3-oxobutan-1-aminium iodide (16)

A mixture of methanol (18mL), dimethylamine hydrochloride (20g, 245mmol), acetone (54.8mL, 736mmol), paraformaldehyde (11g, 368mmol) and 37%HCl (45μL) was stirred at 80 °C for 8h. The mixture was concentrated under reduced pressure to give white solid. The solid was added water and basified to pH 10 with 15%NaOH, extracted with EA. The organic layers were combined, dried and concentrated under reduced pressure. The residue was purified by reduced pressure distillation. The fraction boiling at 76–78 °C at 16mm. was collected to give colorless liquid(10.11g, 35.8%). The liquid was dissolved in acetone (48mL) and cooled to 0 °C, methyl iodide (18.7g,

131.67mmol) was added dropwise over a period of 20min at this temperature. The reaction mixture was stirred at room temperature overnight. The precipitate was filtered and washed with DCM to give **16** (20.15g, 89.3%).

2.15. General procedure for the synthesis of 17a, 17b

A mixture of **15** (18.7mmol), **16** (18.7mmol) and methanol (50mL) was stirred at 80 °C for 1.5h. The mixture was concentrated under reduced pressure. The residue was dissolved in DCM (50mL) and washed with water (2×50mL) and brine (50mL). The organic layer was dried and concentrated. The residue was crystallized from ethanol.

2.15.1. 10-(Benzyloxy)-9-methoxy-3,4,6,7-tetrahydro-1H-pyrido[2,1-a]isoquinolin-2 (11bH)-one (17a). Colorless needle; yield: 36.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.25 (m, 5H), 6.65 (s, 1H), 6.56 (s, 1H), 5.09 (s, 2H), 3.87 (s, 3H), 3.45 (d, *J* = 11.8 Hz, 1H), 3.30 – 3.22 (m, 1H), 3.17 – 3.02 (m, 2H), 2.80 – 2.64 (m, 4H), 2.64 – 2.52 (m, 1H), 2.45 – 2.32 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 184.68, 156.10, 151.96, 145.42, 136.60, 132.39, 128.62, 127.91, 127.22, 121.34, 117.06, 109.97, 89.23, 71.03, 56.05, 49.14, 48.97, 36.09, 29.24; MS(ESI)m/z: 338.2(M+H)$^+$.

2.16. General procedure for the synthesis of 18a, 18b

To a solution of **17** (3mmol) in DCM (20mL) was added NBS (6mmol) portionwise and ammonium acetate (0.3mmol). The reaction mixture was stirred at room temperature overnight. The mixture was washed with water and brine. The organic layer was dried and concentrated. The residue was purified by flash chromatography (EA).

2.16.1. 10-(Benzyloxy)-1-bromo-9-methoxy-2-oxo-1,2,3,4,6,7-hexahydropyrido[2,1- a]isoquinolin-5-ium bromide (18a). Yellow solid; yield: 56.2%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.47 – 7.28 (m, 5H), 6.69 (s, 1H), 5.20 (s, 2H), 3.94 (s, 3H), 3.71 – 3.59 (m, 2H), 3.43 (s, 2H), 2.80 (s, 2H), 2.75 – 2.65 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 184.68, 156.10, 151.96, 145.42, 136.60, 132.39, 128.62, 127.91, 127.22, 121.34, 117.06, 109.97, 89.23, 71.03, 56.05, 49.14, 48.97, 36.09, 29.24; MS(ESI)m/z: 415.1 (M+H)$^+$.

2.17. General procedure for the synthesis of 19a, 19b

A mixture of **18**, thiourea and ethanol was stirred at 80 °C for 1h. Then solution was recovered to room temperature, accompanied with yellow precipitate. After storage in refrigerator at 4 °C overnight, the precipitate was filtered, washed with ethanol and DCM to give **19**.

