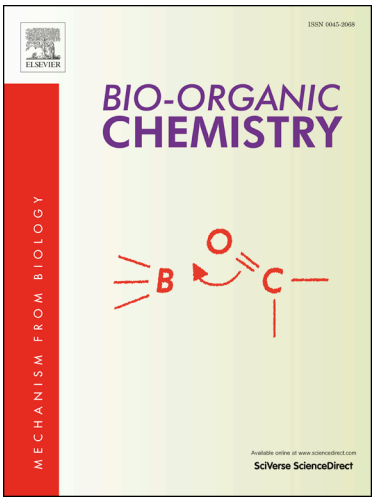

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N-Substituted noscapine derivatives as new antiprotozoal agents: synthesis, antiparasitic activity and molecular docking study

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Abstract

Novel *N*-substituted noscapine derivatives were synthesized by a three-component Strecker reaction of cyclic ether of *N*-nornoscapine with varied aldehydes, in the presence of cyanide ion. Moreover, the corresponding amides were synthesized by the oxidation of cyanide moieties in good yields. The *in vitro* antiprotozoal activity of the products was also investigated. Interestingly, some analogues did put on display promising antiparasitic activity against *Trypanosoma brucei rhodesiense* with IC_{50} values between 2.5-10.0 μM and selectivity index (SI) ranged from 0.8 to 13.2. Eight compounds exhibited activity against *Plasmodium falciparum* K1 strain with IC_{50} ranging 1.7-6.4 μM, and SI values between 2.8 to 10.5 against L6 rat myoblast cell lines. Molecular docking was carried out on trypanothione reductase (TbTR, PDB ID: 2WOW) and UDP-galactose 4' epimerase (TbUDPGE PDB: 1GY8) as targets for studying the envisaged mechanism of action. Compounds **$6j_2$** and **$6b_2$** displayed excellent docking scores with -8.59 and -8.86 kcal/mol for TbTR and TbUDPGE, respectively.

Keywords: Noscapine; Strecker reaction; *Trypanosoma brucei*; *Plasmodium falciparum*; Molecular docking; Induced fit docking.

1. Introduction

Currently, millions of people worldwide suffer from protozoal diseases such as human African trypanosomiasis, malaria, Chagas disease, and leishmaniasis caused by single-celled parasites. These illnesses occur in developing regions of Asia, Africa and Latin America [1]. Human African Trypanosomiasis (HAT) or sleeping sickness is caused by one of the two subspecies of *Trypanosoma brucei* (*Trypanosoma brucei gambiense* and *T. brucei rhodesiense*) transmitted via tsetse fly [2,3]. *T. b. rhodesiense* infection is found in East Africa and ignites an acute infection whereas *T. b. gambiense* is endemic in the West and central Africa and present a chronic type of infection. HTA is deadly if not treated in a timely manner [4,5]. Malaria is the first responsible for the deaths caused by parasitic diseases by which over 219 million infections and 435000 deaths were reported in 2017 [6,7]. *Plasmodium falciparum* is the most widespread one among six *Plasmodium* species (infecting the man) and nearly amongst all malaria-related deaths reasons [7,8]. Leishmaniasis is the second widespread parasitic disease, that is caused by *Leishmania* species [10,11]. WHO (World Health Organization) reports appraised that there are 12 million cases infected with *Leishmania* species worldwide, and over 2 million people get the infection each year [12,13]. WHO report in 2014 revealed that 72% of Iran's population live in the endemic areas of *Leishmania* [14]. *Trypanosoma cruzi* is responsible for Chagas diseases or American trypanosomiasis, which is found in tropical and subtropical countries in Latin America [15,16]. Due to the lack of vaccination against parasitic infections, low efficacy, significant side effects, alongside with the high costs of current treatments, plus emerging resistance to them, development of new chemotherapeutic agents are indispensable [17,18]. Natural products and their semisynthetic derivatives serve an essential role in discovering and expansion of new antiprotozoal drugs namely artemisinin and artesunate [19–21]

Noscapine **1** is a phthalideisoquinoline alkaloid possessing the second highest abundance in opium which has been isolated from *Papaver somniferum*. It is an oral nontoxic cough suppressant prescribed since 1960 [22]. In the last decade, noscapine has attracted the attention of many research groups for its anticancer capabilities[23–25].

Opium was in use as an antimalarial drug for many years in the late 19th century [26]. In the last decades, there have been several reports regarding the investigation of alkaloids as antiprotozoal agents. [18,27,28]. As an instance, quinine and its derivatives as a class of quinoline alkaloids have an deciding role in the development of new antimalarial medicines [29]. Having considered such

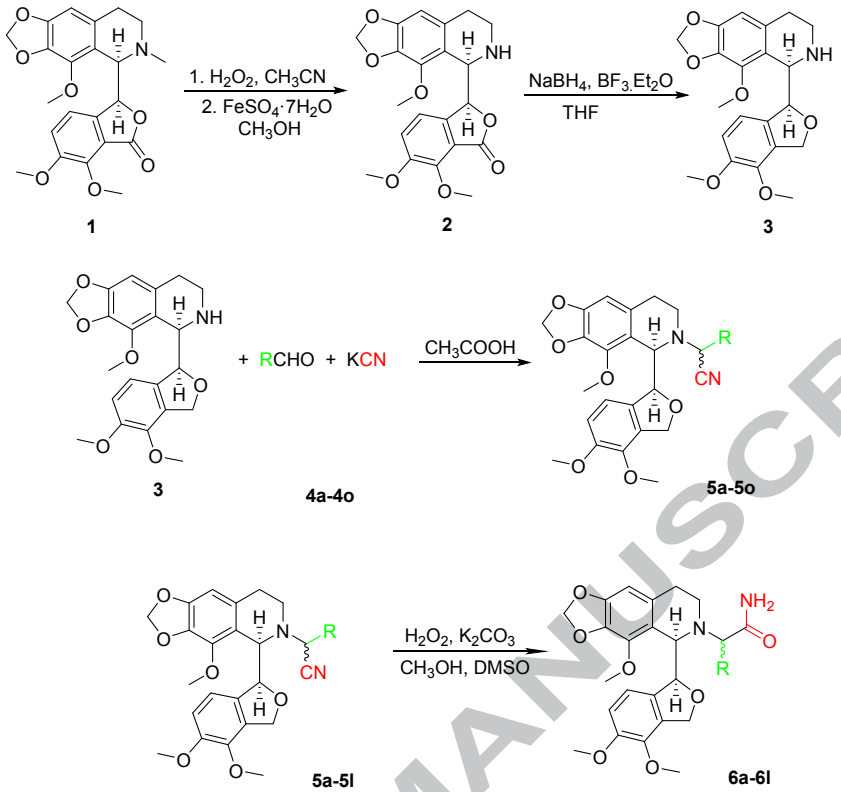
reports, we decide to use noscapine (an isoquinoline alkaloid) as the starting material for the synthesis of new antiparasitic agents.

In the present paper, we report the synthesis of two series of novel *N*-substituted cyclic ether derivatives of *N*-nornoscapine and their antiprotozoal activities against *T. b. rhodesiense*, *T. cruzi*, *L. donovani,* and *P. falciparum*. Furthermore, the cytotoxicity of the products was investigated against L6 cells (rat skeletal myoblasts) to determine their selectivity index. The probable mechanism of action against *T. b. rhodesiense* was studied by molecular docking of the most active compounds in the binding site of two proteins, trypanothione reductase (TbTR) and uridine diphosphate galactose 4′ epimerase (TbUDPGE) by the Glide module (Schrodinger suite).

2. Results and discussion

2.1. Chemistry

To furnish the desired *N*-substituted cyclic ether *N*-nornoscapine analogues, at first commercially available noscapine **1** was converted to *N*-nornoscapine **2** by *N*-demethylation, and then the lactone ring of compound **2** was reduced to the corresponding cyclic ether **3 (**Scheme 1) [30,31]. Secondly, the α-aminonitrile derivatives were prepared employing the three-component Strecker reaction in the presence of **3** as the amine source, different aldehydes **4a-4o** (Fig. 1) and potassium cyanide [32]. A series of aldehydes including aliphatic (**4m** and **4o**), aromatic with electron withdrawing (**4e**, **4d**, and **4f**) plus electron donating groups (**4c**, **4g**, **4i**, **4j**, **4n**, and **4l**), as well as a heterocyclic model compound (**4h**) were used.



Scheme 1. Synthesis of *N*-substituted *N*-nornoscapine derivatives with reduced lactone ring.

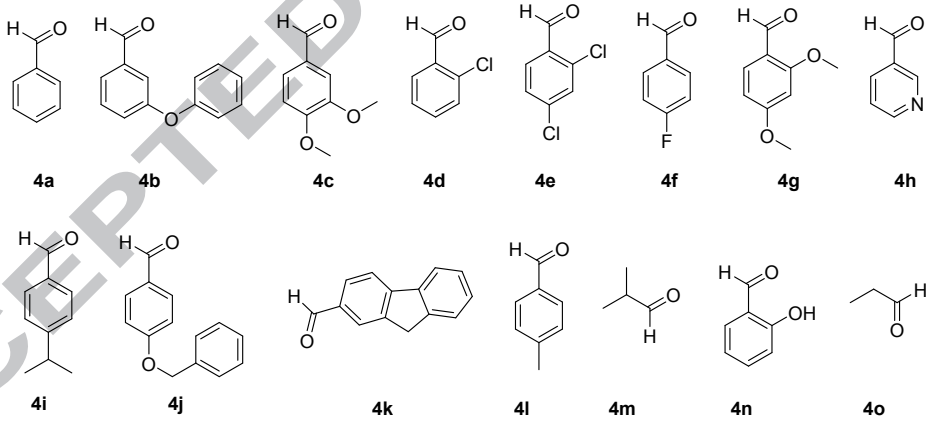


Fig. 1. Series of aromatic and aliphatic aldehydes used in the Strecker reaction.

All of the synthesized compounds **5a-5o** were obtained as a mixture of two diastereomers whose ratio was analyzed by ^{1}H-NMR. Some derivatives were purified by crystallization, from which a single diastereomer was obtained (Table 1). As can be seen, the majority of reactions progressed smoothly with high diastereoselectivity, except for two *ortho*-chloro substituted model compounds.

Throughout the next step, the synthesis of amide derivatives **6a-6l** was considered in the presence of various reagents (KOH, NaOH [33], K_2CO_3 [34], $TiCl_4$ [35], HCl [36], and H_2SO_4 [37]) along with different solvents (MeOH, DMF, DMSO, THF, EtOH, and hexane). The best results were obtained when the α-aminonitrile derivatives **5a-5l** were treated with hydrogen peroxide and potassium carbonate [34]. The two diastereomers of derivatives were separated and purified taking advantage of preparative TLC (Table 1).

Structures of all synthetic compounds were elucidated by the use of ^{1}H-NMR, ^{13}C-NMR, FTIR, and HRMS.

Table 1

Synthesis of compounds **5** and **6**.

Aldehyde	Compound 5			dr[a]	Compound 6		
	Product	Time (h)	Yield%		Product	Time (h)	Yield%
4a	**5a$_1$**	1.0	71	-[b]	**6a**	5.0	65
4b	**5b**	0.5	69	88:12	**6b**	12.0	75
4c	**5c**	5.0	79	84:16	**6c**	24.0	77
4d	**5d**	1.0	50	66:34	**6d**	24.0	55
4e	**5e**	1.0	95	61:39	**6e**	8.0	90
4f	**5f**	1.0	80	81:19	**6f**	24.0	66
4g	**5g**	6.0	65	91:9	**6g**	12.0	58
4h	**5h**	0.5	85	83:17	**6h**	8.0	65
4i	**5i$_1$**	0.5	75	-[b]	**6i$_1$**	48.0	44
4j	**5j**	8.0	75	84:16	**6j**	8.0	65
4k	**5k**	24.0	65	84:16	**6k$_1$**	2.0	54
4l	**5l**	8.0	60	86:14	**6l$_1$**	24.0	50
4m	**5m**	2.0	75	80:20			
4n	**5n$_1$**	2.0	71	-[b]			
4o	**5o**	2.0	65	85:15			

[a]dr: Diastereomeric ratio (calculated by ^{1}H-NMR), [b]Major diastereomer precipitated spontaneously.

The absolute configuration of chiral center generated in the Strecker reaction of compound **5f$_1$** was assigned by single-crystal X-ray diffraction**.** The single crystal of **5f$_1$** was prepared by dissolving it in chloroform followed by slow evaporation of the solvent at room temperature. The ORTEP view (Fig. 2) showed that the configuration of the chiral center at C-1″ was *R*. Thus, **5f$_1$** as the major

diastereomer possesses 3*S*,5'*R*,1″*R* configuration (The X-ray analysis details are reported in supplementary data).

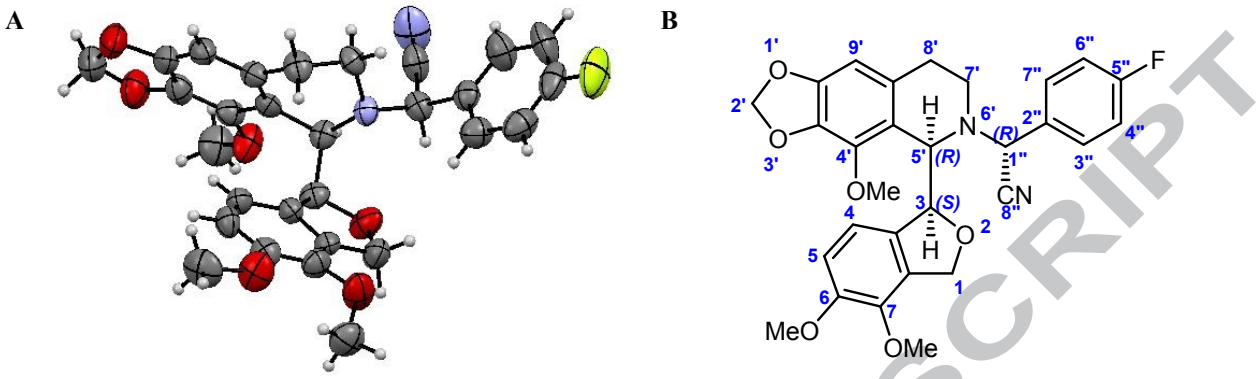


Fig. 2. A) ORTEP view and B) 2D structure of compound **5f$_1$**.

