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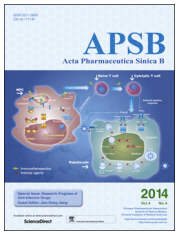

ORIGINAL ARTICLE

Synthesis and antiviral activity of some novel indole-2-carboxylate derivatives

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KEY WORDS

Indole-2-carboxylate;
Broad-spectrum antiviral activity;
DNA and RNA virus;
SAR

Abstract A series of novel indole-2-carboxylate derivatives were synthesized and assayed to determine their *in vitro* broad-spectrum antiviral activities. The biological results showed that some of the synthesized compounds exhibited potent broad-spectrum antiviral activity. Notably, compound **8f** showed the highest SI value (17.1) to Cox B3 virus. Compound **14f** showed both potent inhibitory activity against influenza A (IC_{50} = 7.53 μmol/L) and the highest SI value (12.1). SAR results showed that the alkyloxy at the 4-position of indole ring was not crucial to the antiviral activities. Incorporation of an acetyl substituent at the amino group disfavored antiviral activity towards RNA viruses.

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1. Introduction

Viruses are the most common cause of global infectious disease. Viruses with high infection rates and rapid propagation can cause worldwide human and animal pandemics.

Coxsackie B viruses are single-strand RNA viruses; infection with Cox B can cause fever, headache, chest pain and other problems. Cardiac infection with Cox B3 can result in acute myocarditis that is spontaneously resolved or chronic myocarditis with prolonged viral persistence[1]. Currently, there is no specific treatment or vaccine available for Coxsackie virus infections.

Influenza is a respiratory disease caused by the influenza virus. Despite the extensive effort invested in attempting to control influenza infection, only two classes of drugs are currently clinically available: inhibitors of the M2 protein (*e.g.*, amantadine and rimantadine), and inhibitors of neuraminidase (*e.g.*, zanamivir and oseltamivir). Clinical applications of amantadine and rimantadine have been limited as a consequence of the increasing incidence of adamantane-resistant viruses in the general population[2–4]. Furthermore, blockers of the M2 ion channel inhibit only the replication of influenza A virus and are associated with neurological side effects. The neuraminidase inhibitors (zanamivir and oseltamivir) were marketed in 1999 for the treatment and prophylaxis of influenza and have been subsequently stockpiled by many countries for use in the event of a pandemic. Unfortunately, recent studies have identified significant increases in the frequency of oseltamivir-resistant seasonal influenza A (H1N1) in Europe, the United States, Oceania and South Africa[5–7].

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are human herpes viruses belonging to family Herpesviridae[8]. These types of HSV are responsible for mucocutaneous infections, mainly in immunocompromised patients. HSV-1 provokes orofacial lesions, while HSV-2 causes mucocutaneous genital infections[9]. Antiviral research on HSV preliminarily focuses on compounds capable of targeting the viral polymerase. Acyclovir, a nucleoside inhibitor of DNA polymerase, was the first selective antiviral agent introduced. Acyclovir is still commonly employed in the treatment of HSV infection[10], but the widespread use of this drug has led to the development of viral resistance. Therefore, the search for new drugs against acyclovir-resistant HSV viruses is highly necessary.

Most current antiviral drugs, including those in development, are direct-acting antiviral (DAA) molecules that specifically target viral proteins. These drugs are narrow in spectrum and are vulnerable to the rapid emergence of viral resistance[11]. The emergence of drug-resistant viruses, especially multidrug-resistant strains, represents a significant problem in current clinical practice that needs to be addressed and should be considered a high priority for new avenues of research[12,13]. To fulfill all of these requirements, novel classes of antivirals are needed[14]. Additionally, due to the high mutation rates that are particularly prevalent in RNA viruses, the lifetime of specific antiviral therapeutics is often severely limited. Broad spectrum antivirals would be one way of circumventing this problem[15]. Therefore, a crucial need for developing of new agents with novel antiviral mechanisms and broad antiviral activities exists.

Our group has long been engaging antivirus drug research[16–18]. Previously, we have done high-throughput screening of self-owned compounds for drugs with anti-influenza activity, and have found a group of indole-2-carboxylate derivatives with potential anti-influenza activity. Analysis proved broad-spectrum antiviral activity of these compounds and suggested the need for further work with these agents. In the current work, a series of indole-2-carboxylate derivatives were synthesized and evaluated for their broad spectrum antiviral activity. Four virus strains, including herpes viruses (HSV-1), Influenza viruses (A/FM/1/47, B/Jifang/13/97) and picorna viruses (Cox B3) were selected to investigate *in vitro* antiviral activities of the synthesized indole-2-carboxylate derivatives. Antiviral activity experiments *in vitro* of hepatitis viruses (HBV and HCV) and retrovirus (HIV-1) are not yet complete.

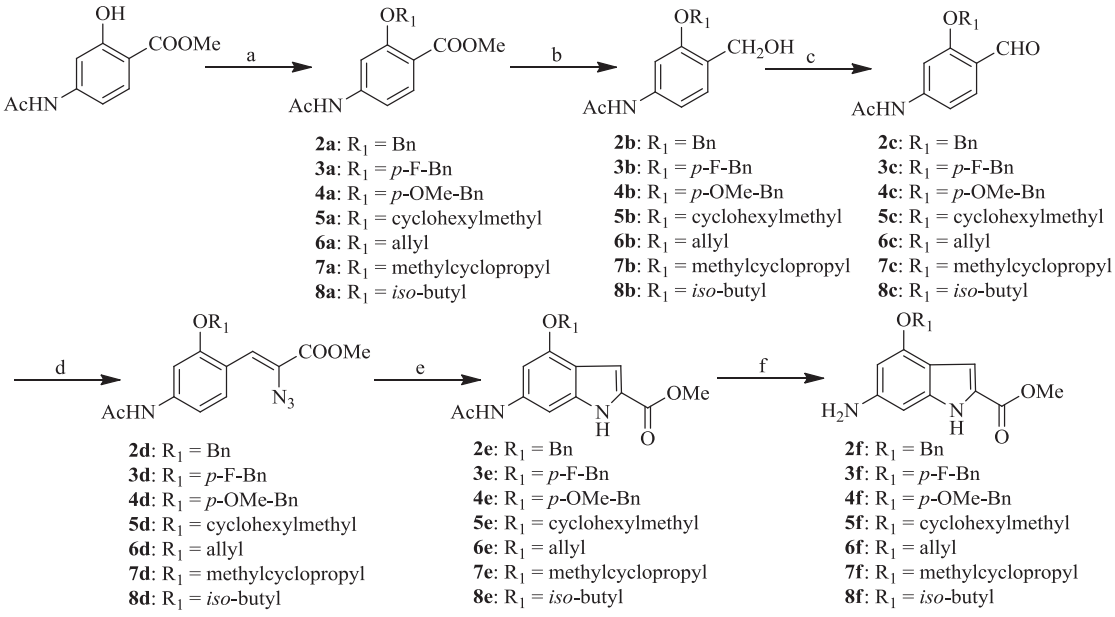
2. Results and discussion

2.1. Chemistry

The structure modification in this work was primarily made to the 4, 6 and 7-position of the indole ring. Compounds **1f–10f** with substituents at the 4 and 6-position of the indole ring were synthesized and screened for the antiviral activity. Compounds **12e** with alkoxyl substituents at the 6-position of indole ring were synthesized and screened for the antiviral activity. Additionally, compounds **11e**, **13e**, **14f**, **15e** without amino or alkoxyl group at the 4-position or the 6-position of indole scaffold were designed to probe the importance of alkoxyl group and amino group for the antiviral activity, respectively. Compounds **16f–18f** with alkoxyl and amino substituents at both 4 and 7-position were designed to gain an insight into the SAR at those positions.