2.17.1. 2-Amino-11-(benzyloxy)-10-methoxy-4,5,7,8-tetrahydrothiazolo[5',4':3,4]py- rido[2,1-a]isoquinolin-6-ium bromide (19a). Yellow solid; yield: 67.5%; ^{1}H NMR (400 MHz, DMSO-d6) δ 9.02 (s, 2H), 7.47 (s, 1H), 7.45 – 7.27 (m, 5H), 7.16 (s, 1H), 5.10 (s, 2H), 4.03 (t, *J* = 8.4 Hz, 2H), 3.87 (s, 3H), 3.79 (t, 2H), 3.05 (t, *J* = 8.5 Hz, 2H), 3.00 (t, *J* = 7.1 Hz, 2H); ^{13}C NMR (400 MHz, DMSO-d6) δ 177.72, 166.60, 155.84, 154.54, 146.70, 136.16, 134.70, 128.65, 128.32, 127.90, 117.68, 113.32, 111.99, 107.09, 70.68, 56.34, 49.88, 48.88, 26.28, 25.16; MS(ESI)m/z: 392.1(M$^+$).

2.18. 10,11-Dimethoxy-5,7,8,12b-tetrahydro-4H-thiazolo[5',4':3,4]pyrido[2,1-a]iso- quinolin-2-amine (20b). White solid; yield: 42.6%; ^{1}H NMR (400 MHz, DMSO) δ 6.75 (s, 1H), 6.65 (s, 1H), 6.62 (s, 2H), 4.97 (s, 1H), 3.72 (s, 3H), 3.69 (s, 3H), 3.20 – 3.07 (m, 2H), 2.93 – 2.68 (m, 4H), 2.67 – 2.52 (m, 2H); MS(ESI)m/z: 318.1(M+H)$^+$.

2.19. 2-Amino-10-methoxy-5,7,8,12b-tetrahydro-4H-thiazolo[5',4':3,4]pyrido[2,1-a] isoquinolin-11-ol (21a). White solid; yield: 47.9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (s, 1H), 6.58 (s, 1H), 5.35 (s, 1H), 5.12 (s, 1H), 4.72 (s, 2H), 3.86 (s, 3H), 3.42 – 3.23 (m, 2H), 3.17 – 2.92 (m, 2H), 2.91 – 2.74 (m, 2H), 2.74 – 2.57 (m, 1H), 2.57 – 2.37 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.82, 145.74, 143.93, 141.96, 129.23, 125.30, 120.77, 112.22, 110.92, 57.02, 55.84, 50.47, 43.42, 28.57, 22.33; MS(ESI)m/z: 304.1(M+H)$^+$.

2.20. 10,11-Dimethoxy-5,7,8,12b-tetrahydro-4H-thiazolo[5',4':3,4]pyrido[2,1-a]iso- quinolin-2-amine (21b). White solid; yield: 38.4%; ^{1}H NMR (400 MHz, D_2O) δ 6.73 (s, 1H), 6.70 (s, 1H), 5.63 (s, 1H), 3.75 – 3.63 (m, 2H), 3.57 – 3.41 (m, 2H), 3.12 – 2.80 (m, 4H); MS(ESI)m/z: 290.1(M+H)$^+$.

2.21. Synthesis of 10,11-Dimethoxy-5,7,8,12b-tetrahydro-4H-thiazolo[5',4':3,4] pyrido[2,1-a]isoquinoline (23)

To a solution of **19b** (100mg, 0.25mmol) in DMF (10mL) at 60 °C was added *t*-Butyl nitrite (52mg, 0.5mmol), the mixture was stirred at 60 °C for 1h and concentrated *in vacuo*. The residue was dissolved in methanol and cooled in an ice bath. $NaBH_4$ was added and the reaction mixture was stirred at this temperature for 10min. The mixture was concentrated and purified by flash chromatography (DCM: MeOH=50:1) to give **23**. Yield: 34.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 6.84 (s, 1H), 6.60 (s, 1H), 5.29 (s, 1H), 3.93 (s, 3H), 3.86 (s, 3H), 3.51 – 3.38 (m, 2H), 3.19 – 2.75 (m, 5H), 2.66 (d, *J* = 15.0 Hz, 1H); MS(ESI)m/z: 303.1(M+H)$^+$.