2.2. Antiparasitic activity

All synthesized compounds went through an investigation for growth inhibition against four parasites; namely, *Trypanosoma brucei rhodesiense* strain STIB900, *T. cruzi* strain Tulahuen C4, *Leishmania donovani axenic amastigotes* strain MHOM-ET-67/L82 and *Plasmodium falciparum* strain NF54 at two concentrations of 2 and 10 µg/mL. Melarsoprol, benznidazole, miltefosine, and artemisinin were used as positive controls respectively for *T. b. rhodesiense*, *T. cruzi*, *L. donovani*, and *P. falciparum* (The results are reported in Table S2 in supplementary data). Twenty-eight derivatives exhibited a high percentage of growth inhibition (higher than 50%) for *T. b. rhodesiense* protozoa. Only **6g$_2$** showed activity against *T. cruzi* with 60% growth inhibition at 2 µg/mL. The most potent synthetic compound against *L. donovani* was **6b$_1$** with 100% growth inhibition at 2 µg/mL.

The *N*-α-aminonitrile derivatives **5a$_1$** and **5m** and the amide derivatives **6b$_1$**, **6b$_2$**,**6d$_2$**, **6e$_1$**, **6e$_2$**, **6g$_2$**, **6i$_1$**, and **6k$_1$** presented higher percentage of growth inhibition than other analogues for *P. falciparum*. It should be mentioned that *N*-nornoscapine **2** displayed poor antiprotozoal activities. Derivatives with the growth inhibition higher than 50% for two parasites *T. b. rhodesiense* and *P. falciparum* at 2 µg/mL were opted for determination of the IC_{50} values. Also, the cytotoxic activity of these analogues was evaluated against the rat myoblast L6 cells. Selectivity indices (SIs) were determined employing the IC_{50} values of cytotoxicity and antiprotozoal activity. The results are presented in Table 2. Fortunately, most of the examined products showed weak cytotoxicity.

Table 2

In vitro antiprotozoal and cytotoxic activities and selectivity index of selected derivatives.

Compound	*T. b. rhodesiense*		*P. falciparum*		*L6 cells*
	IC_{50} [a](μM)	SI[b]	IC_{50} (μM)	SI	IC_{50} (μM)
5a$_1$	-	-	14.9 ± 0.0	0.8	11.2 ± 5.2
5b$_1$	17.6 ± 7.1	5.2	-	-	91.8 ± 8.7
5c	8.9 ± 1.8	2.3	-	-	20.7 ±7.9
5d	9.8 ± 1.0	1.7	-	-	16.8 ± 2.2
5e	23.6 ± 0.1	1.2	-	-	29.5 ± 2.6
5f$_1$	10.2 ± 0.9	6.1	-	-	62.3 ± 29.7
5f$_2$	27.7 ± 5.7	1.8	-	-	50.1 ± 11.4
5g	8.8 ± 1.1	1.6	-	-	13.9 ± 1.8
5h	10.6 ± 1.3	1.9	-	-	20.2 ± 6.3
5i$_1$	29.6 ± 2.0	2.3	-	-	70.6 ± 5.2
5j	13.5 ± 4.6	3.0	-	-	40.5 ± 9.3
5k	13.0 ± 3.4	7.2	-	-	93.7 ± 3.3
5l	18.5 ± 8.5	1.7	-	-	31.6 ± 7.7
5l$_1$	11.6 ± 0.6	2.1	-	-	24.2 ± 11.3
5m	-	-	19.0 ± 0.4	1.4	25.6 ± 10.2
5n$_1$	2.5 ± 1.2	12.9	-	-	32.2 ± 2.5
5o	12.6 ± 0.6	2.0	-	-	25.5 ± 3.5
6a$_2$	33.1 ± 5.9	0.3	-	-	10.7 ± 0.3
6b$_1$	15.3 ± 6.6	1.1	4.1 ± 0.8	4.2	17.2 ± 4.8
6b$_2$	19.7 ± 5.4	1.3	3.5 ± 0.0	7.1	24.9 ± 2.6
6c$_1$	6.5 ± 2.6	13.2	-	-	85.5 ± 2.8
6c$_2$	29.7 ± 6.4	0.6	-	-	18.5 ± 6.7
6d$_2$	-	-	4.7 ± 1.1	4.3	20.4 ± 7.8
6e$_1$	-	-	6.4 ± 1.8	2.8	17.9 ± 9.1
6e$_2$	-	-	2.4 ± 0.5	5.5	13.4 ± 1.9
6f$_1$	26.5 ± 3.1	1.5	-	-	39.0 ± 2.2
6f$_2$	32.6 ± 4.1	0.5	-	-	17.1 ± 4.9
6g$_1$	25.6 ± 5.2	3.0	-	-	76.3 ± 6.1
6g$_2$	10.0 ± 0.6	0.8	1.7 ± 0.2	4.6	7.8 ± 3.2
6h$_2$	53.4 ± 14.2	0.2	-	-	11.5 ± 0.5
6i$_1$	-	-	5.9 ± 0.6	6.3	37.4 ± 1.7
6j$_1$	9.2 ± 1.1	3.2	-	-	29.3 ± 8.3
6k$_1$	45.2 ± 19.3	0.8	3.5 ± 0.1	10.5	36.9 ± 2.0
6l$_1$	42.9 ± 16.1	0.7	-	-	31.2 ± 3.4
Positive control	0.0075[c]	-	0.012[d]	-	0.012[e]

[a]IC_{50}: 50% inhibitory concentration, [b]Selectivity Index (IC_{50} value of cytotoxicity/ IC_{50} value of antiprotozoal activity), [c]Melarsoprol, [d]Chloroquine, [e]Podophyllotoxin used as positive controls.

Among twenty-eight tested compounds against *T. b. rhodesiense*, seven compounds showed good activities with IC_{50} values in the range of 2.5-10.0 μM with the selectivity index in the range of 0.8-13.2. The most active compounds were **5n$_1$** (IC_{50} = 2.5 μM, SI = 12.9) and **6c$_1$** (IC_{50} = 6.5 μM, SI = 13.2) with nitrile and amide function, respectively. Derivatives with hydroxyl, dimethoxy, and benzyloxy moieties on the phenyl ring (**5n$_1$**, **5c, 5g, 6c$_1$**, **6g$_2$,** and **6j$_1$**) were the most potent analogues. Moreover, the monohalogenated α-aminonitrile derivatives (**5d**, **5f$_1$**) displayed better activities

compared to other compounds. In general, the nitrile selectivity than the amide bearing compounds.

All derivatives were tested against *P. falciparum*. From which the amide derivatives **6b$_1$**, **6b$_2$**, **6d$_1$**, **6e$_1$**, **6e$_2$**, **6g$_2$**, **6i$_1$**, and **6k$_1$**, displayed high activity with IC_{50} values between 1.7 – 6.4 μM and selectivity index in the range of 2.8 to 10.5. Compound **6g$_2$** showed the lowest IC_{50} value equal to 1.7 μM and SI= 4.6. The highest selectivity index (SI=10.5) was observed for compound **6k$_1$** (IC_{50} = 3.5 μM). In general amide derivatives were more active than their α-aminonitrile parents for *P. falciparum*.

2.3. Docking studies

For more investigation on the mode of interaction between the synthetic compounds and *T. b. rhodesiense* and the possible mechanism of action, molecular docking studies of all derivatives was performed in the flavin adenine dinucleotide (FAD) binding site of trypanothione reductase (TbTRR) (PDB ID: 2WOW) [38] and nicotinamide adenine dinucleotide (NAD) binding site of UDP-galactose 4′ epimerase (TbUDPGE) (PDB:1GY8) using Glide application in Maestro 10.2 platform (Schrödinger, LLC)[39]. The TbTR has a vital and unique role in maintaining redox balance in these parasites and is an attractive target for drug discovery studies [38]. The *in silico* investigation of antitrypanosomal natural compounds by Setzer et al. has shown that TbUDPGE is an appropriate target for investigation of the mechanism of action in indole alkaloids [39].

The docking scores of the compounds with the best IC_{50} values are listed in Table 3 (details were reported in Table S3 in supplementary data). As shown in results, all derivatives illustrated great affinity toward the binding site of the two target proteins chiefly TbUDPGE by high docking scores (-5.29 to -8.86 kcal/mol). Compound **6j$_2$** showed the most significant docking score (-8.59 kcal/mol) for the ligand-TbTR protein complex (Fig. 3A). This ligand showed two hydrogen bonding interactions between MET333 and THR335 and the ligand amide side chain. Also, the interaction between 4-(benzyloxy) phenyl moiety with hydrophobic pocket include ILE438, ALA 363, PRO 435, PHE367, and ALA 364, was revealed.

Then induced fit docking (IFD) was used to study the insight of the interaction between the ligands **5j$_2$**, **6j$_2$**, and **6k$_2$** and protein in flexible mode (Fig. 3B). The IFD scores -8.01, -9.64, and -8.00 kcal/mol were obtained for these ligands, respectively. The hydrogen bond interactions in ARG287,

CYS52, and THR335 were observed, and Pi-Pi staking in PHE198 was revealed in the **6j$_2$**-TbTR binding site complex. The phenyl ring was involved in the Pi-cation interaction with LYS60. The ligand **6j$_2$** indicated hydrophobic interactions with CYS57, PHE367, ALA365, ALA363, VAL337, LEU334, PRO336, and CYS52 whose residues are present in the FAD binding site. Furthermore, a polar pocket containing GLY56, SER178, ASN179, THR51, and ARG287 interacted with dioxolo[4,5-g]isoquinoline moiety. All the mentioned interactions, especially the hydrophobic ones, were likely to be responsible for the IFD score improving (-9.64 kcal/mol).

Table 3

Docking scores (kcal/mol) of the synthesized and reference compounds at FAD and NAD cavity for TbTR and TbUDPGE, respectively.

Compound[a]	Docking score (kcal/mol)		
	TbTR		TbUDPGE
5a$_1$	-5.94		-7.06
5a$_2$	-5.91		-6.59
5b$_2$	-6.71		-8.24
5c$_1$	-6.54		-6.91
5c$_2$	-5.91		-6.94
5d$_1$	-5.61		-7.68
5d$_2$	-6.41		-6.8
5f$_2$	-6.08		-6.75
5g$_1$	-6.06		-6.96
5g$_2$	-5.25		-7.15
5h$_1$	-7.15		-6.81
5h$_2$	-6.72		-6.8
5j$_1$	-6.37		-8.03
5j$_2$	-7.66		-6.69
5k$_2$	-7.18		-8.74
5l$_2$	-6.18		-6.91
5n$_1$	-7.02		-6.81
5n$_2$	-6.65		-6.91
6b$_1$	-6.02		-8.08
6b$_2$	-7.18		-8.86
6c$_2$	-7.52		-7.6
6g$_2$	-7.1		-6.79
6j$_1$	-7.44		-7.82
6j$_2$	-8.59		-8.65
6k$_2$	-7.94		-5.29
FAD	-13.82	NAD	-17.34

[a]Diastereomers of derivatives were docked separately.

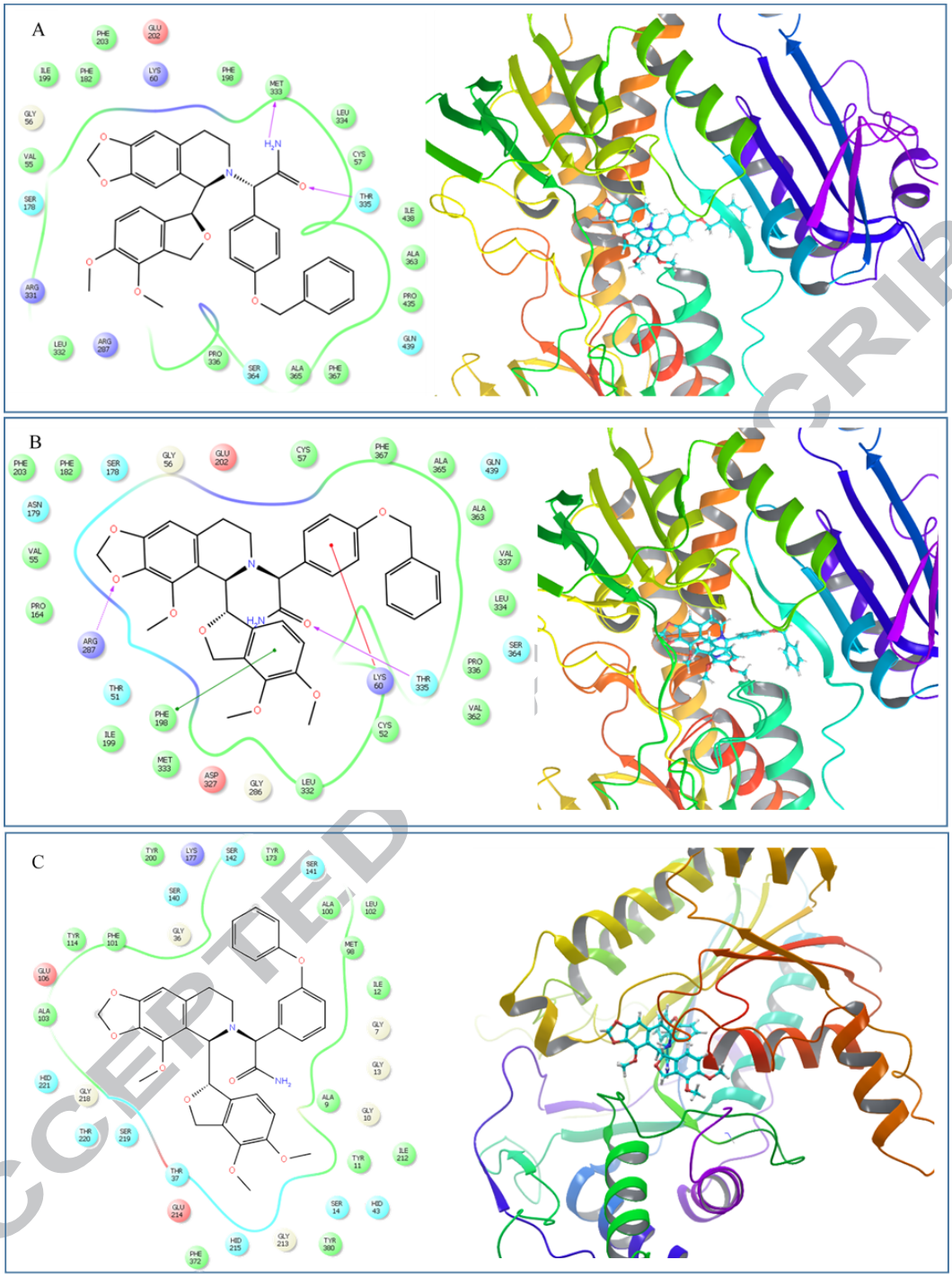


Fig. 3. The 2D and 3D docking of A) the complex of **6j$_2$** with the binding site of TbTR; B) IFD of the complex **6j$_2$** with the binding site of TbTR; C) the complex **6b$_2$** with the binding site of TbUDPGE.