As shown in Scheme 1, compounds **2f–8f** were synthesized according to a reported method with some modifications[19]. Methyl *p*-acetaminosalicylate was used as the starting material to react with a variety of alkyl halide affording compounds **2a–8a**. When substituted benzyl bromide or allyl bromide was used (**2b–4b**, **6b**), a mild base (anhydrous K_2CO_3) was potent enough to drive the reaction to completion. However, in the case of saturated alkyl iodide (**5b**, **7b**, **8b**), a strong base (NaH) was employed for a reasonable yield. Reduction of compounds **2a–8a** by lithium aluminum hydride ($LiAlH_4$) in tetrahydrofuran (THF) yielded corresponding alcohol derivatives **2b–8b**, which were further oxidized to afford compounds in the presence of a mild oxidation reagent ($BaMnO_4$). However, the use of $BaMnO_4$ required a prolonged reaction time (24 h) and resulted in moderate yields. The use of an alternative oxidant pyridinium dichromate (PDC)[20] reduced the reaction time to 2 h, and compounds **2c–8c** were obtained in high yields (85%–90%). During the aldehyde (**2c–8c**) condensing steps with methyl azidoacetate, the intermediate compounds with high polarity (*i.e.*, small Rf values of TLC) was observed, which were subsequently transformed into compounds **2d–8d**. Compounds **2d–8d** were not characterized in this work due to their instability. Thermolysis of compounds **2d–8d** in boiling xylene afforded the expected indole compounds **2e–8e** in 60%–70% yield over two steps. The acid cleavage of acetamide in a boiling hydrogen chloride solution of methanol produced compounds **2f–8f**.

As shown in Scheme 2, compounds **1e, 9e–18e** with various alkoxyl substituents at 4, 6 and 7 position of the indole scaffold were obtained using a method similar to that employed to synthesize compounds **2e–8e**, and compounds **1e, 9e, 10e, 14e, 16e–18e** were further deacetylated to afford compound **1f, 9f, 10f, 14f, 16f–18f**.



2a: R_1 = Bn
3a: R_1 = *p*-F-Bn
4a: R_1 = *p*-OMe-Bn
5a: R_1 = cyclohexylmethyl
6a: R_1 = allyl
7a: R_1 = methylcyclopropyl
8a: R_1 = *iso*-butyl

2b: R_1 = Bn
3b: R_1 = *p*-F-Bn
4b: R_1 = *p*-OMe-Bn
5b: R_1 = cyclohexylmethyl
6b: R_1 = allyl
7b: R_1 = methylcyclopropyl
8b: R_1 = *iso*-butyl

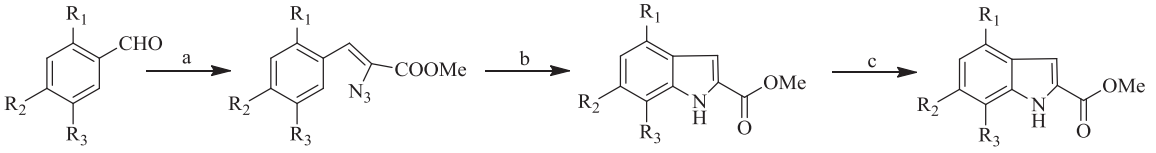
2c: R_1 = Bn
3c: R_1 = *p*-F-Bn
4c: R_1 = *p*-OMe-Bn
5c: R_1 = cyclohexylmethyl
6c: R_1 = allyl
7c: R_1 = methylcyclopropyl
8c: R_1 = *iso*-butyl

2d: R_1 = Bn
3d: R_1 = *p*-F-Bn
4d: R_1 = *p*-OMe-Bn
5d: R_1 = cyclohexylmethyl
6d: R_1 = allyl
7d: R_1 = methylcyclopropyl
8d: R_1 = *iso*-butyl

2e: R_1 = Bn
3e: R_1 = *p*-F-Bn
4e: R_1 = *p*-OMe-Bn
5e: R_1 = cyclohexylmethyl
6e: R_1 = allyl
7e: R_1 = methylcyclopropyl
8e: R_1 = *iso*-butyl

2f: R_1 = Bn
3f: R_1 = *p*-F-Bn
4f: R_1 = *p*-OMe-Bn
5f: R_1 = cyclohexylmethyl
6f: R_1 = allyl
7f: R_1 = methylcyclopropyl
8f: R_1 = *iso*-butyl

Scheme 1 Synthetic route of the target compounds **2f–8f**. Reagents and conditions: (a) K_2CO_3, acetone, R_3X (X = Br, I), reflux, 4–8 h, or NaH, DMF, R_3X, 80 °C, 8 h; 70%-90%; (b) $LiAlH_4$, dry THF, –5 °C, 2 h, 80%–90%; (c) PDC, CH_2Cl_2, reflux, 3 h, 90%; (d) N_3CH_2COOEt, MeONa/MeOH, −15 °C, 1 h, then, 0 °C, 20 h; (e) xylene, reflux, 2 h, 85%; (f) HCl (g)/MeOH, reflux, 3 h, 78%;



1d:R_1 = OMe, R_2 = NHAc, R_3 = H;
9d:R_1 = ethoxyl, R_2 = NHAc, R_3 = H;
10d:R_1 = *iso*-propoxyl, R_2 = NHAc, R_3 = H;
11d: R_1 = OMe, R_2 = H, R_3 = H;
12d: R_1 = OBn, R_2 = OMe, R_3 = H;
13d: R_1 = OBn, R_2 = H, R_3 = H;
14d: R_1 = H, R_2 = NHAc, R_3 = H;
15d: R_1 = H, R_2 = $N(Me)_2$, R_3 = H;
16d: R_1 = OMe, R_2 = H, R_3 = NHAc;
17d: R_1 = ethoxyl, R_2 = H, R_3 = NHAc;
18d: R_1 = *n*-propoxyl, R_2 = H, R_3 = NHAc;

1e:R_1 = OMe, R_2 = NHAc, R_3 = H;
9e:R_1 = ethoxyl, R_2 = NHAc, R_3 = H;
10e:R_1 = *iso*-propoxyl, R_2 = NHAc, R_3 = H;
11e: R_1 = OMe, R_2 = H, R_3 = H;
12e: R_1 = OBn, R_2 = OMe, R_3 = H;
13e: R_1 = OBn, R_2 = H, R_3 = H;
14e: R_1 = H, R_2 = NHAc, R_3 = H;
15e: R_1 = H, R_2 = $N(Me)_2$, R_3 = H;
16e: R_1 = OMe, R_2 = H, R_3 = NHAc;
17e: R_1 = ethoxyl, R_2 = H, R_3 = NHAc;
18e: R_1 = *n*-propoxyl, R_2 = H, R_3 = NHAc;

1f:R_1 = OMe, R_2 = NH_2, R_3 = H;
9f:R_1 = ethoxyl, R_2 = NH_2, R_3 = H;
10f:R_1 = *iso*-propoxyl, R_2 = NH_2, R_3 = H;
14f: R_1 = H, R_2 = NH_2, R_3 = H;
16f: R_1 = OMe, R_2 = H, R_3 = NH_2;
17f: R_1 = ethoxyl, R_2 = H, R_3 = NH_2;
18f: R_1 = *n*-propoxyl, R_2 = H, R_3 = NH_2;

Scheme 2 Synthetic route of the target compounds **1f, 9f–10f, 14f, 16f–18f**. Reagents and conditions: (a) N_3CH_2COOEt, MeONa/MeOH, −15 °C, 1 h, then, 0 °C, 20 h; (b): xylene, reflux, 2 h, 85%; (c) HCl(g)/MeOH, reflux, 3 h, 78%.