3. Binding assay

The affinity of compounds to the D_1, D_2 and D_3 dopamine receptors were determined by competition binding assay. Membrane homogenates of D1-, D2-, and D3-HEK293 cells were prepared as described previously. Duplicated tubes were incubated at 30 °C for 50 min with increasing concentrations (1 nM–100 μM) of respective compound and with 0.7 nM [^{3}H]SCH23390 (for D_1 receptor), or [^{3}H]Spiperone (for D_2 and D_3 receptors) in a final volume of 200 μL binding buffer containing 50 mM Tris, 4 mM $MgCl_2$, pH 7.4. Nonspecific binding was determined by parallel incubations with either 10 μM SCH23390 for D_1 or Spiperone for D_2, D_3 dopamine receptors, respectively. The reaction was started by addition of membranes (15 μg/tube) and stopped by rapid filtration through Whatman GF/B glass fiber filter and subsequent washing with cold buffer (50 mM Tris, 5 mM ethylenediaminetetraacetic acid (EDTA, pH 7.4) using a Brandel 24-well cell harvester. Scintillation cocktail was added and the radioactivity was determined in a MicroBeta liquid scintillation counter. The IC_{50} and K_i values were calculated by nonlinear

regression (PRISM, Graphpad, San Diego, CA) using a sigmoidal function.

4. Molecular modeling

4.1. D_1 receptor homology modeling

The D_1 receptor model was built based on homology modeling with the newly resolved β_2-adrenergic receptor with an agonistic conformation (PBD code: 3P0G) [21] 42.4%. Sequence alignment of β_2-adrenergic receptor and D_1 receptor was generated by ClustalW. The sequence identity was 42.4% in the TM helix region between the two receptors. The Modeller 9v2 program was employed to assemble the 3D model of D1 receptor by using the X-ray crystal structure of β_2-adrenergic receptor as a template. Stepwise energy minimizations of the D_1 homology model was carried out for 500 steps with Kollman All-Atom force field using the SYBYL 6.9 program. The final model was verified by a professional structure validation program Procheck 3.5.4. The Procheck statistics showed that 94.9% of the residues of the D_1 model were either in the most favored or in the additionally allowed regions of the Ramachandran map, suggesting that the overall main chain and side chain structures are all reasonable.

4.2. Molecular Docking

Docking of compounds **10a**, **10b**, and **10c** (constructed and energy minimized in vacuum using SYBYL 6.9 with the MMFF94s force field) in the D1 receptor binding site was performed using the GOLD Suite v5.0.1. The binding pocket was defined as amino acids within 15 Å of the Cγ carbon atom of D3.32. A distance constraint was used to preserve the known salt bridge between D3.32 and the protonated nitrogen of ligand. Ten conformations were produced for each ligand. Other parameters were set as default. 30 docking orientations were obtained. The best one was chosen based on the GOLD score, the reported binding mode between *l*-SPD and D_1 receptor, and the mutation experiments.

Results and Discussion

Design of analogs and structure-activity relationships

Using hybrid drug development approach, we combined SPD (Figure 1，2) and Pramipexole (Figure 1，3) to design a series of novel aminothiazole-containing SPD derivatives aiming to develop novel molecules as D_1 and D_3 agonists with better bioavailability. Specifically, we keep rings A, B and C of SPD (**2**) and replace D-ring by drug-like 2-aminothiazole ring lent from Pramipexole **3** in the light of bioisosterism, thus a series of SPD analogues bearing different aminothiazole substituents (Figure 2) were prepared. To obtain potent thiazole-SPD derivatives, the substituents -OH and -OCH_3 at positions C2 and C3, the substituents -H, -NH2 and -N(CH3)2 at position C10 were designed and synthesized, and the effect of these substituents on activity were examined. Meanwhile, several 5,7,8,12b-tetrahydro-4H-thiazolo[5',4':3,4]pyrido[2,1-a]isoquinolin-2-amines were synthesized and assayed for verifying the effect of position of hybrid pattern on activity , and further studying the structure and activity relationship (SAR) and interaction mode of new designed compounds.