The docking studies of the synthesized compounds in the FAD-binding site of TbTR exhibited that in most cases the conversion of nitrile group to amide (i.e., **5b** vs. **6b**, **5c** vs. **6c$_1$**, and **5j** vs. **6j**) and addition of hydroxyl group (**5n$_1$**) or heteroatom in aromatic ring (**5h**) caused additional hydrogen

bonding and greater docking score. Insertion of groups with a bulky side chain at C-7' position (**5b$_2$**, **5j$_2$**, **5k$_2$**, **6b$_2$**, **6j$_2$**, and **6k$_2$**) afforded more hydrophobic interactions with the binding site. Comparison between two diastereomers of the synthesized derivatives docking scores did not show a notable variation. The docking scores of the ligands-TbTR complex were in good agreement with IC_{50} values.

Compound **6b$_2$** showed the highest docking score (-8.86 kcal/mol) with the TbUDPGE enzyme (Fig 3C). This ligand revealed no hydrogen bond, Pi-Pi or Pi-cation interaction with the protein residues. The primary interaction was among the 3-phenoxyphenyl group of the ligand **6b$_2$** and hydrophobic residues containing TYR173, ALA100, LEU102, MET98, ILE12, and ALA9. Interestingly these residues also play an essential role in the binding of NAD as the native ligand by hydrophobic interaction. Moreover, polar interactions of the benzofuran group with HID221, THR220, SER219, THR37, HID215, HID43, and Ser14 and hydrophobic interactions of isoquinoline moiety with PHE101, TYR114, and AlA103 were all revealed in the ligand-protein complex.

Molecular docking of the ligands with NAD binding site in TbUDPGE demonstrated that bulky groups in the C-7' position of the ligands (**5b$_2$**, **5j$_2$**, **5k$_2$**, **6b$_1$**, **6b$_2$**, and **6k$_2$**) and their orientation in the binding site had a vital role in the formation of hydrophobic interactions.

Finally, for the evaluation of drug-likeness and molecular properties of all the active compounds, the consistency with Lipinski's rules of five was investigated through ADMET prediction by Qikprop 4.4. This rule recommends some criteria for molecular weight (MW<500), H acceptor bonds (HA<10), H donor bonds (HD<5), and octanol/water partition coefficient (QPlogPo/w<5). The orally active compounds mostly adhere to three of the four Lipinski's rules. The results proved that the synthesized derivatives were compatible with the Lipinski's rules (Table 4).

Table 4

Calculated theoretical parameters of Lipinski's rules for the active compounds.

Compound	mol_MW	donorHB	accptHB	QPlogPo/w	Rule Of Five(<4)
$5a_1$	500.55	0	8.95	3.331	1
$5b_1$	592.647	0	9.45	4.372	1
5c	560.602	0	10.45	3.013	1
5d	534.995	0	8.95	3.786	1
$5f_1$	518.54	0	8.95	3.497	1
$5f_2$	518.54	0	8.95	3.118	1
5g	560.602	0	10.45	3.099	1
5h	501.538	0	10.45	2.501	1
$5i_1$	542.63	0	8.95	4.56	1
5j	606.674	0	9.7	4.95	1
5l	514.577	0	8.95	3.374	1
5m	466.533	0	8.95	2.621	0
$5n_1$	516.549	1	9.7	2.805	1
5o	452.506	0	8.95	2.507	0
$6a_1$	518.565	2	9.95	2.539	1
$6a_2$	610.662	2	10.45	3.493	1
$6b_1$	610.662	2	10.45	4.099	1
$6b_2$	610.662	2	10.45	3.493	1
$6d_2$	553.01	2	9.95	2.938	1
$6e_1$	587.455	2	9.95	3.283	1
$6e_2$	587.455	2	9.95	3.103	1
$6f_1$	536.556	2	9.95	2.579	1
$6f_2$	536.556	2	9.95	2.715	1
$6h_2$	519.553	2	11.45	1.649	1
$6i_1$	560.646	2	9.95	2.995	1
$6j_1$	624.689	2	10.7	3.613	1
$6k_1$	606.674	2	9.95	3.57	1
$6l_1$	532.592	2	9.95	2.941	1

3. Conclusion

In this paper, the synthesis procedure of two novel classes of noscapine derivatives was described. The antiparasitic activities of new noscapinoids were evaluated and for the first time antipasitic activity from noscapinoids were reported. Most derivatives displayed promising activity against *T. b. rhodesiense* and *P. falciparum*. Compound **$5n_1$** showed high potency and selectivity for *T. b. rhodesiense* with IC_{50}= 2.5µM and SI=13. Compound **$6g_2$** was identified as the most active against *P. falciparum* with IC_{50}= 1.7µM but had toxicity. Molecular docking studies for the most active compounds against *T. b. rhodesiense* were performed on two proteins TbTR and TbUDPGE, and the results were in good agreement with the experimental data showing good affinity of the synthesized compounds to the binding site of the target receptors. As we assumed, the noscapine derivatives

showed plausible antiparasitic activity but we need more manipulation for obtaining better selectivities. Therefore, these novel semi-synthetic analogues could be explored as the new lead compounds for protozoal diseases, and deserve more investigation for *in vivo* studies.

4. Experimental

4.1. General

Noscapine was purchased from Faran Shimi Pharmaceutical Co. Miltefosine, artemisinin, chloroquine, and podophyllotoxin were purchased from Sigma, and benznidazole and melarsoprol were received from WHO (with purity >95% according to the suppliers). Other chemicals and solvents were provided from Merck, Sigma-Aldrich, and Kimia Exir chemical companies without further purification. Pre-coated silica gel F_{254} sheets for TLC from Merck Co. were used for reaction monitoring. Products were purified by preparative TLC plates coated with silica gel 60 PF_{254} containing gypsum. Melting points were measured using a Branstead/Electrothermal 9200 apparatus and were uncorrected. ^{1}HNMR and ^{13}CNMR spectra were recorded in $CDCl_3$ and DMSO-d_6 as solvents on Bruker Avance III spectrometers at 600, 500 and 300 MHz and TMS was employed as internal standard. Chemical shifts were reported by ppm. HRESIMS data were acquired on a Bruker micro TOF ESIMS in positive mode. FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer.

*4.1.2. N-Nornoscapine; (3S)-6,7-Dimethoxy-3-((5R)-4-methoxy-5,6,7,8 tetrahydro[1,3]dioxolo[4,5-g] isoquinolin-5-yl)-2-benzofuran-1(3H)-one (**2**)*

N-Nornoscapine was synthesized by the literature procedure with some modification [30]. 10.0g noscapine (24.0 mmol) was added to a flask containing 200 mL H_2O_2 30% and 200 mL CH_3CN. The reaction mixture was stirred overnight at room temperature. When the reaction completed and noscapine-*N*-oxide formed, the mixture was cooled to 0 °C and then solid MnO_2 was slowly added to remove excess amount of H_2O_2. The mixture was filtered and concentrated in vacuo. The reaction mixture was acidified to pH 1with 1M HCl and extracted with $CHCl_3$ (3×300). The organic layer was removed under the reduced pressure and the noscapine-*N*-oxide·as hydrochloride salt was isolated. 13.34 g of $FeSO_4.7H_2O$ (48.0 mmol) was added to a solution of noscapine-*N*-oxide·HCl in 750 mL CH_3OH at -8 °C and stirred. Having passed 8 hours, the TLC showed that the noscapine-*N*-oxide was consumed to the full, thereafter the solvent was evaporated under reduced pressure. The residue was

dissolved in $CHCl_3$ and then washed with a saturated solution of EDTA in 1M HCl. The organic layer was washed with 1M NaOH and dried over Na_2SO_4. The crude product was recrystalized from dichloromethane and hexane to give pure *N*-nornoscapine.

*4.1.3. (5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinoline (**3**)*

Reduction of *N*-nornoscapine lactone moiety was performed according to previous reports [23,22]. 1.0 g *N*-nornoscapine (2.5 mmol) was dissolved in 12 mL $BF_3.Et_2O$ and then added dropwise to a solution of 0.19 g $NaBH_4$ (5.0 mmol) in 20 mL dry THF at -5°C. After 1h, the reaction mixture was warmed to room temperature and stirred overnight. To quench the reaction, 10 mL of HCl (10%) was added dropwise in an ice bath. After stirring for 1h, the reaction was extracted with $CHCl_3$ (3×50) and the organic layer was separated and dried over anhydrous Na_2SO_4. Concentration under reduced pressure gave the free base of **3** which was purified by preparative TLC.

4.1.4. General Procedure for the Synthesis of N-α-Aminonitrile Derivatives

To a solution of compound **3** (1.0 mmol, 385.4 mg) in 5 mL acetic acid was added aldehyde (**4**) (1.1 mmol). After stirring for 30 min at room temperature, 2.0 mmol KCN was added to the reaction mixture. After complete consumption of compound **3** approved by TLC, water was added to the reaction mixture and extracted with $CHCl_3$ (3×30 mL). The organic layer was separated and dried with Na_2SO_4 and solvent was evaporated in vacuo. The crude product was purified by preparative TLC or recrystallization by methanol and chloroform.

*4.1.4.1. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(phenyl)acetonitrile (**5a**)*

Aldehyde substrate: Benzaldehyde (**4a**), reaction time: 1h. The major diastereomer was crystalized from methanol (**5a$_1$**: major diastereomer). Yield: 71%; white crystal; m.p. 158 °C; IR (KBr): 1042, 1275, 1488, 1620, 2845, 2949 cm^{-1}; ^1H NMR (300 MHz, chloroform-*d*) δ 7.29 – 7.43 (m, 5H), 6.70 (d, *J* = 8.2 Hz, 1H), 6.36 (s, 1H), 6.02 (s, 1H), 5.98 (s, 2H), 5.88 (d, *J* = 8.2 Hz, 1H), 5.63 (s, 1H), 5.20 (dd, *J* = 12.5, 2.9 Hz, 1H), 5.10 (d, *J* = 12.5 Hz, 1H), 4.89 (d, *J* = 2.9 Hz, 1H), 4.12 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 2.56 (dt, *J* = 10.0, 3.7 Hz, 1H), 2.47 (td, *J* = 10.0, 3.7 Hz, 1H), 2.38 – 2.21 (m, 2H) ppm; ^{13}C NMR (75 MHz, chloroform-*d*) δ 151.2, 148.3, 142.6, 140.5, 134.8, 134.5, 133.0, 132.8,

132.4, 128.7, 128.4, 126.8, 117.7, 117.6, 117.5, 112.0, 102.2, 100.9, 88.1, 71.6, 63.1, 59.9, 59.5, 56.1, 43.6, 29.9 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{29}N_2O_6$ calcd 501.1981, found 501.2043.

*4.1.4.2. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(3-phenoxyphenyl) acetonitrile (**5b**)*

Aldehyde substrate: 3-Phenoxybenzaldehyde (**4b**), reaction time: 0.5 h. The mixture of two diastereomers (**5b**) (88:12) were purified by preparative TLC, yield: 69%.

5b: Off white powder; m.p. 139 °C; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.44 – 6.86 (m, 18H, mixture of two diastereomers), 6.67 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 6.64 (d, *J* = 8.2 Hz, 1H, major diastereomer), 6.36 (s, 1H, major diastereomer), 6.32 (s, 1H, minor diastereomer), 6.05 – 6.01 (m, 2H, minor diastereomer), 6.00 (s, 1H, major diastereomer), 5.97 (s, 2H, major diastereomer), 5.91 (brs, 2H, minor diastereomer), 5.85 (d, *J* = 8.2 Hz, 1H, major diastereomer), 5.60 (s, 1H, major diastereomer), 5.41 (s, 1H, minor diastereomer), 5.33 (m, 4H, minor diastereomer), 5.18 (dd, *J* = 12.4, 2.9 Hz, 1H, major diastereomer), 5.08 (d, *J* = 12.4 Hz, 1H, major diastereomer), 4.87 (d, *J* = 3.0 Hz, 1H, major diastereomer), 4.74 (d, *J* = 3.8 Hz, 1H, minor diastereomer), 4.11 (s, 3H, major diastereomer), 3.88 (s, 3H, minor diastereomer), 3.87 (s, 3H, minor diastereomer), 3.84 (s, 3H, minor diastereomer), 3.83 (s, 3H, major diastereomer), 3.81 (s, 3H, major diastereomer), 2.64 – 2.19 (m, 8H, mixture of two diastereomers); HRMS (ESI): $[M+H]^+$ $C_{35}H_{33}N_2O_7$ calcd 593.6560, found 593.2278.

5b$_1$:Off white powder; m.p. 137 °C; IR (KBr): 1033, 1083, 1270, 1488, 1620, 2925 cm^{-1}; ^{1}H NMR (600 MHz, chloroform-*d*) δ 7.36-7.33 (m, 2H), 7.27 (t, *J* = 7.9 Hz, 1H), 7.15 (d, *J*= 7.9Hz, 1H), 7.13 (t, *J* = 7.4 Hz, 1H), 7.09 (s, 1H), 6.97 (d, *J* = 8.0 Hz, 2H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.64 (d, *J* = 8.2 Hz, 1H), 6.36 (s, 1H), 5.99 (s, 1H), 5.98 (s, 2H), 5.85 (d, *J* = 8.2 Hz, 1H), 5.61 (br s, 1H), 5.17 (dd, *J* = 12.4, 2.6 Hz, 1H), 5.07 (d, *J* = 12.3 Hz, 1H), 4.86 (d, *J* = 2.7 Hz, 1H), 4.11 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 2.61 (dt, *J* = 10.0, 3.4 Hz, 1H), 2.50 (td, *J* = 10.4, 2.5 Hz, 1H), 2.32 (d, *J* = 15.2 Hz, 1H), 2.31-2.28 (m. 1H) ppm; ^{13}C NMR (151 MHz, chloroform-*d*) δ 157.6, 156.7, 151.2, 148.3, 142.7, 140.5, 136.9, 134.5, 132.8, 132.7, 132.3, 129.9, 129.7, 123.4, 121.6, 118.7, 118.4, 117.7, 117.7, 117.4, 117.2, 112.1, 102.1, 100.8, 88.0, 71.6, 62.7, 60.6, 59.9, 59.5, 56.1, 43.7, 29.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{35}H_{33}N_2O_7$ calcd 593.6560, found 593.2278.