2.2. *Antiviral activity*

The synthesized compounds were assayed for their broad spectrum antiviral activity towards influenza A, influenza B, HSV-1 and Cox B3 virus *in vitro*. Specifically, the activity against influenza A and Cox B3 was determined by the cytopathic effect (CPE) inhibitory assay, and oseltamivir and ribavirin (RBV) were used as positive controls, respectively. A total of 22 compounds were evaluated, and the results are summarized in Table 1.

2.2.1. *Anti Cox B3 virus activity*

As shown in Table 1, 50% of the synthesized compounds showed potent inhibitory activity towards Cox B3 at low micromolar concentrations, especially compounds **2f** (IC_{50} = 1.59 μmol/L), **3f** (IC_{50} = 4.55 μmol/L), **8f** (IC_{50} = 7.18 μmol/L) and **17f** (IC_{50} = 10.56 μmol/L). The IC_{50} value of RBV (the positive control) was 1058.68 μmol/L. Of all the compounds tested, compound **8f** showed the highest SI value (17.1). Compounds **15e**, **6f** and **18f** exhibited moderate levels of inhibitory activity against the Cox B3 virus, with IC_{50} values less then 50 μmol/L.

Given that compounds **14e**, **14f** and **15e** showed anti-Cox B3 activity, we concluded that the 4-alkyloxy substituent of the indole ring was not crucial to antiviral activity. Relocation of the 6-amino substituent to the 7-position retained activity. Acetyl substituent at the amino group (**1e** and **2e**) disfavored the antiviral activities.

2.2.2. *Anti HSV-1 virus activity*

HSV-1 was less sensitive to the presently-synthesized compounds compared to influenza A and Cox B3 virus. Only compounds **15e** and **17f** showed activity towards HSV-1.

Table 1 The antiviral activity and cytotoxicity of the compounds.

Compd.	Cox B3			HSV-1			A/FM/1/47			B/Jifang/13/97		
	TC_{50} (μmol/L)	$158TCID_{50}$		TC_{50} (μmol/L)	$100TCID_{50}$		TC_{50} (μmol/L)	$158TCID_{50}$		TC_{50} (μmol/L)	$100TCID_{50}$	
		IC_{50} (μmol/L)	SI		IC_{50} (μmol/L)	SI		IC_{50} (μmol/L)	SI		IC_{50} (μmol/L)	SI
1e	763.36	>254.47	–	763.36	>254.47	–	763.36	254.47	3.0	763.36	254.47	3.0
2e	45.59	>21.92	–	45.59	>21.92	–	341.63	>65.73	–	NT	NT	NT
8e	50.69	>24.38	–	50.69	>24.38	–	24.38	8.13	3.0	24.38	>8.13	–
11e	225.46	108.39	2.1	225.46	>108.39	–	225.46	62.59	3.6	225.46	>108.39	–
12e	49.55	>7.94	–	49.55	>7.94	–	92.06	23.83	3.9	92.06	>23.83	–
13e	54.84	>8.79	–	54.84	>8.79	–	136.98	26.37	5.2	136.98	>26.37	–
14e	597.71	72.80	8.2	597.71	>287.37	–	414.44	95.78	4.3	414.44	>95.78	–
15e	212.02	44.72	4.7	212.02	101.93	2.1	711.97	101.93	7.0	711.97	>101.93	–
1f	174.95	>33.68	–	174.95	>33.68	–	174.95	33.68	5.2	174.95	>33.68	–
2f	14.51	1.59	9.1	14.51	>2.78	–	108.64	>25.12	–	NT	NT	NT
3f	16.37	4.55	3.6	16.37	>7.87	–	273.57	>70.76	–	NT	NT	NT
4f	52.91	>7.58	–	52.91	>7.58	–	354.20	>204.51	–	NT	NT	NT
5f	11.75	>2.71	–	11.75	>8.15	–	14.13	>8.15	–	NT	NT	NT
6f	210.33	30.12	7.0	210.33	>30.12	–	130.28	30.12	4.3	130.28	>30.12	–
7f	177.77	49.35	3.6	177.77	>85.46	–	177.77	>85.46	-	NT	NT	NT
8f	122.33	7.18	17.1	122.33	>28.28	–	40.76	9.43	4.3	40.76	>9.43	–
9f	94.96	>31.67	–	94.96	>31.67	–	65.85	18.29	3.6	65.85	>31.67	–
10f	625.85	>89.60	–	625.85	>89.60	–	465.60	51.73	9.0	465.60	>89.60	–
14f	243.26	67.53	3.6	243.26	>116.95	–	90.79	7.53	12.1	90.79	13.00	7.0
16f	145.68	>33.68	–	145.68	>33.68	–	11.23	>3.73	–	NT	NT	NT
17f	18.29	10.56	1.7	31.67	10.56	3.0	31.67	>10.56	–	NT	NT	NT
18f	115.44	29.88	3.9	115.44	>29.88	-	5.77	>3.31	–	NT	NT	NT
RBV	8190.01	1058.68	7.7	NT	NT	NT	4766.99	2.58	1847.8	4766.99	10.11	471.3
ACV	NT	NT	NT	>444.05	1.82	>243.9	NT	NT	NT	NT	NT	NT
Oseltamivir	NT	NT	NT	NT	NT	NT	4033.29	6.43	626.9	4033.29	115.04	35.1

TC_{50}, 50% cytotoxic concentration; IC_{50}, 50% inhibition concentration; SI, the selectivity index. NT means not tested.

2.2.3. *Anti-influenza virus activity*

As shown in Table 1, the majority of the synthesized compounds exhibited potent antiviral activity towards influenza A/FM/1/47 virus. This was particularly true for compounds **8e** (IC_{50} = 8.13 μmol/L), **8f** (IC_{50} = 9.43 μmol/L) and **14f** (IC_{50} = 7.53 μmol/L). These IC_{50} values were comparable to those of the positive control drugs RBV (IC_{50} = 2.58 μmol/L) and oseltamivir (IC_{50} = 6.43 μmol/L). Of all the compounds tested, compound **14f** showed both potent inhibitory activity against influenza A (IC_{50} = 7.53 μmol/L) and the highest SI value (12.1). Compounds **12e**, **13e**, **1f**, **6f** and **10f** exhibited moderate levels of inhibitory activity against the A/FM/1/47 strain of the influenza virus, with IC_{50} values less then 52 μmol/L.