The inhibition and K_i values of synthesized compounds against the D_1, D_2 and D_3 receptors are summarized in Table 1. From Table 1 we can see that when the two substituents at C2 and C3 are the same, they are –OH (compounds **14a** and **14b**) or –OCH3 (compounds **9f** and **13**), the compounds show no activities. However, when the substituent at C2 is –OH and the substituent at C3 is –OCH_3, all compounds exhibit good activity toward dopamine D_1 receptor (**10a**, **10b** and **10c**). Also, the contribution of substituent at C10 position to the activity of D_1 receptor is: 10-dimethylamino group > 10-amino> 10-H. Further study shows that the substituent at C10 has great impact on the selectivity of dopamine D3 receptor: the binding inhibitions are 54.79%, 74.06% and 93.47% (Ki = 430.23 nM) when the substituents at C10 are –H, -NH_2 and –$N(CH_3)_2$, respectively. In comparison to the lead compound SPD, none of them show activity toward dopamine D_2 receptor. These results were useful for developing SPD-derivatives in the treatment of Parkinson disease (PD), since our original purpose is to eliminate the D_2 antagonistic activity which limits the application of SPD to treat PD. Therefore, this study provides a viable method to block D_2 antagonistic action of SPD through replacement of the phenolic D-ring of SPD by 2-aminothiazole functionality. We have reported that there was an electrostatic attraction between the protonated side chain of H6.55 in D_2 receptor and the electron-rich group of D-ring and oxygen of hydroxyl group of SPD [22]. Therefore, we presumed that absence of -OH at D-ring of our compounds resulted in the loss of D_2 binding. It also demonstrates that the D-ring of SPD is very essential for its antagonistic activity against D_2 receptor.

In addition, **12a–12d** were designed as the prodrugs of designed compounds by introducing four kinds of acyl groups to improve the bioavailability. **12b** with substituent –CH_3CH_2CO was found to display higher binding inhibition of 84.76% toward D_1 receptor. It provides a clue for aminothiazole-SPD prodrug study.

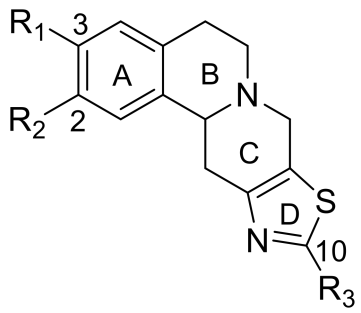
To thoroughly study the effect of aminothiazole ring on dopamine activity, the aminothiazole ring were fused on the C1'-C2' bond to examine the effect of position of aminothiazole ring on activity. The results show that all of these C1'-C2' fused aminothiazole-SPD derivatives do not show activity toward three dopamine receptor subtypes. From this study we can conclude that ring B, C and D should be in a line to keep dopaminergic activities.

Molecular modeling

To elucidate the structure characteristic of active aminothiazole-SPD derivetives (**10a-10c**), the active molecules **10a**, **10b**, and **10c** were docked into the binding-pocket of D_1 receptor (Figure 3).

The docking results revealed that **10a**, **10b**, and **10c** shared common interaction modes with D_1 receptor: (i) Electrostatic interaction between the protonated nitrogen (N1) and carboxyl groups of conserved D3.32; (ii) Stable hydrogen bonds between hydroxy group of ring A and S5.42, methoxyl group of ring A

Table 1: Binding affinities of synthesized compounds against the D_1, D_2 and D_3 receptors (binding affinities of compounds **20b**, **21a**, **21b** and **23** are listed in **Table S1**)