*4.1.4.3. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(3,4-dimethoxyphenyl)acetonitrile (**5c**)*

Aldehyde substrate: 3, 4-Dimethoxybenzaldehyde (**4c**), reaction time: 5h. The mixture of two diastereomers (84:16) were purified by preparative TLC. Yield: 79 %, white powder; m.p. 83 °C; IR (KBr): 1030, 1082, 1269, 1619, 2932 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.07 (d, *J* = 8.4 Hz, 1H, minor diastereomer), 7.00 (d, *J* = 7.8 Hz, 1H, major diastereomer), 6.90 – 6.76 (m, 4H, mixture of two diastereomers), 6.69 (d, *J* = 8.2 Hz, 2H, mixture of two diastereomers), 6.36 (s, 1H, major diastereomer), 6.32 (s, 1H, minor diastereomer), 6.09 (d, *J* = 6.6 Hz, 1H, minor diastereomer), 5.98 (s, 4H, mixture of two diastereomers), 5.95 (s, 2H, mixture of two diastereomers), 5.91 (d, *J* = 7.9 Hz, 1H, major diastereomer), 5.64 (s, 1H, major diastereomer), 5.35 (s, 1H, minor diastereomer), 5.25 (dd, *J* = 12.6, 2.9 Hz, 2H, mixture of two diastereomers), 5.12 (d, *J* = 12.5 Hz, 2H, mixture of two diastereomers), 4.87 (d, *J* = 2.8 Hz, 1H, major diastereomer), 4.77 (d, *J* = 3.5 Hz, 1H, minor diastereomer), 4.12 (s, 3H, major diastereomer), 4.02 (s, 3H, minor diastereomer), 4.00 (s, 3H, minor diastereomer), 3.98 (s, 3H, minor diastereomer), 3.90 (s, 6H, minor diastereomer), 3.88 (s, 3H, major diastereomer), 3.85 (s, 3H, major diastereomer), 3.83 (s, 3H, major diastereomer), 3.73 (s, 3H, major diastereomer), 2.70 – 2.27 (m, 8H, mixture of two diastereomer) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.1, 148.3, 142.6, 140.6, 133.0, 133.0, 132.4, 127.1, 119.2, 117.5, 117.5, 112.2, 112.1, 110.8, 110.7, 109.6, 109.5, 102.1, 100.8, 88.2, 88.1, 71.3, 62.5, 60.5, 59.8, 59.5, 56.1, 55.9, 55.6, 43.4, 30.0, 28.0 ppm; HRMS (ESI): $[M+H]^+$ $C_{31}H_{33}N_2O_8$ calcd 561.6110, found 561.2258.

*4.1.4.4. (2-Chlorophenyl)((5R)-5-((1S)-4,5-dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)acetonitrile (**5d**)*

Aldehyde substrate: 2-Chlorobenzaldehyde (**4d**), reaction time: 1h. The mixture of two diastereomers (66:34) were purified by preparative TLC. Yield: 50 %, white powder; m.p. 92 °C; IR (KBr): 1037, 1269,1483, 1621, 2930 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.73 – 7.64 (m, 1H, major diastereomer), 7.52 – 7.29 (m, 6H, mixture of two diastereomer), 7.26 – 7.14 (m, 1H, major diastereomer), 6.68 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 6.63 (d, *J* = 8.3 Hz, 1H, major diastereomer), 6.39 (s, 1H, minor diastereomer), 6.36 (s, 1H, major diastereomer), 6.11 (d, *J* = 8.1 Hz, 1H, major diastereomer), 6.01 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 5.95 (s, 2H, minor diastereomer), 5.92 (s, 2H, major diastereomer), 5.80 (s, 1H, minor diastereomer), 5.55 (s, 1H, minor diastereomer), 5.47 (s, 1H, major diastereomer), 5.39 (dd, *J* = 12.4, 2.9 Hz, 1H, minor diastereomer), 5.27 (s, 1H, major diastereomer), 5.15 (dd, *J* = 12.4, 2.8 Hz, 1H, major diastereomer), 4.95 (d, *J* = 12.0 Hz, 1H, major diastereomer), 4.92 (d, *J* = 12.0 Hz, 1H, minor diastereomer), 4.76 – 4.64 (m, 2H,

mixture of two diastereomers), 3.99 (s, 3H, minor diastereomer), 3.91 (s, 3H, major diastereomer), 3.84 (s, 3H, minor diastereomer), 3.83 (s, 6H, major diastereomer), 3.67 (s, 3H, minor diastereomer), 3.02 – 2.28 (m, 8H, mixture of two diastereomers) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.1, 148.3, 148.2, 142.6, 140.6, 134.4, 134.3, 133.5, 133.0, 132.7, 132.5, 132.3, 131.6, 130.7, 130.2, 130.2, 129.8, 129.8, 129.3, 126.9, 126.9, 118.0, 117.5, 117.3, 117.3, 116.6, 111.9, 111.7, 103.0, 102.5, 102.3, 100.7, 100.6, 87.3, 86.3, 71.7, 71.4, 71.3, 61.0, 59.9, 59.7, 59.3, 59.2, 58.6, 57.6, 57.5, 56.2, 45.1, 44.4, 28.5, 26.9 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{28}ClN_2O_6$ calcd 535.1636, found 535.2175.

*4.1.4.5. (2,4-Dichlorophenyl)((5R)-5-((1S)-4,5-dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)acetonitrile (**5e**)*

Aldehyde substrate: 2,4-Dichlorobenzaldehyde (**4e**), reaction time: 1h. The mixture of two diastereomers (61:39) were purified by preparative TLC. Yield: 95 %, white powder; m.p. 144 °C; IR (KBr): 1037, 1083, 1269,1483, 1621, 2930 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.59 (d, *J* = 8.3 Hz, 1H, minor diastereomer), 7.46 (dd, *J* = 6.9, 1.9 Hz, 1H, major diastereomer), 7.33 (dd, *J* = 8.3, 2.1 Hz, 1H, minor diastereomer), 7.26 – 7.17 (m, 3H, mixture of two diastereomer), 6.68 (d, *J* = 8.2 Hz, 1H, major diastereomer), 6.62 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 6.39 (s, 1H, major diastereomer), 6.36 (s, 1H, minor diastereomer), 6.09 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 5.99 (d, *J* = 8.0 Hz, 1H, major diastereomer), 5.97 (d, *J* = 1.5 Hz, 1H, major diastereomer), 5.95 (d, *J* = 1.4 Hz, 1H, major diastereomer), 5.94 (d, *J* = 1.4 Hz, 1H, minor diastereomer), 5.93 (d, *J* = 1.5 Hz, 1H, minor diastereomer), 5.74 (s, 1H, major diastereomer), 5.55 (s, 1H, major diastereomer), 5.40 (s, 1H, minor diastereomer), 5.30 (s, 1H, minor diastereomer), 5.19 (dd, *J* = 12.1, 2.7 Hz, 1H, minor diastereomer), 5.02 – 4.88 (m, 2H, mixture of two diastereomers), 4.71 (dd, *J* = 8.1, 2.9 Hz, 1H, major diastereomer), 4.65 (s, 1H, major diastereomer), 4.64 (s, 1H, minor diastereomer), 4.01 (s, 3H, major diastereomer), 3.95 (s, 3H, minor diastereomer), 3.85 (s, 3H, major diastereomr), 3.84 (s, 6H, minor diastereomer), 3.70 (s, 3H, major diastereomer), 2.98 – 2.36 (m, 8H, mixture of two diastereomers) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.1, 148.4, 148.3, 140.5, 135.5, 135.1, 135.1, 134.3, 134.1, 133.2, 132.8, 132.7, 132.5, 131.3, 131.2, 131.0, 130.5, 130.4, 130.2, 130.1, 130.0, 127.3, 127.2, 117.6, 117.4, 117.3, 116.2, 112.1, 111.7, 102.5, 102.3, 100.8, 100.7, 87.3, 86.0, 71.4, 71.3, 60.94, 59.9, 59.7, 59.3, 59.3, 58.2, 57.4, 57.3, 56.2, 56.2, 45.2, 44.5, 28.4, 26.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{27}Cl_2N_2O_6$ calcd 569.1246, found 569.1238.

*4.1.4.6. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(4-fluorophenyl)acetonitrile (**5f**)*

Aldehyde substrate: 4-Fluorobenzaldehyde (**4f**), reaction time: 1h. The two diastereomers (81:19) were separated and purified by preparative TLC.

5f$_1$: Yield: 65%, white powder; m.p. 166 °C; IR (KBr): 1042, 1086, 1275, 1489, 1620, 2898,3056 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.43 – 7.29 (m, 2H), 7.12 – 6.81 (m, 2H), 6.70 (d, *J* = 8.2 Hz, 1H), 6.36 (s, 1H), 5.98 (s, 2H), 5.96 (s, 1H), 5.88 (d, *J* = 8.2 Hz, 1H), 5.63 (s, 1H), 5.15 (dd, *J* = 12.6, 2.8 Hz, 1H), 5.09 (d, *J* = 12.0 Hz, 1H), 4.86 (d, *J* = 2.9 Hz, 1H), 4.12 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 2.56 – 2.41 (m, 2H), 2.39 – 2.22 (m, 2H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 163.6, 161.6, 151.3, 148.4, 140.5, 134.6, 133.0, 132.8, 132.3, 130.5, 128.6, 128.5, 117.5, 117.3, 115.7, 115.5, 112.2, 102.1, 100.8, 88.1, 71.5, 62.3, 60.6, 59.9, 59.5, 56.2, 43.5, 29.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{28}FN_2O_6$ calcd 519.1931, found 519.1944

5f$_2$: Yield: 15%, white powder; m.p. 127 °C; IR (KBr): 1042, 1086, 1275, 1489, 1620, 2898,3056 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.43 – 7.29 (m, 4H), 6.69 (d, *J* = 8.1 Hz, 1H), 6.35 (s, 1H), 6.00 (s, 1H), 5.96 (s, 2H), 5.87 (d, *J* = 8.2 Hz, 1H), 5.62 (s, 1H), 5.18 (dd, *J* = 12.6, 3.2 Hz, 1H), 5.08 (d, *J* = 12.6 Hz, 1H), 4.88 (d, *J* = 3.2 Hz, 1H), 4.11 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 2.60 – 2.41 (m, 2H), 2.38 – 2.23 (m, 2H).ppm; ^{13}C NMR (126 MHz, chloroform-*d*) 163.5, 161.5, 151.3, 148.4, 140.5, 134.6, 133.0, 132.8, 132.3, 130.5, 128.6, 128.6, 117.6, 117.3, 115.7, 115.6, 112.2, 102.2, 100.8, 88.1, 71.5, 62.3, 60.7, 60.0, 59. 6, 56.2, 43.5, 29.9 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{28}FN_2O_6$ calcd 519.1931, found 519.2072.

*4.1.4.7. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(2,4-dimethoxyphenyl)acetonitrile (**5g**)*

Aldehyde substrate: 2, 4-Dimethoxybenzaldehyde (**4g**), reaction time: 6h. The mixture of two diastereomers (91:9) were purified by preparative TLC. Yield: 65 %, yellow oil; IR (KBr): 1030, 1082, 1269, 1619, 2932 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.08 – 6.95 (m, 4H, mixture of two diastereomers), 6.86 (d, *J* = 8.2 Hz, 2H, mixture of two diastereomers), 6.78 (s, 1H, minor diastereomer), 6.77 (s, 1H, major diastereomer), 6.68 (d, *J* = 8.3 Hz, 1H, major diastereomer), 6.34 (s, 1H, major diastereomer), 6.30 (s, 1H, minor diastereomer), 6.07 (d, *J* = 8.1 Hz, 1H, minor diastereomer), 5.95 (s, 2H, major diastereomer), 5.89 (s, 2H, minor diastereomer), 5.87 (s, 1H, minor diastereomer), 5.62 (s, 1H, major diastereomer), 5.47 (s, 1H, major diastereomer), 5.30 (s, 1H, minor diastereomer), 5.22 (dd, *J* = 12.5, 2.8 Hz, 2H, mixture of two diastereomers), 5.09 (d, *J* = 12.5 Hz,

1H, major diastereomer), 5.04 (d, *J* = 12.2 Hz, 1H, minor diastereomer), 4.84 (d, *J* = 2.8 Hz, 1H, major diastereomer), 4.74 (d, *J* = 4.0 Hz, 1H, minor diastereomer), 4.10 (s, 6H, mixture of two diastereomers), 3.84 (s, 6H, mixture of two diastereomers), 3.83 (s, 6H, mixture of two diastereomers), 3.81 (s, 6H, mixture of two diastereomers), 3.71 (s, 6H, mixture of two diastereomers), 2.58 – 2.25 (m, 8H, mixture of two diastereomers) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.2, 148.2, 142.5, 140.3, 134.4, 133.4, 132.4, 131.8, 130.7, 129.0, 117.6, 117.4, 116.87, 114.9, 111.7, 105.7, 104.6, 102.1, 100.8, 99.0, 97.9, 87.5, 71.5, 60.5, 59.9, 59.7, 59.5, 56.2, 55.7, 55.6, 55.5, 48.8, 46.9, 29.6 ppm; HRMS (ESI): $[M+H]^+$ $C_{31}H_{33}N_2O_8$ calcd 561.6110, found 561.2693.