The compounds exhibiting activity against the A/FM/1/47 (H1N1) strain of the influenza virus were further evaluated for their potential antiviral activity against strain of the influenza B virus, also using the CPE method. Compounds **1e** and **14f** also displayed antiviral activity against the influenza B strain. Compound **14f** (IC_{50} = 13.00 μmol/L) was found to be the most effective compound against the B/Jingfang/13/97 strain of the influenza virus, with an IC_{50} value comparable to that of the positive control RBV (IC_{50} = 10.11 μmol/L).

In terms of the structure-activity relationships, substitution of the benzyloxy (**2f–4f**) for the alkyloxy (**8f–10f**) at the 4-position of the indole ring decreased the anti-influenza A activity. Replacement of the amino group at the 6-position of the indole ring (**1f, 9f**) with hydrogen (**11e, 16f** and **17f**) resulted in a dramatic decrease in the anti-influenza A activity. Thus, the 6-amino group in the indole ring is indispensable to the antiviral activities. Additionally, introduction of the acetyl substituent at the amino group (**1e** and **2e**) disfavored the anti-influenza A activity. Given that compounds **14e**, **14f** and **15e** showed anti-influenza A activity, we concluded that the alkyloxy at the 4-position of indole ring was not crucial to the anti-influenza A activities. In addition, as the activity of compound **8f** was superior to that of **10f**, it can be concluded that a more bulky alkyloxy substituent at the 4-position of indole ring favored the anti-influenza A activity.

3. Conclusions

In summary, a total of 22 indole-2-carboxylate derivatives were designed, synthesized and screened for antiviral activities towards influenza A, influenza B, HSV-1 and Cox B3. In general, the target compounds were more effective against RNA viruses (influenza A and Cox B3) than against the DNA virus (HSV-1). The majority of the synthesized compounds simultaneously exhibited potent activity against the influenza A and Cox B3 viruses. A similar trend was observed among the SARs of the synthesized compounds towards the RNA virus. For example, the alkyloxy at the 4-position of indole ring was not crucial to the antiviral activities. Acetyl substituent at the amino group also disfavored the antiviral activities. Notably, compounds **8f** and **14f** showed potent antiviral activity towards the RNA virus at low micromolar concentrations. Compound **8f** showed the highest SI value (17.1) to Cox B3 virus. Compound **14f** showed both potent inhibitory activity against influenza A (IC_{50} = 7.53 μmol/L) and the highest SI value (12.1). The detailed structure optimization of compound **8f** and **14f** and mechanistic studies are ongoing in our laboratory.

4. Experimental

4.1. *Synthesis and characterization*

^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as the internal standard in DMSO-d_6 or $CDCl_3$ with a Bruker BioSpin GmbH spectrometer. The mass spectra (MS) were recorded on a Thermo Scientific LTQ ORBITRAP instrument with an ESI mass selective detector. Melting points (m.p.) were determined using an SRS-OptiMelt automated melting point instrument, without correction. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co., Ltd.

4.1.1. *General procedure A for the preparation of **2a–8a***

Methyl 4-acetamido-2-hydroxybenzoate (20 mmol) was dissolved in anhydrous DMF (50 mL), followed by the addition of anhydrous K_2CO_3 (30 mmol) or NaH (30 mmol) and alkylation agents (30 mmol). The mixture was heated to 60 °C for 4–6 h, and the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated *in vacuum*, and the resulting residue was recrystallized from ethyl acetate and petroleum ether to afford the target compounds.

Methyl 4-acetamido-2-(benzyloxy)benzoate (**2a**): white solid, yield, 90%; m.p. 107–108 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.18 (s, 3H), 3.88 (s, 3 H), 5.18 (s, 2 H), 6.82 (d, 1H, J = 8.4 Hz), 7.30 (t, 1H, J = 7.6 Hz), 7.38 (t, 2H, J = 7.6 Hz), 7.51 (d, 2H, J = 7.6 Hz), 7.69 (s, 1H), 7.83 (d, 1H, J = 8.4 Hz).

Methyl 4-acetamido-2-(4-fluorobenzyloxy)benzoate (**3a**): white solid, yield, 85%; m.p. 132–133 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 3H), 3.88 (s, 3H), 5.15 (s, 2H), 6.76 (dd, 1H, J = 8.4, 1.6 Hz), 7.06 (m, 2H), 7.50 (m, 2H), 7.76 (s, 1H), 7.83 (d, 1H, J = 8.4 Hz)

Methyl 4-acetamido-2-(4-methoxybenzyloxy)benzoate (**4a**): white solid, yield, 90%. m.p. 124–125 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.05 (s, 3H), 3.73 (s, 3H), 3.74 (s, 3H), 5.03 (s, 2H), 6.94 (d, 2H, J = 8.4 Hz), 7.16 (d, 1H, J = 8.4 Hz), 7.40 (d, 2H, J = 8.4 Hz), 7.56 (s, 1H), 7.66 (d, 1H, J = 8.4 Hz), 10.19 (s, 1H).

Methyl 4-acetamido-2-(cyclohexylmethoxy)benzoate (**5a**): white solid, yield, 65%; m.p. 132–133 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05–1.32 (m, 5H), 1.68–1.90 (m, 6H), 2.23 (s, 3H), 3.83 (d, 2H, J = 6.0 Hz), 3.87 (s, 3H), 6.79 (d, 1H, J = 8.4 Hz), 7.29 (s, 1H), 7.53 (s, 1H), 7.80 (d, 1H, J = 8.4 Hz).

Methyl 4-acetamido-2-(allyloxy)benzoate (**6a**): white solid, yield, 90%; m.p. 114–115 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.06 (s, 3H), 3.74 (s, 3H), 4.54 (d, 2H, J = 6.4 Hz), 5.25 (d, 1H, J = 6.4 Hz), 5.51 (d, 1H, J = 16.0 Hz), 6.02 (m, 1H), 7.16 (dd, 1H, J = 8.4, 1.6 Hz), 7.47 (d, 1H, J = 1.6 Hz), 7.66 (d, 1H, J = 8.4 Hz), 10.18 (s, 1H).

Methyl 4-acetamido-2-(cyclopropylmethoxy)benzoate (**7a**): white solid, yield 78%; m.p. 127–128 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.38 (t, 2H, J = 4.8 Hz), 0.59–0.64 (m, 2H), 1.30 (t, 1H, J = 6.0 Hz), 2.19 (s, 3H), 3.87 (s, 3H), 3.92 (d, 2H, J = 6.4 Hz), 6.79 (d, 1H, J = 8.4 Hz), 7.33 (s, 1H), 7.59 (s, 1H), 7.78 (d, 1H, J = 8.4 Hz).

Methyl 4-acetamido-2-isobutoxybenzoate (**8a**): white solid, yield, 70%; m.p. 112–113 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00 (d, 6H, J = 6.8 Hz), 2.01 (s, 3H), 2.03–2.05 (m, 1H), 3.72 (d, 2H, J = 7.2 Hz), 3.74 (s, 3H), 7.15 (dd, 1H, J = 8.4, 2.0 Hz), 7.44 (d, 1H, J = 2.0 Hz), 7.64 (d, 1H, J = 8.4 Hz), 10.16 (s, 1H).