Cmp.	R1	R2	R3	Inhibition% or K_i (±SEM, nM)		
				D_1	D_2	D_3
				[^{3}H]SCH23390	[^{3}H]Spiperone	[^{3}H]Spiperone
9f	OCH3	OCH3	H	-2.14%	4.36%	1.65%
10a	OCH3	OH	NH2	354 ±7	35.94%	74.06%
10b	OCH3	OH	H	85.17%	31.18%	59.97%
10c	OCH3	OH	N(CH3)2	201 ±26	54.38%	430±62
12a	OCH3	PhCOO	NHFmoc	–	–	–
12b	OCH3	CH3CH2COO	NH2	84.76%	21.78%	46.70%
12c	OCH3	PhCOO	NH2	38.40%	10.80%	10.06%
12d	OCH3	CH3SO2O	NH2	-6.40%	-3.62%	21.83%
13	OCH3	OCH3	NH2	-15.51%	-1.65%	7.09%
14a	OH	OH	H	45.75%	-9.80%	54.79%
14b	OH	OH	NH2	58.39%	10.33%	65.03%
l-SPD				13[b]	85[c]	15.2±3.2[d]
pramipexole				ND	3	0.5
SCH23390				1.69±0.12	ND	ND
Spiperone				ND	1.08±0.09	0.48±0.07

[a]Dashed lines indicate that compound can't dissolve, so the binding assay was not determined.

[b]ND denotes that the activity was not determined.

and S5.46; (iii) Hydrophobic interaction between the A, B-rings and F6.52, W6.48; (iv) A hydrogen bond between the nitrogen (N2) of thiazole ring and S188 in EL-2. The first three interactions have been reported in dopaminergic ligands [22,23] and mutation experiments have demonstrated the important roles of above residues[24,25]. The contribution of S188 to binding has also been recently reported by Malo *et al.*[26]. It indicates that a DHX aza-analogue formed a hydrogen bond with S188 in EL-2 in the D_1 receptor, which may be a reason for D_1/D_2 agonist selectivity. The subtle difference among three ligands **10a-10c** existed in that the amino group of **10a** acts as a hydrogen bond donor forming an extra hydrogen bond with S188. However, it can be speculated that this kind of interaction didn't have a significant impact on the binding strength between **10a-10c** and D_1 receptor; this is in agreement with our pharmacological results, **10a-10c** display similar binding affinities. S188 is an important residue for the binding of **10a-10c** with D1 receptor. Above interaction modes indicate that the protonated nitrogen, 3-methoxyl and 2-hydroxyl groups at A-ring are critical for keeping D_1 activities of the described molecules derived from the chimera of SPD and pramipexole. The aminothiazole ring should be jointed in a line with B, C rings for obtaining activity by hydrogen-bonding interaction with S188, and substituent at C10 has an effect on the D_1 activities as a result of its electron-donating ability. Further modifications will be carried out through exchanging the nitrogen and sulfur atoms of D-ring for verifying the contribution of hydrogen-bonding interaction with S188 to activity, and introducing other electron-donating and withdrawing groups at C10 for exploring its effect on the hydrogen-bonding interaction and activity.

Conclusion

In this paper we presented the design and syntheses of a set of 6,8,12,12a -tetrahydro-5H-thiazolo[4',5':4,5]pyrido[2,1-a]isoquinolines which are aminothiazole bioisosteres of the natural product stepholidine. Several 5,7,8,12b-tetrahydro-4H- thiazolo[5',4':3,4]pyrido[2,1-a]isoquinolin-2-amines were also synthesized for exploring the jointing position of thiazole ring (D-ring) to the piperidine ring (C-ring). Competition binding assays revealed four compounds (**10a**, **10b**, **10c** and **12b**) with binding inhibitions above 80% for D_1 receptor (**10a** with a K_i value of 354 nM and **10c** with 201 nM), and one compound (**10c**) with a binding inhibition of 93.47% and a K_i value of 430 nM for D_3 receptor. **10a**, **10b** and **12b** turned out to be selective D_1 inhibitors, and **10c** had remarkably improved binding selectivity for the D_3 over D_2 receptor which has always been considered as a big challenge in developing selective SPD-like D_3 inhibitors. All the active