*4.1.4.8. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(pyridin-3-yl)acetonitrile (**5h**)*

Aldehyde substrate: 3-Pyridinecarbaldehyde (**4h**), reaction time: 0.5 h. The mixture of diastereomers (83:17) were purified by preparative TLC. Yield: 85%; off white powder; m.p. 87 °C; IR (KBr): 1032, 1083, 1271, 1486, 1621, 2939 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.77 (d, *J* = 2.4 Hz, 1H, minor diastereomer), 8.61 (d, *J* = 7.2 Hz, 1H, minor diastereomer), 8.63 – 8.53 (m, 2H, major diastereomer), 7.85 (d, *J* = 8.0 Hz, 1H, minor diastereomer), 7.67 (dd, *J* = 7.9, 2.3 Hz, 1H, major diastereomer), 7.35 (dd, *J* = 8.0, 4.9 Hz, 1H, minor diastereomer), 7.28 - 7.25 (m, 1H, major diastereomer), 6.69 (d, *J* = 8.2 Hz, 1H, major diastereomer), 6.62 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 6.36 (s, 1H, major diastereomer), 6.32 (s, 1H, minor diastereomer), 6.07 (s, 1H, major diastereomer), 5.98 (s, 2H, major diastereomer), 5.95 (s, 1H, minor diastereomer), 5.92 (s, 2H, minor diastereomer), 5.88 (d, *J* = 8.2 Hz, 2H, mixture of two diastereomers), 5.80 (s, 1H, minor diastereomer), 5.64 (s, 1H, major diastereomer), 5.51 (s, 1H, minor diastereomer), 5.38 (d, *J* = 11.7 Hz, 1H, minor diastereomer), 5.19 – 5.02 (m, 2H, major diastereomer), 4.88 (d, *J* = 2.9 Hz, 1H, major diastereomer), 4.69 (dd, *J* = 8.9, 3.8 Hz, 1H, minor diastereomer), 4.12 (s, 3H, major diastereomer), 3.94 (s, 3H, minor diastereomer), 3.87 (s, 3H, major diastereomer), 3.86 (s, 3H, major diastereomer), 3.74 (s, 6H, minor diastereomer), 2.72 – 2.29 (m, 8H, mixture of two diastereomers) ppm; ^{13}C NMR (151 MHz, chloroform-*d*) δ 151.4, 150.0, 149.7, 149.3, 148.5, 148.4, 142.7, 140.4, 135.7, 134.7, 134.6, 132.9, 132.7, 132.5, 132.1, 130.9, 130.7, 123.4, 117.9, 117.5, 117.4, 117.4, 116.4, 112.3, 111.9, 102.9, 102.3, 102.1, 100.9, 100.7, 88.0, 86.8, 71.6, 71.4, 63.6, 60.9, 60.6, 60.1, 60.0, 59.5,

59.2, 58.2, 58.2, 56.2, 56.1, 43.7, 29.8, 27.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{28}H_{28}N_3O_6$ calcd 502.1978, found 502.1974.

*4.1.4.9. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(4-(propan-2-yl)phenyl)acetonitrile (**5i**)*

Aldehyde substrate: 4-(Propan-2-yl)benzaldehyde (**4i**), reaction time: 1h. The major diastereomer was crystalized from methanol and hexane (**5i$_1$**). Yield: 75%; white crystal; mp : 154 °C; IR (KBr): 1033, 1083, 1272, 1486, 1620, 2926 cm^{-1}; ^1H NMR (300 MHz, chloroform-*d*) δ 7.31 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 2H), 6.69 (d, *J* = 8.2 Hz, 1H), 6.36 (s, 1H), 5.98 (s, 2H), 5.96 (s, 1H), 5.88 (d, *J* = 8.2 Hz, 1H), 5.62 (br s, 1H), 5.24 (dd, *J* = 12.5, 2.7 Hz, 1H), 5.11 (d, *J* = 12.3 Hz, 1H), 4.88 (d, *J* = 2.8 Hz, 1H), 4.12 (s, 3H), 3.88 (s, 6H), 2.90 (hept, *J* = 6.9 Hz, 1H), 2.57 (dt, *J* = 6.7, 3.3 Hz, 1H), 2.45 (td, *J* = 10.4, 3.3 Hz, 1H), 2.37 – 2.19 (m, 2H), 1.24 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.3, 149.2, 148.3, 142.7, 140.5, 134.6, 133.1, 132.9, 132.5, 132.05, 126.9, 126.7, 117.9, 117.6, 117.5, 112.1, 102.2, 100.8, 88.1, 71.6, 62.9, 60.8, 59.9, 59.5, 56.2, 43.60, 33.7, 29.9, 23.9, 23.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{32}H_{35}N_2O_6$ calcd 543.2495, found 543.2497.

*4.1.4.10. (4-(Benzyloxy)phenyl)((5R)-5-((1S)-4,5-dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)acetonitrile (**5j**)*

Aldehyde substrate: 4-Benzyloxybenzaldehyde (**4j**), reaction time: 8h. The mixture of two diastereomers (84:16) were purified by preparative TLC. Yield: 75 %, off white powder; m.p. 98 °C; IR (KBr): 1033, 1083, 1270, 1488, 1620, 2925 cm^{-1}; ^1H NMR (300 MHz, chloroform-*d*) δ 7.87 (d, *J* = 8.4 Hz, 2H, minor diastereomer), 7.48 – 7.28 (m, 10H, mixture of two diastereomers), 7.10 (d, *J* = 8.4 Hz, 2H, minor diastereomer), 6.99 (d, *J* = 8.4 Hz, 2H, minor diastereomer), 6.92 (d, *J* = 8.3 Hz, 2H, major diastereomer), 6.69 (d, *J* = 8.3 Hz, 1H, major diastereomer), 6.63 (d, *J* = 8.1 Hz, 1H, minor diastereomer), 6.36 (s, 1H, major diastereomer), 6.32 (s, 1H, minor diastereomer), 6.05 (d, *J* = 8.4 Hz, 1H, minor diastereomer), 5.98 (s, 2H, major diastereomer), 5.94 (s, 2H, minor diastereomer), 5.92 (s, 1H, major diastereomer), 5.88 (d, *J* = 8.5 Hz, 1H, major diastereomer), 5.84 (s, 1H, minor diastereomer), 5.81 (s, 1H, minor diastereomer), 5.62 (s, 1H, major diastereomer), 5.46 – 5.31 (m, 4H, minor diastereomer), 5.19 (d, *J* = 12.34, 2.7 Hz, 1H, major diastereomer), 5.10 (d, *J* = 12.4 Hz, 1H, major diastereomer), 5.06 (s, 2H, major diastereomer), 4.86 (d, *J* = 2.9 Hz, 1H, major diastereomer), 4.75 (d, *J* = 3.0 Hz, 1H, minor diastereomer), 4.11 (s, 3H, major diastereomer), 4.02 (s, 3H, minor diastereomer), 3.87 (s, 6H, mixture of two diastereomers), 3.84 (s, 6H, mixture of two

diastereomers), 3.10 – 2.95 (m, 1H, minor diastereomer), 2.61 – 2.22 (m, 7H, mixture of two diastereomers) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 158.8, 151.2, 148.3, 134.6, 133.1, 132.9, 132.4, 128.6, 128.2, 128.0, 127.4, 127.4, 126.9, 117.9, 117.6, 117.6, 114.9, 112.1, 102.1, 100.8, 88.1, 71.5, 70.0, 62.4, 60.7, 59.9, 59.5, 56.2, 43.4, 29.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{36}H_{35}N_2O_7$ calcd 607.2744, found 607.2747.

*4.1.4.11. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(9H-fluoren-2-yl)acetonitrile (**5k**)*

Aldehyde substrate: 9H-fluoren-2-carbaldehyde (**4k**), reaction time: 24h. The mixture of two diastereomers (**5k**) (84:16) were purified by preparative TLC, yield: 65 %.

5k: Off white powder; m.p. 120 °C; IR (KBr): 1042, 1086, 1275, 1488, 1620, 2898 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.80 (d, *J* = 7.2 Hz, 2H, minor diastereomer), 7.78 (d, *J* = 7.2 Hz, 2H, major diastereomer), 7.77 – 7.67 (m, 4H, mixture of two diastereomers), 7.57 (d, *J* = 6.9 Hz, 2H, mixture of two diastereomers), 7.56 (s, 2H, mixture of two diastereomer), 7.47 – 7.28 (m, 6H, mixture of two diastereomers), 6.73 (d, *J* = 8.2 Hz, 1H, major diastereomer), 6.67 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 6.37 (s, 1H, major diastereomer), 6.32 (s, 1H, minor diastereomer), 6.09 (s, 1H, minor diastereomer), 6.07 (s, 1H, major diastereomer), 5.99 (s, 2H, major diastereomer), 5.93 (s, 2H, minor diastereomer), 5.89 (d, *J* = 8.1 Hz, 2H, mixture of two diastereomer), 5.67 (s, 1H, major diastereomer), 5.60 (s, 1H, minor diastereomer), 5.35 (dd, *J* = 12.4, 2.6 Hz, 1H, minor diastereomer), 5.26 (dd, *J* = 12.4, 2.9 Hz, 1H, major diastereomer), 5.13 (d, *J* = 12.4 Hz, 1H, major diastereomer), 5.11 (d, 12.4 Hz, 1H, minor diastereomer), 4.92 (d, *J* = 2.8 Hz, 1H, major diastereomer), 4.83 (d, *J* = 2.6 Hz, 1H, minor diastereomer), 4.13 (s, 3H, major diastereomer), 4.11 (s, 3H, minor diastereomer), 3.91 (s, 6H, mixture of two diastereomers), 3.89 (s, 6H, mixture of two diastereomers), 3.88 (s, 2H, major diastereomer), 3.84 (s, 2H, minor diastereomer), 2.64-2.60 (m, 2H, mixture of two diastereomers), 2.58 – 2.46 (m, 2H, mixture of two diastereomers), 2.40 – 2.30 (m, 4H, mixture of two diastereomers) ppm; ^{13}C NMR (151 MHz, chloroform-*d*) δ 151.3, 148.4,143.8, 143.4, 142.7, 142.2, 140.9, 140.6, 134.5, 132.9, 132.2, 129.7, 128.4, 127.1, 127.0, 126.8, 125.7, 125.3, 125.1, 123.8, 121.1, 120.1, 120.0, 119.9, 117.5, 112.2, 102.2, 100.8, 88.0, 71.6, 62.9, 60.7, 59.9, 59.5, 56.2, 43.6, 36.8, 29.6 ppm; HRMS (ESI): $[M+H]^+$ $C_{36}H_{33}N_2O_6$ calcd 589.2339, found 589.2320.

5k$_1$ (major diastereomer): White powder; m.p. 104 °C; IR (KBr): 1042, 1086, 1275, 1488, 1620, 2898 cm-1; ^{1}H NMR (300 MHz, chloroform-d) δ 7.78 (d, *J* = 7.4 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 6.5 Hz, 1H), 7.55 (s, 1H), 7.45- 7.31 (m, 3H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.37 (s, 1H), 6.08

(s, 1H), 5.99 (s, 2H), 5.94 (d, *J* = 8.3 Hz, 1H), 5.67 (s, 1H), 5.27 (dd, *J* = 12.6, 2.8 Hz, 1H), 5.14 (d, *J* = 12.4 Hz, 1H), 4.92 (d, *J* = 2.8 Hz, 1H), 4.14 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.88 (s, 2H), 2.68 – 2.45 (m, 2H), 2.42 – 2.30 (m, 2H) ppm; ^{13}C NMR (75 MHz, chloroform-*d*) δ 151.3, 148.3, 143.8, 143.4, 142.7, 142.1, 140.9, 140.6, 134.6, 133.2, 133.0, 132.4, 127.0, 126.8, 125.5, 125.1, 123.7, 120.0, 119.9, 117.9, 117.7, 117.5, 112.1, 102.2, 100.8, 88.2, 71.6, 63.1, 60.8, 59.9, 59.5, 56.2, 43.6, 36.8, 29.9 ppm; HRMS (ESI): [M+H]+ $C_{36}H_{33}N_2O_6$ calcd 589.2339, found 589.2320.

*4.1.4.12. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(4-methylphenyl)acetonitrile (**5l**)*

Aldehyde substrate: 4-Methylbenzaldehyde (**4l**), reaction time: 8h. The mixture of two diastereomers (**5l**) (86:14) were purified by preparative TLC, yield: 60 %.

5l: Off white powder; m.p. 74 °C; IR (KBr): 1033, 1083, 1272, 1486, 1620, 2926 cm^{-1}; ^{1}H NMR (600 MHz, chloroform-*d*) δ 7.25 (d, *J* = 7.9 Hz, 4H, mixture of two diastereomer), 7.01 (d, *J* = 7.8 Hz, 4H, mixture of two diastereomers), 6.67 (d, *J* = 8.2 Hz, 1H, major diastereomer), 6.65 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 6.31 (s, 2H, mixture of two diastereomers), 6.03 (s, 2H, minor diastereomer), 5.92 (s, 2H, major diastereomer), 5.90 (d, *J* = 7.8 Hz, 2H, mixture of two diastereomers), 5.83 (s, 1H, major diastereomer), 5.78 (s, 1H, minor diastereomer), 5.63 (s, 1H, major diastereomer), 5.56 (s, 1H, minor diastereomer), 5.10 (d, *J* = 12.0, Hz, 2H, mixture of two diastereomers), 5.04 (d, *J* = 12.4 Hz, 2H, mixture of two diastereomers), 4.81 (d, *J* = 2.1 Hz, 1H, major diastereomer), 4.80 (d, *J* = 2.0 Hz, 1H, minor diastereomer), 4.00 (s, 3H, major diastereomer), 3.84 (s, 3H, minor diastereomer), 3.83 (s, 6H, mixture of two diastereomers), 3.82 (s, 6H, mixture of two diastereomers), 2.70-2.61 (m, 2H, mixture of two diastereomers), 2.58 – 2.46 (m, 2H, mixture of two diastereomers), 2.40 – 2.32 (m, 4H, mixture of two diastereomers), 2.30 (s, 6H, mixture of two diastereomers) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.2, 148.3, 140.5, 138.2, 134.5, 133.1, 132.9, 132.5, 131.7, 129.3, 129.3, 128.3, 126.8, 117.9, 117.6, 117.5, 112.1, 111.9, 102.3, 102.1, 101.6, 100.8, 88.1, 71.6, 70.7, 62.8, 60.8, 60.7, 60.0, 59.9, 59.5, 56.2, 43.8, 43.5, 29.9, 29.6, 28.1, 21.0 ppm; HRMS (ESI): $[M+H]^+$ $C_{30}H_{31}N_2O_6$ calcd 515.2182, found 515.2192.

5l$_1$: Off white powder; m.p. 91 °C; IR (KBr): 1033, 1083, 1272, 1486, 1620, 2926 cm^{-1}; ^{1}H NMR (600 MHz, chloroform-*d*) δ 7.30 (d, *J* = 7.6 Hz, 2H), 7.15 (d, *J* = 7.8 Hz, 2H), 6.740 (d, *J* = 8.2 Hz, 1H), 6.37 (s, 1H), 6.00 - 5.94 (m, 3H), 5.84 (s, 1H), 5.70 (s, 1H), 5.11 (d, *J* = 12.6, 1H), 5.07 (d, *J* = 12.1 Hz, 1H), 4.85 (s, 1H), 4.02 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 2.76 – 2.73 (m, 1H), 2.64 – 2.60

(m, 1H), 2.42 – 2.37 (m, 2H), 2.35 (s, 3H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.2, 148.3, 138.2, 134.6, 133.1, 132.9, 132.5, 131.7, 129.4, 129.3, 126.8, 117.5, 112.1, 102.1, 100.8, 88.1, 71.6, 62.8, 60.8, 59.9, 59.5, 56.2, 43.5, 29.9, 21.0 ppm; HRMS (ESI): $[M+H]^+$ $C_{30}H_{31}N_2O_6$ calcd 515.2182, found 515.2192.