4.1.2. General procedure B for the preparation of compounds **2b–8b**

A suspension of $LiAlH_4$ (25 mmol) in anhydrous THF (100 mL) was added dropwise a solution of compounds **2a–8a** (20 mmol) in anhydrous THF (50 mL), maintaining the reaction temperature blow 0 °C. After completion of the addition, the reaction mixture was warmed up to room temperature for another 2 h, and was cooled again with ice bath. $Na_2SO_4 \cdot 7H_2O$ was added to quench the reaction, and the resulting mixture was stirred for another 2 h at room temperature. After that, the mixture was filtered, and the filtrate was concentrated *in vacuum* to give a white solid, which was triturated with ether to afford the target compounds.

N-(3-(Benzyloxy)-4-(hydroxymethyl)phenyl)acetamide (**2b**): white solid, yield, 85%; m.p. 115–116 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.02 (s, 3 H), 4.48 (d, 2H, J=5.6 Hz), 4.89 (t, 1H, J=5.6 Hz), 5.05 (s, 2 H), 7.12 (dd, 1H, J=1.6, 8.0 Hz), 7.26 (d, 1H, J=8.0 Hz), 7.32–7.46 (m, 6H), 9.87 (s, 1H).

N-(3-(4-Fluorobenzyloxy)-4-(hydroxymethyl)phenyl)acetamide (**3b**): white solid, yield, 80%; m.p. 124–125 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.01 (s, 3H), 4.46 (d, 2 H, J=5.6 Hz), 4.87 (t, 1H, J=5.6 Hz), 5.02 (s, 2 H), 7.09 (d, 1H, J=7.6 Hz), 7.22 (m, 3H), 7.37 (s, 1H), 7.49 (dd, 2H, J=8.0, 5.6 Hz), 9.86 (s, 1H).

N-(4-(Hydroxymethyl)-3-(4-methoxybenzyloxy)phenyl)acetamide (**4b**): white solid, yield, 85%. m.p. 140–142 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.01 (s, 3H), 3.75 (s, 3H), 4.43 (d, 2H, J=5.6 Hz), 4.85 (t, 1H, J=5.6 Hz), 4.95 (s, 2 H), 6.93 (d, 2H, J=8.4 Hz), 7.08 (d, 1H, J=8.4 Hz), 7.23 (d, 1H, J=8.4 Hz), 7.36 (d, 3H, J=8.4 Hz), 9.85 (s, 1H).

N-(3-(Cyclohexylmethoxy)-4-(hydroxymethyl)phenyl)acetamide (**5b**): white solid, yield, 79%; m.p. 112–114 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.03–1.29 (m, 5H), 1.68–1.80 (m, 6H), 2.00 (s, 3H), 3.68 (d, 2H, J=6.0 Hz), 4.42 (d, 2H, J=5.2 Hz), 4.83 (t, 1H, J=5.2 Hz), 7.07 (dd, 1H, J= 8.4, 1.6 Hz), 7.21 (d, 1H, J=8.4 Hz), 7.24 (d, 1H, J=1.6 Hz), 9.82 (s, 1H).

N-(3-(Allyloxy)-4-(hydroxymethyl)phenyl)acetamide (**6b**): white solid, yield, 75%; m.p. 129–131 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.01 (s, 3H), 4.44 (d, 2H, J=6.4 Hz), 4.49 (d, 2H, J=5.6 Hz), 4.86 (t, 1H, J=5.6 Hz), 5.25 (d, 1H, J=6.4 Hz), 5.40 (d, 1H, J=17.2 Hz), 6.01–6.07 (m, 1H), 7.08 (dd, 1H, J=8.4, 2.0 Hz), 7.23 (d, 1H, J=8.4 Hz), 7.28 (d, 1H, J=1.6 Hz), 9.85 (s, 1H).

N-(3-(Cyclopropylmethoxy)-4-(hydroxymethyl)phenyl)acetamide (**7b**): white solid, yield, 79%. m.p. 120–121 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.30–0.34 (m, 2H), 0.52-0.57 (m, 2H), 1.19-1.22 (m, 1H), 2.20 (s, 3H), 3.76 (d, 2H, J=6.8 Hz), 4.43 (d, 2H, J=5.6 Hz), 4.83 (t, 1H, J=5.6 Hz), 7.06 (dd, 1H, J=8.4, 1.6 Hz), 7.22 (d, 1H, J=8.4 Hz), 7.25 (d, 1H, J=1.6 Hz), 9.82 (s, 1H).

N-(4-(Hydroxymethyl)-3-isobutoxyphenyl)acetamide (**8b**): white solid, yield, 85%; m.p. 120–121 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.97 (d, 6H, J=6.8 Hz), 2.00 (s, 3H), 2.02-2.03 (m, 1H), 3.65 (d, 2H, J=6.4 Hz), 4.44 (d, 2H, J=5.6 Hz), 4.84 (t, 1H, J=5.6 Hz), 7.06 (dd, 1H, J=8.4, 2.0 Hz), 7.22 (d, 1H, J=8.4 Hz), 7.26 (d, 1H, J=2.0 Hz), 9.83 (s, 1H).

4.1.3. General procedure C for the preparation of compounds **2c–8c**

Compounds **2b**-**8b** (20 mmol) was dissolved in CH_2Cl_2 (100 mL), and PDC (30 mmol) was added. The resulting mixture was heated under reflux for 2 h. The reaction mixture was cooled and filtered through a thin layer of silica gel, and the filtrate was concentrated *in vacuum*. The residue was recrystallized from ethyl acetate and petroleum ether to afford the target compounds as white solid.

N-(3-(Benzyloxy)-4-formylphenyl)acetamide (**2c**): white solid, yield, 90%; m.p. 126-128 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.06 (s, 3H), 5.21 (s, 2H), 7.21 (d, 1H, J=8.4 Hz), 7.35 (t, 1H, J=7.6 Hz), 7.42 (t, 2H, J=7.6 Hz), 7.51 (d, 2H, J=7.6 Hz), 7.65 (s, 1H), 7.68 (d, 1H, J=8.4 Hz), 10.25 (s, 1H), 10.34 (s,1H).

N-(3-(4-Fluorobenzyloxy)-4-formylphenyl)acetamide (**3c**): white solid, yield, 87%; m.p. 158-160 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.22 (s, 3H), 5.16 (s, 2H), 6.69 (d, 1H, J=8.0 Hz), 7.08 (m, 2H), 7.35 (s, 1H), 7.43 (dd, 2H, J=8.4, 5.6 Hz), 7.79 (d, 1H, J=8.0 Hz), 7.93 (s, 1H), 10.34 (s, 1H).

N-(4-Formyl-3-(4-methoxybenzyloxy)phenyl)acetamide (**4c**): white solid, yield, 86%. m.p. 158-160 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.08 (s, 3H), 3.74 (s, 3H), 5.11 (s, 2H), 6.94 (d, 2H, J=8.4 Hz), 7.18 (d, 1H, J=8.4 Hz), 7.42 (d, 2H, J=8.4 Hz), 7.63 (d, 1H, J=8.4 Hz), 7.69 (s, 1H), 10.20 (s, 1H), 10.33 (s, 1H).