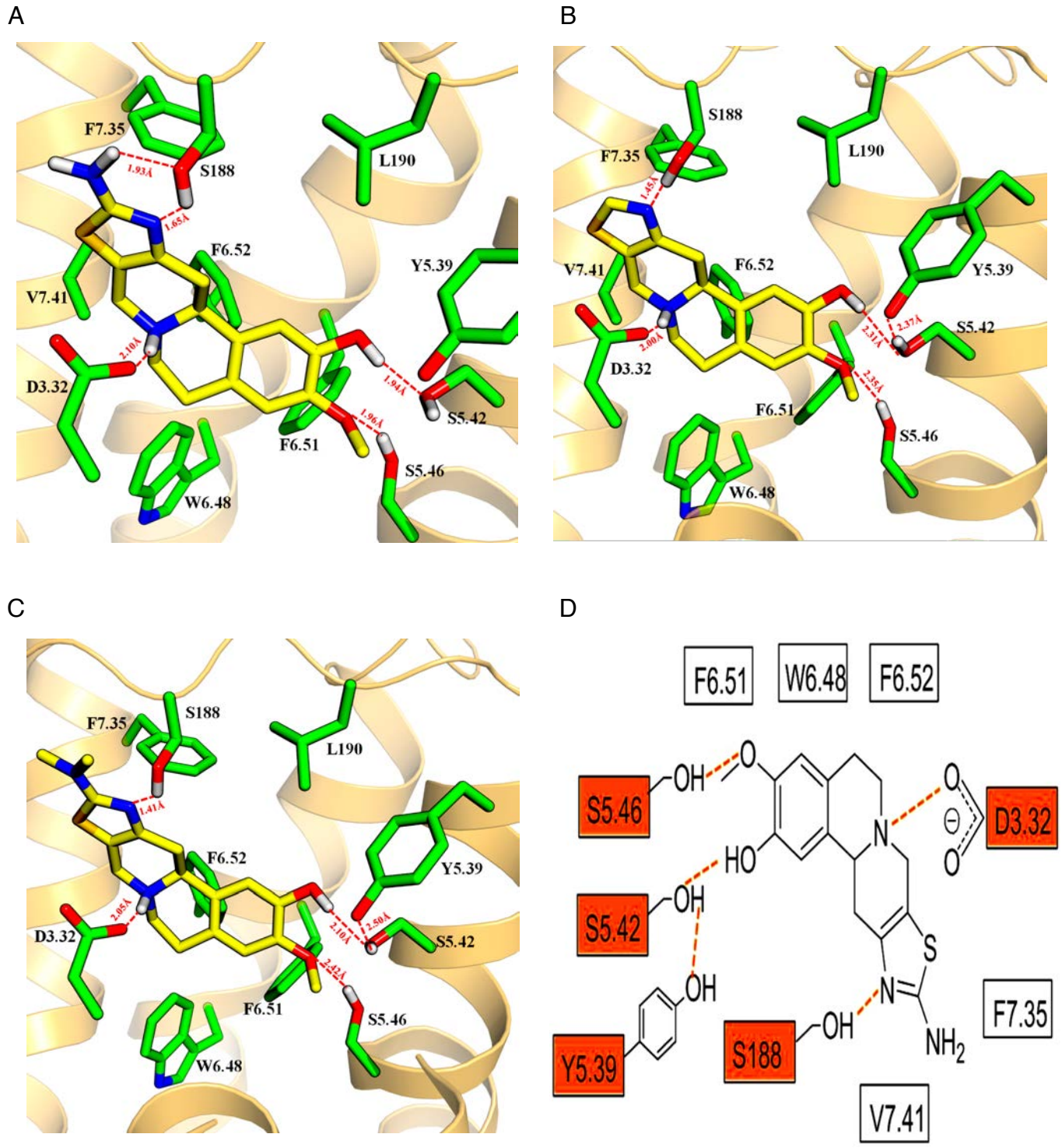


Figure 3. 3D views of the interaction modes of **10a (A)**, **10b (B)** and **10c (C)** with D_1 receptor, respectively. Residues within 4 Å of ligands are shown as sticks. In all panels, red and blue represent oxygen and nitrogen respectively. Hydrogen bonds are shown in red dashed lines. **(D)** Schematic representation of interactions between **10a** and D_1 receptor. The residues shown here are within 4 Å of the ligands in the D_1 receptor. Orange residues and lines indicate polar interactions.

compounds need to bear 3-methoxyl and 2-hydroxyl groups at ring A, which were explained to form hydrogen-bonding interactions with S5.42 and S5.46 of the D_1 receptor through docking analysis. The thiazole ring should be jointed in a line with rings B and C to obtain a proper conformation for maintaining hydrogen-bonding interaction between the nitrogen atom within the thiazole ring and S188 located in the second extracellular loop of D_1 receptor. The N-substituents at C10 have little effect on the activity against D_1 receptor.