*4.1.4.13. 2-((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)-3-methylbutanenitrile (**5m**)*

Aldehyde substrate: 2-Methylpropanal (**4m**), reaction time: 2h. The mixture of two diastereomers (80:20) were purified by preparative TLC. Yield: 75 %, off white powder; m.p. 68 °C; IR (KBr): 1035, 1083, 1270, 1487, 1622, 2962 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 6.68 (d, *J* = 8.2 Hz, 1H, minor diastereomer) 6.64 (d, *J* = 8.2 Hz, 1H, major diastereomer), 6.35 (s, 2H, mixture of two diastereomers), 6.03 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 5.94 (s, 4H, mixture of two diastereomers), 5.93 (d, *J* = 8.2 Hz, 1H, major diastereomer), 5.47 (s, 1H, minor diastereomer), 5.43 (s, 1H, major diastereomer), 5.23 (dd, *J* = 12.2, 2.7 Hz, 2H, mixture of two diastereomers), 5.12 (d, *J* = 12.5 Hz, 1H, minor diastereomer), 5.05 (d, *J* = 12.5 Hz, 1H, major diastereomer), 4.60 (d, *J* = 5.2 Hz, 1H, major diastereomer), 4.55 (d, *J* = 4.3 Hz, 1H, minor diastereomer), 4.05 (s, 3H, major diastereomer), 3.94 (d, *J* = 5.7 Hz, 2H, mixture of two diastereomers), 3.90 (s, 3H, minor diastereomer), 3.86 (s, 6H, minor diastereomer), 3.82 (s, 6H, major diastereomer), 2.87 – 2.69 (m, 2H, mixture of two diastereomers), 2.66 – 2.55 (m, 1H, minor diastereomer), 2.49 – 2.19 (m, 6H, mixture of two diastereomers), 2.07 – 1.86 (m, 1H, major diastereomer), 1.12 (d, *J* = 6.6 Hz, 6H, mixture of two diastereomers), 0.87 (d, *J* = 6.6 Hz, 6H, mixture of two diastereomers) ppm; ^{13}C NMR (151 MHz, chloroform-*d*) δ 151.2, 148.3, 142.6, 140.5, 134.6, 133.1, 132.8, 132.0, 117.5, 112.0, 102.1, 100.8, 87.5, 71.6, 71.1, 66.2, 61.4, 60.0, 59.9, 59.5, 56.3, 56.2, 43.1, 29.1, 20.3, 18.7 ppm; HRMS (ESI): $[M+H]^+$ $C_{26}H_{31}N_2O_6$ calcd 467.2182, found 467.2180.

*4.1.4.14. ((5R)-5-((1S)-4,5-dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(2-hydroxyphenyl)acetonitrile (**5n**)*

Aldehyde substrate: 2-Hydroxybenzaldehyde (**4n**), reaction time: 2h. The major diastereomer was crystalized from methanol (**5n$_1$**). Yield: 71%; orange crystal; mp : 186 °C; IR (KBr): 1081, 1276, 1488, 1618, 2912, 3269 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 9.22 (s, 1H), 7.46 (d, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 7.0 Hz, 1H), 6.89 (td, *J* = 7.5, 1.2 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.40 (s, 1H), 6.18 (s, 1H), 5.99 (s, 2H), 5.82 (d, *J* = 8.2 Hz, 1H), 5.71 (s, 1H), 5.41 (dd, *J* =

12.3, 2.7 Hz, 1H), 5.06 (d, *J* = 12.3 Hz, 1H), 4.95 (d, *J* = 3.3 Hz, 1H), 4.13 (s, 3H), 3.93 (s, 3H), 3.85 (s, 3H), 2.73 (dt, *J* = 10.4, 3.6 Hz, 1H), 2.66 – 2.38 (m, 3H) ppm; ^{13}C NMR (75 MHz, chloroform-*d*) δ 156.2, 151.8, 148.8, 143.1, 140.5, 134.7, 133.9, 131.6, 130.8, 130.3, 127.9, 119.7, 117.4, 116.8, 116.5, 116.1, 115.7, 112.4, 102.1, 101.0, 86.5, 71.0, 62.1, 61.2, 59.9, 59.6, 56.1, 44.2, 29.0 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{29}N_2O_7$ calcd 517.1975, found 517.1987.

*4.1.4.15. 2-((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)butanenitrile (**5o**)*

Aldehyde substrate: Propanal (**4o**), reaction time: 2h. The mixture of two diastereomers (85:15) were purified by preparative TLC. Yield: 65 %, yellow powder; m.p. 84 °C; IR (KBr): 1035, 1083, 1270, 1487, 1622, 2962 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 6.65 (d, *J* = 8.2 Hz, 1H, minor diastereomer), 6.61 (d, *J* = 8.1 Hz, 1H, major diastereomer),6.34 (s, 1H, minor diastereomer), 6.32 (s, 1H, major diastereomer), 5.99 (d, *J* = 8.4 Hz, 1H, minor diastereomer), 5.94 (s, 2H, major diastereomer), 5.92 (s, 2H, minor diastereomer), 5.83 (d, *J* = 8.2 Hz, 1H, major diastereomer), 5.38 (s, 1H, major diastereomer), 5.35 (s, 1H, minor diastereomer), 5.19 (d, *J* = 12.2 Hz, 2H, mixture of two diastereomer), 5.09 (d, *J* = 12.1 Hz, 2H, mixture of two diastereomer), 4.63 (d, *J* = 4.1 Hz, 1H, minor diastereomer), 4.56 (d, *J* = 3.9 Hz, 1H, major diastereomer), 4.50 (dd, *J* = 9.8, 4.1 Hz, 1H, minor diastereomer), 4.25 (dd, *J* = 9.8, 6.0 Hz, 1H, major diastereomer), 4.05 (s, 3H, major diastereomer), 3.99 (s, 3H, minor diastereomer), 3.86 (s, 6H, minor diastereomer), 3.84 (s, 3H, major diastereomer), 3.82 (s, 3H, major diastereomer), 2.87 – 2.75 (m, 2H, mixture of two diastereomers), 2.69 – 2.49 (m, 2H, mixture of two diastereomers), 2.48 – 2.30 (m, 2H, mixture of two diastereomers), 2.05 (td, *J* = 15.0, 13.8, 4.1 Hz, 2H, mixture of two diastereomers), 1.94 – 1.76 (m, 2H, mixture of two diastereomers), 1.75 – 1.61 (m, 2H, mixture of two diastereomers), 1.06 (t, *J* = 7.4 Hz, 6H, mixture of two diastereomers) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 151.2, 148.1, 140.4, 134.5, 133.3, 132.7, 132.3, 119.1, 118.0, 117.54, 117.3, 111.6, 102.4, 102.0, 100.8, 100.6, 87.6, 87.30, 8.7, 71.6, 71.5, 61.5, 61.4, 59.9, 59.5, 59.0, 58.4, 56.2, 56.1, 44.5, 43.1, 42.6, 41.6, 29.7, 29.5, 28.1, 25.8, 23.5, 10.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{25}H_{29}N_2O_6$ calcd 453.2026, found 453.2038.

4.1.5. General Procedure for the Synthesis of Amide Derivatives

H_2O_2 30% (2mL) was added dropwise to a mixture of *N*-*α*-aminonitrile derivative (1.0 mmol) and K_2CO_3 (5.0 mmol) in 5 mL CH_3OH and 500 µL DMSO. The reaction was stirred at room

temperature for the specified time. The reaction was monitored by TLC and after completion, the solvent was evaporated and water (5 mL) was added. The mixture was extracted with $CHCl_3$ (3×30). Organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Finally, the two diastereomers were purified by preparative TLC.

*4.1.5.1. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(phenyl)acetamide (**6a**)*

Yield: 65 %, reaction time: 5h

6a$_1$ (major diastereomer): White powder; m.p. 78 °C; IR (KBr): 1036, 1078, 1267, 1484, 1684, 2931, 3436 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 7.91 (s, 1H), 7.19 (s, 5H), 6.68 (d, *J* = 8.2 Hz, 1H), 6.27 (s, 1H), 6.09 (d, *J* = 8.1 Hz, 1H), 5.93 (s, 1H), 5.88 (s, 1H), 5.84 (s, 1H), 5.47 (s, 1H), 5.13 (s, 2H), 4.29 (s, 1H), 4.27 (d, *J* = 5.5 Hz, 1H), 3.84 (s, 3H), 3.84 (s, 3H), 3.50 (s, 3H), 2.92 – 2.75 (m, 3H), 2.35 – 2.20 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 175.8, 153.3, 149.3, 147.9, 140.1, 136.8, 134.1, 129.7, 129.0, 128.2, 127.9, 118.0, 117.2, 112.2, 112.1, 102.7, 100.4, 85.5, 72.3, 71.4, 63.7, 60.0, 58.9, 56.2, 44.0, 23.6 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{31}N_2O_7$ calcd 519.2131, found 519.2191.

6a$_2$ (minor diastereomer): White powder; m.p. 82 °C; IR (KBr): 1033, 1075, 1267, 1485, 1684, 2928, 3432 cm^{-1}; ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.94 (s, 1H), 7.19 (s, 5H), 6.67 (d, *J* = 8.1 Hz, 1H), 6.26 (s, 1H), 6.08 (d, *J* = 8.2 Hz, 1H), 5.97 (s, 1H), 5.87 (s, 1H), 5.84 (s, 1H), 5.47 (s, 1H), 5.12 (s, 2H), 4.28 (s, 1H), 4.27 (s, 1H), 3.83 (s, 6H), 3.49 (s, 3H), 2.94 – 2.74 (m, 3H), 2.27 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 175.6, 149.9, 140.8, 136.6, 129.9, 128.8, 128.4, 128.1, 117.2, 112.3, 112.2, 102.6, 100.6, 84.9, 71.9, 71.8, 60.0, 59.7, 59.3, 56.2, 42.6, 40.4, 23.6 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{31}N_2O_7$ calcd 519.2131, found 519.2191.

*4.1.5.2. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(3-phenoxyphenyl)acetamide (**6b**)*

Yield: 75 %, reaction time: 12h

6b$_1$ (major diastereomer): Off white powder; m.p. 54 °C; IR (KBr): 1038, 1080, 1263, 1485, 1699, 2926, 3237 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-*d*) δ 8.09 (s, 1H), 7.39 – 7.23 (m, 2H), 7.16 (t, *J* = 7.8 Hz, 1H), 7.08 (t, *J* = 7.4 Hz, 1H), 7.02 – 6.77 (m, 5H), 6.65 (d, *J* = 8.2 Hz, 1H), 6.25 (s, 1H), 6.06 (s, 1H), 6.05 (d, *J* = 8.2 Hz, 1H), 5.87 (s, 1H), 5.86 (s, 1H), 5.50 (s, 1H), 5.15 (t, *J* = 14.2 Hz, 2H),

4.37 (d, *J* = 4.7 Hz, 1H), 4.26 (s, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.64 (s, 3H), 2.91 – 2.67 (m, 3H), 2.33 – 2.11 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 175.3, 156.8, 151.2, 147.9, 139.0, 133.9, 133.7, 131.8, 129.7, 129.6, 129.3, 123.9, 123.1, 119.8, 118.7, 118.2, 117.8, 117.2, 112.2, 102.7, 100.4, 85.5, 72.4, 71.4, 60.0, 58.9, 56.3, 56.2, 44.4, 42.6, 23.7 ppm; HRMS (ESI): $[M+H]^+$ $C_{35}H_{35}N_2O_8$ calcd 611.2393, found 611.2427.

6b$_2$ (minor diastereomer): Off white powder; m.p. 76 °C; IR (KBr): 1038, 1080, 1263, 1486, 1698, 2928, 3417 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.22 (d, *J* = 3.7 Hz, 1H), 7.34 (t, *J* = 7.9 Hz, 2H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.14 (s, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 7.00 (d, *J* = 7.8 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 6.29 (s, 1H), 5.95 (d, *J* = 1.5 Hz, 1H), 5.94 (d, *J* = 1.5 Hz, 1H), 5.88 (d, *J* = 8.1 Hz, 1H), 5.77 (s, 1H), 5.53 (d, *J* = 3.7 Hz, 1H), 5.41 (dd, *J* = 12.2, 2.4 Hz, 1H), 5.24 (d, *J* = 12.2 Hz, 1H), 4.56 (d, *J* = 4.9 Hz, 1H), 4.27 (s, 1H), 4.02 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 2.51 – 2.32 (m, 2H), 2.24 – 2.08 (m, 1H), 2.00 – 1.82 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 175.1, 157.3, 156.9, 148.3, 143.3, 140.8, 138.7, 134.0, 133.3, 129.8, 129.7, 125.2, 123.8, 123.3, 118.9, 118.9, 118.3, 117.2, 112.3, 102.6, 101.6, 100.6, 84.8, 77.2, 77.0, 76.7, 71.8, 71.5, 60.0, 59.6, 59.3, 56.2, 40.4, 23.6 ppm; HRMS (ESI): $[M+H]^+$ $C_{35}H_{35}N_2O_8$ calcd 611.2393, found 611.2444.

*4.1.5.3. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(3,4-dimethoxyphenyl)acetamide (**6c**)*

Yield: 77 %, reaction time: 24h

6c$_1$ (major diastereomer): Yellow powder; m.p. 114 °C; IR (KBr): 1032, 1079, 1264, 1478, 1683, 2944, 3446 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 7.84 (s, 1H), 6.83 – 6.59 (m, 4H), 6.32 (s, 1H), 6.30 (s, 1H), 6.14 (d, *J* = 8.1 Hz, 1H), 5.87 (s, 1H), 5.85 (s, 1H), 5.48 (s, 1H), 5.16 (t, *J* = 12.9 Hz, 2H), 4.25 (d, *J* = 5.3 Hz, 1H), 4.21 (s, 1H), 3.84 (s, 3H), 3.83 (s, 6H), 3.69 (s, 3H), 3.55 (s, 3H), 2.95 – 2.73 (m, 3H), 2.48 – 2.15 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 175.8, 151.2, 148.6, 147.9, 143.0, 140.3, 134.2, 133.8, 131.9, 129.6, 129.4, 121.4, 118.2, 117.1, 112.2, 112.0, 110.9, 102.7, 100.4, 85.5, 71.8, 71.3, 59.9, 58.8, 56.2, 56.1, 55.9, 55.5, 43.7, 23.4 ppm; HRMS (ESI): $[M+H]^+$ $C_{31}H_{35}N_2O_9$ calcd 579.2343, found 579.2389.