N-(3-(Cyclohexylmethoxy)-4-formylphenyl)acetamide (**5c**): white solid, yield, 90%, m.p. 156-157 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06-1.27 (m, 5H), 1.70-1.83 (m, 6H), 2.07 (s, 3H), 3.85 (d, 2H, J=6.0 Hz), 7.17 (d, 1H, J=8.4 Hz), 7.53 (s, 1H), 7.62 (d, 1H, J=8.4 Hz), 10.22 (s, 1H), 10.30 (s, 1H).

N-(3-(Allyloxy)-4-formylphenyl)acetamide (**6c**): white solid. yield, 90%; m.p. 121-122 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.08 (s, 3H), 4.65 (d, 2H, J=6.8 Hz), 5.30 (d, 1H, J=6.4 Hz), 5.48 (d, 1H, J=16.0 Hz), 6.05-6.12 (m, 1H), 7.17 (dd, 1H, J=8.4, 1.6 Hz), 7.58 (d, 1H, J=1.6 Hz), 7.64 (d, 1H, J=8.4 Hz), 10.24 (s, 1H), 10.32 (s, 1H).

N-(3-(Cyclopropylmethoxy)-4-formylphenyl)acetamide (**7c**): white solid, yield, 82%. m.p. 152–153 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.37 (t, 2H, J=4.8 Hz), 0.56-0.60 (m, 2H), 1.26-1.29 (m, 1H), 2.07 (s, 3H), 3.92 (d, 2H, J=6.8 Hz), 7.15 (d, 1H, J=8.4 Hz), 7.55 (s, 1H), 7.62 (d, 1H, J=8.4 Hz), 10.24 (s, 1H), 10.30 (s, 1H).

N-(4-Formyl-3-isobutoxyphenyl)acetamide (**8c**): white solid, yield, 84%, m.p. 133-134 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00 (d, 6H, J=6.8 Hz), 2.07 (s, 3H), 2.10-2.11 (m, 1H), 3.82 (d, 2H, J=6.4 Hz), 7.15 (d, 1H, J=8.4 Hz), 7.56 (s, 1H), 7.63 (d, 1H, J=8.4 Hz), 10.24 (s, 1H), 10.31 (s, 1H).

4.1.4. General procedure D for the preparation of compounds **1e–18e**

To a solution of MeONa (30 mmol) in MeOH (80 mL) were added aromatic aldehydes (20 mmol) and methyl 2-azidoacetate (200 mmol) in anhydrous THF (80 mL) at –15 °C, and the mixture was stirred for another 4 h. After that, the reaction was quenched by the addition of saturated ammonium chloride solution, and extracted with ethyl acetate (3 × 100 mL). The combined organic layer was washed with brine, and dried over anhydrous $MgSO_4$, filtered and concentrated, and the residue was triturated with MeOH to give the intermediate compounds **1d–18d** as yellow solids. The crude compounds **1d–18d** were dissolved in xylene, and heated under reflux for 2 h. After cooling, white solids were crystallized. Filtration afforded the target compounds.

Methyl 6-acetimidamido-4-methoxy-1*H*-indole-2-carboxylate (**1e**): white solid, yield, 84%. m.p. 237–239 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.05 (s, 3H), 3.82 (s, 3H), 3.85 (s, 3H), 6.76 (s, 1H), 7.01 (s, 1H), 7.60 (s, 1H), 9.93 (s, 1H), 11.77 (s, 1H). HRMS (ESI$^+$): found 263.10238 (Calcd. for $C_{13}H_{15}O_4N_2$ $[M+H]^+$: 263.10263).

Methyl 6-acetamido-4-(benzyloxy)-1*H*-indole-2-carboxylate (**2e**): white solid, yield, 60%; m.p. 264-265 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.04 (s, 3H), 3.83 (s, 3H), 5.18 (s, 2H), 6.78 (s, 1H), 7.07 (s, 1H), 7.36 (t, 1H, J=7.6 Hz), 7.42 (t, 2H, J =7.6 Hz), 7.51 (d, 2H, J=7.6 Hz), 7.62 (s, 1H), 9.92 (s, 1H), 11.79 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 24.62, 52.05, 69.50, 95.76, 105.79, 115.05, 125.86, 127.83, 128.27, 128.93, 137.45, 138.16, 139.37, 152.81, 161.90, 168.60. HRMS (ESI$^+$): found 339.13379 (Calcd. for $C_{19}H_{19}O_4N_2$ [M+H]$^+$: 339.13393).

Methyl 6-acetamido-4-(4-fluorobenzyloxy)-1*H*-indole-2-carboxylate (**3e**): white solid, yield, 60%; m.p. 273-273 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.03 (s, 3H), 3.82 (s, 3H), 5.15 (s, 2H), 6.78 (s, 1H), 7.05 (s, 1H), 7.23 (t, 2H, J=8.4 Hz), 7.55 (dd, 2H, J= 5.6, 8.4 Hz), 7.60 (s, 1H), 9.92 (s, 1H), 11.79 (s, 1H). HRMS (ESI$^+$): found 357.12429 (Calcd. for $C_{19}H_{18}O_4N_2F$ [M+H]$^+$: 357.12451).

Methyl 6-acetimidamido-4-(4-methoxyphenoxy)-1*H*-indole-2-carboxylate (**4e**): pale yellow solid, yield, 80%. m.p. 268-270 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.03 (s, 3H), 3.76 (s, 3H), 3.81 (s, 3H), 5.08 (s, 2H), 6.77 (s, 1H), 6.95 (d, 1H, J=8.4 Hz), 7.01 (s, 1H), 7.42 (d, 1H, J=8.4 Hz), 7.59 (s, 1H), 9.91 (s, 1H), 11.77 (s, 1H). HRMS (ESI$^+$): found 391.12626 (Calcd. for $C_{20}H_{20}O_5N_2Na$ [M+Na]$^+$: 391.12644).

Methyl 6-acetamido-4-(cyclohexylmethoxy)-1*H*-indole-2-carboxylate (**5e**): pale yellow solid, yield, 63%; m.p. 253-255 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.04-1.30 (m, 5H), 1.65-1.75 (m, 6H), 2.02 (s, 3H), 3.81 (s, 3H), 3.82 (d, 2H, J=6.0 Hz), 6.63 (s, 1H), 7.01 (s, 1H), 7.57 (s, 1H), 9.87 (s, 1H), 11.74 (s, 1H). HRMS (ESI$^+$): found 345.18094 (Calcd. for $C_{19}H_{25}O_4N_2$ [M+H]$^+$: 345.18088).

Methyl 6-acetamido-4-(allyloxy)-1*H*-indole-2-carboxylate (**6e**): pale yellow solid, yield, 62%; m.p. 200-201 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.01 (s, 3H), 3.82 (s, 3H), 4.61 (d, 2H, J=5.2 Hz), 5.28 (d, 1H, J=6.4 Hz), 5.45 (d, 1H, J=17.2 Hz), 6.06-6.14 (m, 1H), 6.68 (s, 1H), 7.03 (s, 1H), 7.59 (s, 1H), 9.90 (s, 1H), 11.77 (s, 1H). HRMS (ESI$^+$): found 289.11805 (Calcd. for $C_{15}H_{17}O_4N_2$ [M+H]$^+$: 289.11828).