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References

1. Payne SL, Johansson AM, Strange PG: **Mechanisms of ligand binding and efficacy at the human D2(short) dopamine receptor**. *J Neurochem* 2002, **82**:1106-1117.
2. Lovenberg TW, Brewster WK, Mottola DM, Lee RC, Riggs RM, Nichols DE, Lewis MH, Mailman RB: **Dihydrexidine, a novel selective high potency full dopamine D-1 receptor agonist**. *Eur J Pharmacol* 1989, **166**:111-113.
3. Jin GZ, Zhu ZT, Fu Y: **(-)-Stepholidine: a potential novel antipsychotic drug with dual D1 receptor agonist and D2 receptor antagonist actions**. *Trends Pharmacol Sci* 2002, **23**:4-7.
4. Sun Y, Dai J, Hu Z, Du F, Niu W, Wang F, Liu F, Jin G, Li C: **Oral bioavailability and brain penetration of (-)-stepholidine, a tetrahydroprotoberberine agonist at dopamine D(1) and antagonist at D(2) receptors, in rats**. *Br J Pharmacol* 2009, **158**:1302-1312.
5. Newman-Tancredi A, Cussac D, Quentric Y, Touzard M, Verriele L, Carpentier N, Millan MJ: **Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes**. *J Pharmacol Exp Ther* 2002, **303**:815-822.
6. van Vliet LA, Rodenhuis N, Wikstrom H, Pugsley TA, Serpa KA, Meltzer LT, Heffner TG, Wise LD, Lajiness ME, Huff RM, et al.: **Thiazoloindans and thiazolobenzopyrans: a novel class of orally active central dopamine (partial) agonists**. *J Med Chem* 2000, **43**:3549-3557.
7. Batra S, Sabnis YA, Rosenthal PJ, Avery MA: **Structure-based approach to falcipain-2 inhibitors: synthesis and biological evaluation of 1,6,7-trisubstituted dihydroisoquinolines and isoquinolines**. *Bioorg Med Chem* 2003, **11**:2293-2299.
8. Lerchner A, Machauer R, Betschart C, Veenstra S, Rueeger H, McCarthy C, Tintelnot-Blomley M, Jaton AL, Rabe S, Desrayaud S, et al.: **Macrocyclic BACE-1 inhibitors acutely reduce Abeta in brain after po application**. *Bioorg Med Chem Lett* 2010, **20**:603-607.
9. Kim KS, Kimball SD, Misra RN, Rawlins DB, Hunt JT, Xiao HY, Lu S, Qian L, Han WC, Shan W, et al.: **Discovery of aminothiazole inhibitors of cyclin-dependent kinase 2: synthesis, X-ray crystallographic analysis, and biological activities**. *J Med Chem* 2002, **45**:3905-3927.
10. Waser J, Gaspar B, Nambu H, Carreira EM: **Hydrazines and azides via the metal-catalyzed hydrohydrazination and hydroazidation of olefins**. *J Am Chem Soc* 2006, **128**:11693-11712.
11. Chiang HC, Brochmann-Hanssen E: **Total synthesis of (+/-)-discretamine and (+/-)-stepholidine**. *J Org Chem* 1977, **42**:3190-3194.
12. Cheng JJ, Yang YS: **Enantioselective total synthesis of (-)-(S)-stepholidine**. *J Org Chem* 2009, **74**:9225-9228.
13. Ueda H, Satoh H, Matsumoto K, Sugimoto K, Fukuyama T, Tokuyama H: **Total synthesis of (+)-haplophytine**. *Angew Chem Int Ed Engl* 2009, **48**:7600-7603.
14. Jung ME, Lowe JA: **Synthetic approaches to adriamycin involving Diels-Alder reactions of photochemically generated bisketenes. Total synthesis of islandicin and digitopurpone**. *J Org Chem* 1977, **42**:2371-2373.
15. Brossi A, Teitel S: **Selective O-demethylation of papaverine**. *J Org Chem* 1970, **35**:1684-1687.
16. Pettit GR, Hoffmann H, Herald DL, McNulty J, Murphy A, Higgs KC, Hamel E, Lewin NE, Pearce LV, Blumberg PM, et al.: **Antineoplastic agents 491. Synthetic conversion of aaptamine to isoaaptamine, 9-demethylaaptamine, and 4-methylaaptamine**. *J Org Chem* 2004, **69**:2251-2256.
17. Pelletier JC, Cava MP: **SYNTHESIS OF THE MARINE ALKALOIDS AAPTAMINE AND DEMETHYLOXYAAPTAMINE AND OF THE PARENT STRUCTURE DIDEMETHOXYAAPTAMINE**. *Journal of Organic Chemistry* 1987, **52**:616-622.
18. Gano KW: **New substituted 3-isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-pyrido(2,1-a) isoquinolin-2-ol compounds are vesicular monoamine transporter-2 inhibitors useful to treat hyperkinetic disorder e.g. Huntington's disease**. US Patent, WO2008058261-A1.
19. Hagemeyer HJ: **PREPARATION OF ANHYDROUS METHYL VINYL KETONE AND METHYL ISOPROPENYL KETONE**. *Journal of the American Chemical Society* 1949, **71**:1119-1120.
20. Willand N, Folleas B, Boutillon C, Verbraeken L, Gesquiere J-C, Tartar A, Deprez B: **Efficient, two-step synthesis of N-substituted nortropinone derivatives**. *Tetrahedron Letters* 2007, **48**:5007-5011.
21. Rasmussen SG, Choi HJ, Fung JJ, Pardon E, Casarosa P, Chae PS, Devree BT, Rosenbaum DM, Thian FS, Kobilka TS, et al.: **Structure of a nanobody-stabilized active state of the beta(2) adrenoceptor**. *Nature* 2011, **469**:175-180.
22. Fu W, Shen J, Luo X, Zhu W, Cheng J, Yu K, Briggs JM, Jin G, Chen K, Jiang H: **Dopamine D1 receptor agonist and D2 receptor antagonist effects of the natural product (-)-stepholidine: molecular modeling and**