6c$_2$ (minor diastereomer): Yellow powder; m.p. 85 °C; IR (KBr): 1032, 1079, 1264, 1478, 1683, 2944, 3446 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.27 (s, 1H), 6.96 (dd, *J* = 8.2, 1.9 Hz, 1H),

6.91 (d, *J* = 1.9 Hz, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.63 (d, *J* = 8.2 Hz, 1H), 6.30 (s, 1H), 5.96 (s, 1H), 5.94 (s, 1H), 5.89 (d, *J* = 8.2 Hz, 1H), 5.79 (s, 1H), 5.62 (s, 1H), 5.48 (dd, *J* = 12.0, 2.9 Hz, 1H), 5.28 (d, *J* = 12.1 Hz, 1H), 4.58 (d, *J* = 5.0 Hz, 1H), 4.24 (s, 1H), 4.02 (s, 3H), 3.88 (s, 6H), 3.87 (s, 6H), 3.82 (s, 3H), 2.47 – 2.32 (m, 2H), 2.23 – 2.05 (m, 1H), 1.97 – 1.86 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 175.7, 159.9, 141.7, 140.8, 131.0, 130.0, 129.2, 129.1, 121.8, 121.5, 117.3, 117.2, 116.3, 112.3, 111.9, 111.1, 102.6, 100.6, 90.5, 85.0, 71.8, 71.4, 60.0, 59.6, 59.3, 56.2, 55.8, 42.6, 40.4, 23.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{31}H_{35}N_2O_9$ calcd 579.2343, found 579.2384.

*4.1.5.4. (2-Chlorophenyl)((5R)-5-((1S)-4,5-dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)acetamide (**6d**)*

Yield: 55 %, reaction time: 24h

6d$_1$ (major diastereomer): Off white powder; m.p. 66 °C; IR (KBr): 1034, 1081, 1267, 1482, 1689, 2902, 3413 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.13 (s, 1H), 7.35 – 7.29 (m, 1H), 7.27 – 7.21 (m, 1H), 7.16 – 7.02 (m, 2H), 6.65 (d, *J* = 8.2 Hz, 1H), 6.17 (s, 2H), 6.00 (d, *J* = 8.2 Hz, 1H), 5.87 (d, *J* = 1.5 Hz, 1H), 5.84 (d, *J* = 1.5 Hz, 1H), 5.51 (s, 1H), 5.17 (dd, *J* = 12.4, 4.9 Hz, 1H), 5.11 (d, *J* = 12.2 Hz, 1H), 5.03 (s, 1H), 4.46 (d, *J* = 4.8 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.72 (s, 3H), 2.93 – 2.61 (m, 3H), 2.30 – 2.09 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 174.8, 151.2, 147.9, 139.8, 135.4, 135.0, 133.7, 133.5, 131.8, 130.3, 130.1, 129.4, 128.9, 126.4, 117.8, 117.3, 112.1, 102.6, 100.4, 85.5, 71.4, 70.6, 68.1, 60.0, 58.9, 56.4, 56.2, 44.5, 24.3 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{30}ClN_2O_7$ calcd 553.1742, found 553.1792.

6d$_2$ (minor diastereomer): Off white powder; m.p. 67 °C; IR (KBr): 1034, 1081, 1267, 1482, 1689, 2942, 3413 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.38 (s, 1H), 7.50 (dd, *J* = 7.4, 2.1 Hz, 1H), 7.36 (dd, *J* = 7.4, 1.9 Hz, 1H), 7.22 (td, *J* = 7.4, 1.9 Hz, 2H), 6.62 (d, *J* = 8.3 Hz, 1H), 6.29 (s, 1H), 5.95 (d, *J* = 1.5 Hz, 1H), 5.94 (d, *J* = 1.5 Hz, 1H), 5.88 (d, *J* = 8.1 Hz, 1H), 5.80 (s, 1H), 5.76 (s, 1H), 5.50 (dd, *J* = 12.1, 2.9 Hz, 1H), 5.28 (d, *J* = 12.1 Hz, 1H), 4.99 (s, 1H), 4.70 (d, *J* = 5.0 Hz, 1H), 4.04 (s, 3H), 3.88 (s, 3H), 3.81 (s, 3H), 2.45 (ddd, *J* = 16.7, 11.2, 5.7 Hz, 1H), 2.36 – 2.03 (m, 2H), 1.92 (d, *J* = 16.6 Hz, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 175.3, 151.3, 148.3, 140.7, 135.0, 134.6, 134.0, 133.7, 133.2, 132.1, 130.1, 130.1, 129.0, 126.8, 117.3, 116.1, 112.3, 102.7, 102.6, 100.6, 84.9, 71.9, 71.5, 60.1, 59.8, 59.3, 56.1, 40.9, 24.2 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{30}ClN_2O_7$ calcd 553.1742, found 553.1773.

*4.1.5.5. (2,4-Dichlorophenyl)((5R)-5-((1S)-4,5-dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)acetamide (**6e**)*

Yield: 90 %, reaction time: 8h

6e$_1$ (major diastereomer): Off white powder; m.p. 76 °C; IR (KBr): 1034, 1081, 1267, 1482, 1689, 2902, 3413 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.14 (s, 1H), 7.29 – 7.18 (m, 2H), 7.03 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.64 (d, *J* = 8.3 Hz, 1H), 6.50 (s, 1H), 6.16 (s, 1H), 5.97 (d, *J* = 8.1 Hz, 1H), 5.86 (s, 2H), 5.48 (s, 1H), 5.17 (dd, *J* = 12.4, 2.6 Hz, 1H), 5.11 (d, *J* = 12.5 Hz, 1H), 4.97 (s, 1H), 4.44 (d, *J* = 4.8 Hz, 1H), 3.82 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 2.95 – 2.51 (m, 3H), 2.24 – 2.05 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 174.8, 151.3, 148.1, 142.9, 139.8, 134.3, 133.9, 133.5, 133.4, 131.1, 130.0, 129.0, 126.7, 117.4, 117.2, 112.2, 102.6, 100.4, 85.3, 71.4, 60.0, 59.0, 56.4, 56.2, 44.6, 42.6, 24.3 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{29}Cl_2N_2O_7$ calcd 587.1352, found 587.1373.

6e$_2$ (minor diastereomer): Off white powder; m.p. 79 °C; IR (KBr): 1034, 1081, 1267, 1482, 1689, 2942, 3413 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.33 (s, 1H), 7.41 (d, *J* = 11.4 Hz, 1H), 7.28 (s, 1H), 7.23 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 6.29 (s, 1H), 5.95 (s, 1H), 5.94 (s, 1H), 5.92 (s, 1H), 5.87 (d, *J* = 8.2 Hz, 1H), 5.78 (s, 1H), 5.48 (dd, *J* = 12.2, 2.8 Hz, 1H), 5.26 (d, *J* = 12.2 Hz, 1H), 4.98 (s, 1H), 4.70 (d, *J* = 4.8 Hz, 1H), 4.04 (s, 3H), 3.88 (s, 3H), 3.81 (s, 3H), 2.40 (ddd, *J* = 16.5, 10.9, 5.8 Hz, 1H), 2.31 – 2.08 (m, 2H), 1.94 (dt, *J* = 16.8, 3.8 Hz, 1H) ppm; ^{13}C NMR (125 MHz, chloroform-*d*) δ 172.3, 151.4, 148.3, 143.0, 140.7, 135.7, 134.1, 134.0, 133.3, 133.0, 132.2, 131.1, 129.9, 129.8, 127.2, 117.3, 116.0, 112.3, 102.5, 100.6, 85.0, 71.8, 60.0, 59.8, 59.3, 56.1, 41.1, 24.5 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{29}Cl_2N_2O_7$ calcd 587.1352, found 587.1363.

*4.1.5.6. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(4-fluorophenyl)acetamide (**6f**)*

Yield: 66 %, reaction time: 24h

6f$_1$ (major diastereomer): Yellow powder; m.p. 64 °C; IR (KBr): 1035, 1080, 1268, 1488, 1679, 2927, 3430 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.00 (s, 1H), 7.21 – 7.09 (m, 2H), 6.93 – 6.85 (m, 2H), 6.67 (d, *J* = 8.2 Hz, 1H), 6.25 (s, 1H), 6.05 (d, *J* = 8.2 Hz, 1H), 5.97 (d, *J* = 4.4 Hz, 1H), 5.88 (d, *J* = 1.5 Hz, 1H), 5.86 (d, *J* = 1.4 Hz, 1H), 5.48 (s, 1H), 5.15 (m, 2H), 4.27 (s, 1H), 4.25 (d, *J* = 5.2 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.60 (s, 3H), 2.88 – 2.71 (m, 3H), 2.33 – 2.18 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 176.1, 163.3, 161.3, 151.2, 148.0, 139.9, 133.8, 133.7, 132.8,

130.7, 130.7, 129.6, 117.6, 117.2, 115.0, 114.9, 112.2, 102.6, 100.5, 85.43, 71.6, 71.4, 60.0, 58.9, 56.2, 56.1, 44.2, 42.6, 23.5 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{30}FN_2O_7$ calcd 537.2037, found 537.2067.

6f$_2$ (minor diastereomer): Off white powder; m.p. 67 °C; IR (KBr): 1033, 1082, 12678, 1490, 1686, 2926, 3425 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.24 (s, 1H), 7.39 – 7.33 (m, 2H), 7.06 – 6.96 (m, 2H), 6.63 (d, *J* = 8.2 Hz, 1H), 6.29 (s, 1H), 5.96 (d, *J* = 1.5 Hz, 1H), 5.94 (d, *J* = 1.5 Hz, 1H), 5.88 (d, *J* = 8.2 Hz, 1H), 5.79 (s, 1H), 5.57 – 5.45 (m, 2H), 5.28 (d, *J* = 12.2 Hz, 1H), 4.58 (d, *J* = 4.8 Hz, 1H), 4.32 (s, 1H), 4.03 (s, 3H), 3.88 (s, 3H), 3.81 (s, 3H), 2.42 – 2.08 (m, 3H), 1.98 – 1.85 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-d) δ 174.1, 163.5, 161.5, 151.4, 148.3, 134.0, 133.1, 132.2, 132.1, 130.5, 130.5, 129.8, 127.4, 117.2, 116.9, 116.0, 115.5, 115.3, 112.3, 102.6, 100.6, 85.0, 71.8, 70.8, 60.1, 59.7, 59.3, 40.5, 23.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{29}H_{30}FN_2O_7$ calcd 537.2037, found 537.2064.

*4.1.5.7. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(2,4-dimethoxyphenyl)acetamide (**6g**)*

Yield: 58 %, reaction time: 12h

6g$_1$ (major diastereomer): Yellow powder; m.p. 65 °C; IR (KBr): 1032, 1079, 1264, 1478, 1683, 2944, 3446 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.49 (d, *J* = 5.0 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.64 (d, *J* = 8.2 Hz, 1H), 6.38 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.23 (s, 2H), 6.01 (d, *J* = 4.9 Hz, 1H), 5.95 (d, *J* = 8.2 Hz, 1H), 5.85 (d, *J* = 1.5 Hz, 1H), 5.84 (d, *J* = 1.5 Hz, 1H), 5.49 (s, 1H), 5.20 (dd, *J* = 12.3, 2.7 Hz, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 4.73 (s, 1H), 4.38 (d, *J* = 5.0 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.75 (s, 3H), 3.61 (s, 3H), 3.50 (s, 3H), 2.90 – 2.67 (m, 3H), 2.19 – 2.04 (m, 1H) ppm; ^{13}C NMR (75 MHz, chloroform-*d*) δ 175.4, 160.8, 147.5, 134.2, 130.6, 128.3, 120.5, 117.3, 112.2, 105.1, 104.3, 103.1, 102.6, 100.6, 100.3, 98.9, 98.3, 98.1, 85.3, 71.3, 67.9, 60.0, 58.9, 56.2, 55.9, 55.7, 55.4, 55.3, 54.8, 44.7, 24.0 ppm; HRMS (ESI): $[M+H]^+$ $C_{31}H_{35}N_2O_7$ calcd 579.2343, found 579.2383.

6g$_2$ (minor diastereomer): Yellow powder; m.p. 67 °C; IR (KBr): 1032, 1079, 1264, 1478, 1683, 2944, 3446 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.17 (d, *J* = 4.4 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 6.63 (d, *J* = 8.2 Hz, 1H), 6.52 – 6.42 (m, 2H), 6.27 (s, 1H), 5.93 (s, 2H), 5.90 (d, *J* = 8.5 Hz, 1H), 5.73 (br s, 2H), 5.44 (dd, *J* = 12.4, 2.8 Hz, 1H), 5.23 (d, *J* = 12.1 Hz, 1H), 4.91 (s, 1H), 4.69 (d, *J* = 4.7 Hz, 1H), 4.00 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 2.44 – 2.16 (m,

3H), 2.01 – 1.88 (m, 1H) ppm; ^{13}C NMR (125 MHz, chloroform-*d*) δ 176.6, 160.4, 158.9, 151.2, 148.0, 142.9, 140.7, 133.9, 133.6, 132.4, 130.8, 130.6, 117.5, 117.3, 112.2, 104.8, 104.4, 102.4, 100.5, 98.8, 85.6, 71.6, 70.6, 59.9, 59.2, 56.2, 55.5, 55.3, 42.6, 25.2 ppm; HRMS (ESI): $[M+H]^+$ $C_{31}H_{35}N_2O_7$ calcd 579.2343, found 579.2389.