Methyl 6-acetimidamido-4-cyclopropoxy-1*H*-indole-2-carboxylate (**7e**): Pale white solid, yield, 84%. m.p. 240-241 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.35-0.39 (m, 2H), 0.57-0.60 (m, 2H), 1.30-1.33 (m, 1H), 2.02 (s, 3H), 3.82 (s, 3H), 3.88 (d, 2H, J=6.8 Hz), 6.64 (s, 1H), 7.01 (s, 1H), 7.56 (s, 1H), 9.87 (s, 1H), 11.74 (s, 1H). HRMS (ESI$^+$): found 325.11577 (Calcd. for $C_{16}H_{18}O_4N_2Na$ [M+Na]$^+$: 325.11588).

Methyl 6-acetamido-4-isobutoxy-1*H*-indole-2-carboxylate (**8e**): white solid, yield, 66%; m.p. 107–108 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.01 (d, 6H, J=6.8 Hz), 2.03 (s, 3H), 2.06-2.11 (m, 1H), 3.78 (d, 2H, J=6.4 Hz), 3.82 (s, 3H), 6.65 (s, 1H), 7.01 (s, 1H), 7.57 (s, 1H), 9.88 (s, 1H), 11.74 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 19.52, 24.62, 28.19, 52.02, 74.13, 95.33, 105.74, 114.99, 125.69, 138.26, 139.34, 153.31, 161.93, 168.58. HRMS (ESI$^+$): found 305.14938 (Calcd. for $C_{16}H_{21}O_4N_2$ [M+H]$^+$: 305.14958).

Methyl 6-acetimidamido-4-ethoxy-1*H*-indole-2-carboxylate (**9e**): white solid, yield, 76%. m.p. 205-207 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.33 (s, 3H), 2.02 (s, 3H), 3.87 (s, 3H), 3.95 (s, 2H), 6.78 (s, 1H), 7.06 (s, 1H), 7.65 (s, 1H), 9.63 (s, 1H), 11.70 (s, 1H). HRMS (ESI$^+$): found 277.11863, (Calcd. for $C_{14}H_{17}O_4N_2$ [M+H]$^+$: 277.11828).

Methyl 6-acetimidamido-4-isopropoxy-1*H*-indole-2-carboxylate (**10e**): pale white solid, yield, 72%. m.p. 196-197 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38 (s, 6H), 2.03 (s, 3H), 3.88 (s, 3H), 4.04 (s, 1H), 6.88 (s, 1H), 7.12 (s, 1H), 7.63 (s, 1H), 9.69 (s, 1H), 11.65 (s, 1H). HRMS (ESI$^+$): found 291.13396 (Calcd. for $C_{15}H_{19}O_4N_2$ [M+H]$^+$: 291.13393).

Methyl 4-methoxy-1*H*-indole-2-carboxylate (**11e**): white solid, yield, 65%; m.p. 143-145 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 3H), 3.88 (s, 3H), 6.53 (d, 1H, J=7.6 Hz), 7.02 (d, 1H, J=7.6 Hz), 7.09 (s, 1H), 7.18 (t, 1H, J=7.6 Hz), 11.92 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 52.36, 55.34, 100.05, 105.36, 105.99, 118.37, 126.23, 126.33, 139.21, 154.28, 162.05. HRMS (ESI$^+$): found 206.08092 (Calcd. for $C_{11}H_{12}O_3N$ [M+H]$^+$: 206.08117).

Methyl 4-(benzyloxy)-6-methoxy-1*H*-indole-2-carboxylate (**12e**): white solid, yield, 85%. m.p. 182-183 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.74 (s, 3H), 3.81 (s, 3H), 5.20 (s, 2H), 6.28 (s, 1H), 6.46 (s, 1H), 7.03 (s, 1H), 7.32 (t, 1H, J=7.2 Hz), 7.39 (t, 2H, J=7.2 Hz), 7.48 (d, 2H, J=7.2 Hz), 11.69 (s, 1H). HRMS (ESI$^+$): found 312.12275 (Calcd. for $C_{18}H_{18}O_4N$ [M+H]$^+$: 312.12303).

Methyl 4-(benzyloxy)-1*H*-indole-2-carboxylate (**13e**): white solid, yield, 82%. m.p. 192-193 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 3H), 5.24 (s, 2H), 6.63 (d, 1H, J=7.6 Hz), 7.03 (d, 1H, J=7.6 Hz), 7.13 (s, 1H), 7.16 (t, 1H, J=7.6 Hz), 7.36 (t, 1H, J=7.6 Hz), 7.41 (t, 2H, J=7.6 Hz), 7.52 (d, 2H, J=7.6 Hz), 11.94 (s, 1H). HRMS (ESI$^+$): found 282.11227 (Calcd. for $C_{17}H_{16}O_3N$[M+H]$^+$: 282.11247).

Methyl 6-acetamido-1*H*-indole-2-carboxylate (**14e**): white solid, yield, 70%; m.p. 235-237 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.05 (s, 3H), 3.83 (s, 3H), 7.07 (s, 1H), 7.10 (d, 1H, J=8.8 Hz), 7.52 (d, 1H, J=8.8 Hz), 8.01 (s, 1H), 9.95 (s, 1H), 11.75 (s, 1H). HRMS (ESI$^+$): found 233.09207 (Calcd. for $C_{12}H_{13}O_3N_2$ [M+H]$^+$: 233.09188).

Methyl 6-(dimethylamino)-1*H*-indole-2-carboxylate (**15e**): brown solid, yield, 67%; m.p. 177–178 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.91 (s, 6H), 3.80 (s, 3H), 6.56 (s, 1H), 6.74 (dd, 1H, J=8.8, 2.0 Hz), 6.99 (s, 1H), 7.43 (d, 1H, J=8.8 Hz), 11.34 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 41.22, 51.83, 93.62, 108.90, 111.03, 119.10, 122.57, 124.94, 139.97, 149.58, 161.97. HRMS (ESI$^+$): found 219.11261 (Calcd. for $C_{12}H_{15}O_2N_2$ [M+H]$^+$: 219.11280).

*4.1.5. General procedure E for the preparation of compounds **1f–10f, 14f, 16f–18f***

A solution of compounds ***1e–3e, 5e–10e, 14e, 16e–18e*** (10 mmol) in MeOH (50 mL) presaturated with hydrogen chloride was heated under reflux for 2 h. After cooling, the solution was poured into saturated sodium hydrogen carbonate (200 mL), and extracted with ethyl acetate (3 × 80 mL). The combined organic layer was dried over $MgSO_4$. Filtered and concentrated, the residue was recrystallized from anhydrous EtOH to afford the target compounds.

Methyl 6-amino-4-methoxy-1*H*-indole-2-carboxylate (**1f**): pale yellow solid, yield, 78%. m.p. 202-203 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H), 3.88 (s, 3H), 6.40 (s, 1H), 6.92 (s, 1H), 7.06 (s, 1H), 9.49 (br, 2H), 12.01 (s, 1H). HRMS (ESI$^+$): found 221.09183 (Calcd. for $C_{11}H_{13}O_3N_2$ [M+H]$^+$: 221.09207).