dynamics simulations. *Biophys J* 2007, **93**:1431-1441.

23. Bonner LA, Laban U, Chemel BR, Juncosa JI, Lill MA, Watts VJ, Nichols DE: **Mapping the catechol binding site in dopamine D receptors: synthesis and evaluation of two parallel series of bicyclic dopamine analogues**. *ChemMedChem* 2011, **6**:1024-1040.
24. Hibert MF, Trumpp-Kallmeyer S, Bruinvels A, Hoflack J: **Three-dimensional models of neurotransmitter G-binding protein-coupled receptors**. *Mol Pharmacol* 1991, **40**:8-15.
25. Pollock NJ, Manelli AM, Hutchins CW, Steffey ME, MacKenzie RG, Frail DE: **Serine mutations in transmembrane V of the dopamine D1 receptor affect ligand interactions and receptor activation**. *J Biol Chem* 1992, **267**:17780-17786.
26. Malo M, Brive L, Luthman K, Svensson P: **Investigation of D receptor-agonist interactions and D/D agonist selectivity using a combination of pharmacophore and receptor homology modeling**. *ChemMedChem* 2012, **7**:483-494, 338.

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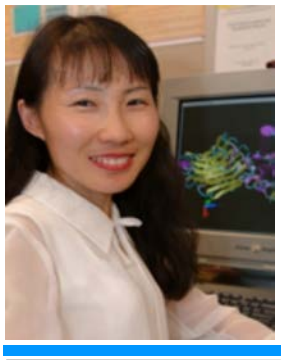

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