*4.1.5.8. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(pyridin-3-yl)acetamide (**6h**)*

Yield: 65 %, reaction time: 8h

6h$_1$ (major diastereomer): White powder; m.p. 69 °C; IR (KBr): 1036, 1080, 1267, 1481, 1686, 2936, 3424 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.37 (s, 2H), 8.33 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.08 (t, *J* = 6.4 Hz, 1H), 6.63 (s, 1H), 6.60 (s, 1H), 6.16 (s, 1H), 5.92 (d, *J* = 8.2 Hz, 1H), 5.83 (s, 2H), 5.48 (s, 1H), 5.21 (d, *J* = 12.8 Hz, 1H), 5.12 (d, *J* = 12.5 Hz, 1H), 4.41 – 4.21 (m, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.68 (s, 3H), 2.85 – 2.59 (m, 3H), 2.24 – 2.01 (m, 1H) ppm; ^{13}C NMR (75 MHz, chloroform-*d*) δ 175.1, 151.3, 150.2, 148.8, 148.1, 143.0, 139.7, 136.3, 133.6, 133.5, 133.3, 131.7, 129.5, 122.8, 117.2, 112.2, 102.6, 100.5, 85.1, 71.4, 70.6, 60.0, 59.0, 56.3, 56.2, 44.7, 23.8 ppm; HRMS (ESI): $[M+H]^+$ $C_{28}H_{30}N_3O_7$ calcd 520.2084, found 520.2108.

6h$_2$ (minor diastereomer): White powder; m.p. 79 °C; IR (KBr): 1036, 1080, 1267, 1481, 1686, 2936, 3424 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.65 (s, 1H), 8.54 (d, *J* = 4.8 Hz, 1H), 8.17 (d, *J* = 3.8 Hz, 1H), 7.71 (d, *J* = 7.9 Hz, 1H), 7.30 – 7.24 (m, 1H), 6.63 (d, *J* = 8.2 Hz, 1H), 6.29 (s, 1H), 5.95 (d, *J* = 1.6 Hz, 1H), 5.94(d, *J* = 1.6 Hz, 1H), 5.88 (d, *J* = 8.4 Hz, 1H), 5.78 (br s, 1H), 5.69 (br s, 1H), 5.50 (dd, *J* = 12.1, 2.9 Hz, 1H), 5.27 (d, *J* = 12.2 Hz, 1H), 4.62 (d, *J* = 4.6 Hz, 1H), 4.49 (s, 1H), 4.05 (s, 3H), 3.88 (s, 3H), 3.81 (s, 3H), 2.37 – 2.20 (m, 3H), 2.02 – 1.93 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-d) δ 174.8, 151.4, 150.3, 149.4, 148.4, 140.7, 136.4, 134.1, 132.9, 132.2, 132.1, 129.8, 123.3, 117.2, 116.0, 112.4, 102.5, 100.7, 85.3, 71.8, 69.4, 60.0, 59.7, 59.4, 56.1, 41.1, 24.4 ppm; HRMS (ESI): $[M+H]^+$ $C_{28}H_{30}N_3O_7$ calcd 520.2084, found 520.2101.

*4.1.5.9. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(4-(propan-2-yl)phenyl)acetamide (**6i$_1$**)*

The major diastereomer was purified by preparative TLC. Yield: 44 %, reaction time: 48h

6i$_1$ (major diastereomer): White powder; m.p. 83 °C; IR (KBr): 1036, 1080, 1267, 1484, 1689, 2949, 3444 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 8.05 (d, *J* = 4.7 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 2H), 7.02 (d, *J* = 8.1 Hz, 2H), 6.65 (d, *J* = 8.1 Hz, 1H), 6.25 (s, 1H), 6.17 (d, *J* = 4.6 Hz, 1H), 6.05 (d, *J* = 8.1 Hz, 1H), 5.85 (d, *J* = 2.0 Hz, 1H), 5.84 (d, *J* = 2.1 Hz, 1H), 5.47 (d, J = 4.9 Hz, 1H), 5.18 (dd, *J* = 12.4, 2.8 Hz, 1H), 5.12 (d, *J* = 12.4 Hz, 1H), 4.28 – 4.21 (m, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 3.50 (s, 3H), 2.89 – 2.75 (m, 4H), 2.30 – 2.16 (m, 1H), 1.16 (d, *J* = 7.0 Hz, 6H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 176.3, 151.2, 148.5, 147.8, 142.9, 140.0, 134.1, 133.8, 131.8, 129.8, 129.0, 126.1, 118.2, 117.2, 112.1, 102.7, 100.4, 85.3, 72.2, 71.4, 60.0, 58.8, 56.2, 56.0, 44.2, 42.6, 33.7, 23.9, 23.8, 23.6 ppm; HRMS (ESI): $[M+H]^+$ $C_{32}H_{37}N_2O_7$ calcd 561.2601, found 561.2640.

*4.1.5.10. (4-(Benzyloxy)phenyl)((5R)-5-((1S)-4,5-dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)acetamide (**6j**)*

Yield: 65 %, reaction time: 8h

6j$_1$ (major diastereomer): White powder; m.p. 68 °C; IR (KBr): 1013, 1179, 1253, 1458, 1651, 2918, 3388 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 7.94 (d, *J* = 4.8 Hz, 1H), 7.45 – 7.30 (m, 5H), 7.10 (d, *J* = 8.2 Hz, 2H), 6.82 (d, *J* = 8.3 Hz, 2H), 6.68 (d, *J* = 8.2 Hz, 1H), 6.28 (s, 1H), 6.13 (s, 1H), 6.10 (d, *J* = 8.5 Hz, 1H), 5.88 (d, *J* = 1.5 Hz, 1H), 5.86 (d, *J* = 1.5 Hz, 1H), 5.49 (d, *J* = 4.8 Hz, 1H), 5.23 – 5.07 (m, 2H), 5.02 (s, 2H), 4.27 (d, *J* = 5.2 Hz, 1H), 4.24 (s, 1H), 3.85 (s, 3H), 3.85 (s, 3H), 3.51 (s, 3H), 2.94 – 2.76 (m, 2H), 2.35 – 2.19 (m, 1H), 2.14 – 1.96 (m, 1H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 176.1, 158.4, 151.1, 147.9, 142.9, 140.3, 137.0, 134.3, 133.9, 131.9, 130.1, 129.7, 129.4, 128.5, 127.9, 127.3, 118.2, 117.2, 114.6, 112.2, 102.8, 100.4, 85.7, 71.4, 71.3, 69.9, 59.9, 58.8, 56.2, 56.2, 43.7, 23.5 ppm; HRMS (ESI): $[M+H]^+$ $C_{36}H_{37}N_2O_8$ calcd 625.2550, found 625.2625.

6j$_2$ (minor diastereomer): White powder; m.p. 88 °C; IR (KBr): 1033, 1180, 1263, 1302, 1683, 2931, 3438 cm^{-1}; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.26 (s, 1H), 7.47 – 7.30 (m, 7H), 6.93 (d, *J* = 8.0 Hz, 2H), 6.63 (d, *J* = 8.2 Hz, 1H), 6.29 (s, 1H), 5.95 (s, 1H), 5.94 (s, 1H), 5.89 (d, *J* = 8.3 Hz, 1H), 5.78 (s, 1H), 5.68 (s, 1H), 5.50 (d, *J* = 11.8 Hz, 1H), 5.28 (d, *J* = 12.1 Hz, 1H), 5.04 (s, 2H), 4.56 (d, *J* = 5.1 Hz, 1H), 4.25 (s, 1H), 4.03 (s, 3H), 3.89 (s, 3H), 3.82 (s, 3H), 3.05 (q, *J* = 7.4 Hz, 1H), 2.56 – 1.94 (m, 3H) ppm; ^{13}C NMR (126 MHz, chloroform-*d*) δ 175.8, 158.7, 151.3, 148.2, 143.1, 140.8, 136.9, 133.4, 132.2, 130.1, 130.0, 130.0, 129.0, 128.5, 127.9, 127.4, 117.2, 117.2, 114.8, 112.4, 102.6, 100.5, 85.0, 71.8, 71.1, 70.0, 60.0, 59.6, 59.3, 56.2, 42.6, 23.7 ppm; HRMS (ESI): $[M+H]^+$ $C_{36}H_{37}N_2O_8$ calcd 625.2550, found 625.2577.

*4.1.5.11. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(9H-fluoren-2-yl)acetamide (**6k**)*

The major diastereomer was purified by preparative TLC. Yield: 54 %, reaction time: 2h

6k$_1$ (major diastereomer): Off white powder; m.p. 92 °C; IR (KBr): 1035, 1078, 1267, 1484, 1684, 2931, 3430 cm^{-1}; ^{1}H NMR (300 MHz, chloroform-d) δ 7.92 (s, 1H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 7.3 Hz, 1H), 7.42 – 7.30 (m, 3H), 7.20 (d, *J* = 7.9 Hz, 1H), 6.69 (d, *J* = 8.0 Hz, 1H), 6.27 (s, 1H), 6.16 (d, *J* = 8.0 Hz, 1H), 6.08 (s, 1H), 5.80 (s, 1H), 5.74 (s, 1H), 5.49 (d, *J* = 4.8 Hz, 1H), 5.13 (s, 2H), 4.35 (s, 1H), 4.33 (d, *J* = 6.9 Hz, 1H), 3.84 (s, 6H), 3.78 (s, 2H), 3.42 (s, 3H), 2.95 – 2.82 (m, 3H), 2.44 – 2.15 (m, 1H) ppm; ^{13}C NMR (75 MHz, chloroform-*d*) δ 175.9, 151.1, 147.9, 143.4, 143.1, 142.9, 141.4, 141.3, 140.2, 135.6, 134.3, 133.7, 131.8, 129.7, 127.7, 126.7, 125.6, 125.0, 119.9, 119.5, 118.1, 117.2, 112.1, 102.7, 100.4, 85.7, 72.4, 71.3, 59.9, 58.8, 56.3, 56.2, 43.9, 36.7, 23.6 ppm; HRMS (ESI): $[M+H]^+$ $C_{36}H_{35}N_2O_7$ calcd 607.2444, found 607.2475.

*4.1.5.12. ((5R)-5-((1S)-4,5-Dimethoxy-1,3-dihydro-2-benzofuran-1-yl)-4-methoxy-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-yl)(4-methylphenyl)acetamide (**6l$_1$**)*

The major diastereomer purified by preparative TLC. Yield: 50 %, reaction time: 24h

6l$_1$ (major diastereomer): White powder; m.p. 76 °C; IR (KBr): 1036, 1080, 1267, 1484, 1684, 2942, 3444 cm^{-1}; ^{1}H NMR (500 MHz, chloroform-d) δ 7.76 (d, *J* = 4.7 Hz, 1H), 7.06 (d, *J* = 7.9 Hz, 2H), 7.00 (d, *J* = 7.8 Hz, 2H), 6.68 (d, *J* = 8.2 Hz, 1H), 6.28 (s, 1H), 6.13 (d, *J* = 8.1 Hz, 1H), 6.01 (d, *J* = 4.7 Hz, 1H), 5.87 (d, *J* = 1.5 Hz, 1H), 5.84 (d, *J* = 1.5 Hz, 1H), 5.46 (d, *J* = 4.8 Hz, 1H), 5.16 – 5.06 (m, 2H), 4.25 (d, *J* = 5.1 Hz, 1H), 4.24 (s, 1H), 3.83 (s, 3H), 3.83 (s, 3H), 3.49 (s, 3H), 2.90 – 2.79 (m, 3H), 2.38 – 2.28 (m, 1H), 2.27 (s, 3H) ppm; ^{13}C NMR (126 MHz, chloroform-d) δ 175.8, 151.1, 147.9, 140.2, 137.5, 134.3, 133.9, 133.8, 131.9, 129.7, 128.9, 128.8, 118.2, 117.2, 112.1, 102.8, 100.4, 85.5, 71.9, 71.4, 60.0, 58.8, 56.2, 43.7, 23.6, 21.0. ppm; HRMS (ESI): $[M+H]^+$ $C_{30}H_{33}N_2O_7$ calcd 533.2288, found 533.2318.

4.2. Antitrypanosomal and Cytotoxicity Assays

The inhibitory activity assays against the protozoan parasites *T. b. rhodesiense* (STIB900) trypomastigotes, *T. cruzi* (Tulahuen C4) amastigotes, L. donovani (MHOM-ET-67/L82) amastigotes, and *P. falciparum* (NF54) IEF stage, and cytotoxicity in the L6 cells (rat skeletal myoblasts) were

performed as previously described [32, 33]. The growth inhibition assay for primary screening of compounds were performed at 2 and 10 μg/mL. For determination IC_{50}s, Alamar Blue assay was used according to that reported in previous [42]. All assays were repeated for at least two independent experiments. The selectivity index was calculated as the ratio of IC_{50} for the L6-cells to IC_{50} for parasites.

4.3. Molecular docking studies and Lipinski's rule of five prediction

The 2D structures of the derivatives were generated by ChemDraw Professional 15.0 and 3D structures were built by Chem3D suite. The structures of products were saved in SDF format and prepared for docking. The ligand structures were minimized using Lig Prep module implemented in Schrodinger 2015-2, using Maestro 10.2 platform. The crystal structure of TbTR (PDB: 2WOW) with a resolution of 2.2 Å and TbUDPGE (PDB: 1GY8) with a resolution of 2.0 Å were selected from previous publications [30, 31] and downloaded from protein data bank (www.rcsb.org). The proteins preparation for docking was performed using Protein Preparation Wizard on Maestro 10.2 Schrodinger suite. All water molecules were deleted. Formal charge and bond order were assigned. All hydrogen atoms and missing loops and missing side chains were added to crystal structures. Then the hydrogen network was refined and hydrogen minimization was performed by OPLS3 force field parameter. The receptor grid box was generated by selecting native ligand in the crystal structure (FAD for TbTR and NAD for TbUDPGE) as a 25 Å ×25 Å ×25 Å box with the Grid generation application. All derivatives and native ligands conformers were docked to grid files of proteins and Glide extra precision (XP) mode were used. The refinement and scoring were performed by default. The drug-likeness properties of the most active compounds were predicted by using Qikprop 4.4 [43]. For more details investigation of molecular docking of selected ligands (**5j$_1$**, **6j$_1$**, and **6k$_1$**) was carried out by Induced-Fit Docking (IFD) protocol using Glide application in Schrodinger [44].

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declarations of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary Data. ^{1}H & ^{13}C NMR, HRMS and FTIR spectra of compounds **5a-5o** and **6a-6l.** X-ray crystallographic analysis, Table S2. Table S3.

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Highlights

- Two series of novel noscapine derivatives were synthesized in good yields.
- Antiprotozoal potency of the noscapine derivatives was reported for the first time.
- Compound **$6g_2$** showed excellent antiplasmodial activity with IC_{50} = 1.7 μM.
- Molecular docking results were in good agreement with experimental data.
- ADMET properties were calculated for most active compounds.

Graphical abstract