Methyl 6-amino-4-(benzyloxy)-1*H*-indole-2-carboxylate (**2f**): pale yellow solid, yield, 75%; m.p. 222-223 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H), 5.12 (s, 2H), 5.16 (s, 2H), 6.06 (s, 1H), 6.14 (s, 1H), 6.95 (s, 1H), 7.36 (t, 1H, J=7.6Hz),7.41 (t, 2H, J=7.6 Hz), 7.49 (d, 2H, J=7.6 Hz),

11.19 (s, 1H). HRMS (EI^+): found 296.1146 (Calcd. for $C_{17}H_{16}O_3N_2$ $[M]^+$: 296.1161).

Methyl 6-amino-4-(4-fluorobenzyloxy)-1*H*-indole-2-carboxylate (**3f**): pale yellow solid, yield, 70%; m.p. 254-256 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H), 5.21 (s, 2H), 6.49 (s, 1H), 6.94 (s, 1H), 7.12 (s, 1H), 7.24 (t, 2H, J=8.4 Hz), 7.56 (dd, 2H, J=5.6, 8.4 Hz), 9.54 (br, 2H), 12.04 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 52.15, 69.05, 97.38, 105.60, 115.72, 115.89, 127.13, 130.17, 130.23, 133.03, 138.45, 153.26, 153.57, 161.34, 161.74, 163.28. HRMS (ESI^+): found 315.11399 (Calcd. for $C_{17}H_{16}O_3N_2F$ $[M+H]^+$: 315.11395).

Methyl 6-amino-4-(4-methoxybenzyloxy)-1*H*-indole-2-carboxylate (**4f**): pale yellow solid, yield, 61%; m.p. 270-272 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.83 (s, 3H), 3.89 (s, 3H),5.13 (s, 2H), 6.28 (s, 1H), 6.77 (s, 1H), 7.09 (s, 1H), 7.28 (t, 2H, J=8.0 Hz), 7.50 (dd, 2H, J=5.2, 8.0 Hz), 9.61 (br, 2H), 11.99 (s, 1H). HRMS (ESI^+): found 327.13388 (Calcd. for $C_{18}H_{19}O_3N_2$ $[M+H]^+$: 327.13393).

Methyl 6-amino-4-(cyclohexylmethoxy)-1*H*-indole-2-carboxylate (**5f**): pale yellow solid, yield, 73%; m.p. 240-242 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08-1.31 (m, 5H), 1.65-1.85 (m, 6H), 3.83 (s, 3H), 3.87 (d, 2H, J=5.6 Hz), 6.34 (s, 1H), 6.82 (s, 1H), 7.06 (s, 1H), 9.18 (br, 2H), 11.92 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 25.78, 26.49, 29.52, 37.12, 52.36, 73.44, 85.82, 96.48, 105.59, 115.00, 116.73, 126.96, 138.44, 154.10, 161.77. HRMS (ESI^+): found 303.17031 (Calcd. for $C_{17}H_{23}O_3N_2$ $[M+H]^+$: 303.17032).

Methyl 4-(allyloxy)-6-amino-1*H*-indole-2-carboxylate (**6f**): pale yellow solid, yield, 65%; m.p. 230-231 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.85 (s, 3H), 4.68 (d, 2H, J=5.2Hz), 5.30 (d, 1H, J=6.4 Hz), 5.48 (d, 1H, J=17.2 Hz), 6.06-6.16 (m, 1H), 6.46 (s, 1H), 6.99 (s, 1H), 7.11 (s, 1H), 9.75 (br, 2H), 12.08 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 52.14, 69.05, 97.11, 100.16, 105.58, 117.14, 118.26, 127.25, 133.59, 138.45, 153.59, 161.69. HRMS (ESI^+): found 247.10758 (Calcd. for $C_{13}H_{15}O_3N_2$ $[M+H]^+$: 247.10772).

Methyl 6-amino-4-cyclopropoxy-1*H*-indole-2-carboxylate (**7f**): pale yellow solid, yield, 90%. m.p. 251-253 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.37-0.40 (m, 2H), 0.58-0.62 (m, 2H), 1.29-1.34 (m, 1H), 3.84 (s, 3H), 3.94 (d, 2H, J=6.8 Hz), 6.38 (s, 1H), 6.91 (s, 1H), 7.07 (s, 1H), 9.50 (br, 2H), 12.00 (s, 1H). HRMS (ESI^+): found 261.12305 (Calcd. for $C_{14}H_{17}O_3N_2$ $[M+H]^+$: 261.12337).

Methyl 6-amino-4-isobutoxy-1*H*-indole-2-carboxylate (**8f**): pale yellow solid, yield, 68%; m.p. 230–233 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (d, 6H, J=6.8 Hz), 2.08-2.14 (m, 1H), 3.84 (d, 2H, J=6.4 Hz), 3.85 (s, 3H), 6.42 (s, 1H), 6.95 (s, 1H), 7.10 (s, 1H), 9.62 (br, 2H), 12.04 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 19.30, 28.20, 52.36, 74.48, 96.49, 105.39, 126.69, 138.44, 149.09, 149.31, 153.90, 161.47. HRMS (ESI^+): found 263.13892 (Calcd. for $C_{14}H_{19}O_3N_2$ $[M+H]^+$: 263.13902).

Methyl 6-amino-4-ethoxy-1*H*-indole-2-carboxylate (**9f**): pale yellow solid, yield, 65%. m.p. 184-186 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.35 (s, 3H), 3.86 (s, 3H), 3.97 (s, 2H), 6.60 (s, 1H), 6.79 (s, 1H), 7.08 (s, 1H), 9.27 (br, 2H), 11.75 (s, 1H). HRMS (ESI^+): found 235.10768 (Calcd. for $C_{12}H_{15}O_3N_2$ $[M+H]^+$: 235.10772).

Methyl 6-amino-4-isopropoxy-1*H*-indole-2-carboxylate (**10f**): pale yellow solid, yield, 70%. m.p. 171-173 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.37 (s, 6H), 3.86 (s, 3H), 4.10 (s, 1H), 6.67 (s, 1H), 6.84 (s, 1H), 7.00 (s, 1H), 9.47 (br, 2H), 11.88 (s, 1H). HRMS (ESI^+): found 249.12330 (Calcd. for $C_{13}H_{17}O_3N_2$ $[M+H]^+$: 249.12337).

Methyl 6-amino-1*H*-indole-2-carboxylate (**14f**): pale yellow solid, yield, 65%; m.p. 240-242 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.87 (s, 3H), 7.01 (s, 1H), 7.19 (s, 1H), 7.40 (s, 1H), 7.72 (d, 1H, J=8.4 Hz), 9.79 (br, 2H), 12.01 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 52.27, 107.22, 107.87, 115.73, 123.32, 126.15, 128.63, 129.81, 137.43, 161.87. HRMS (ESI^+): found 191.08137 (Calcd. for $C_{10}H_{11}O_2N_2$ $[M+H]^+$: 191.08150).

Methyl 7-amino-4-methoxy-1*H*-indole-2-carboxylate (**16f**): pale yellow solid, yield, 70%. m.p. 184-185 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.89-3.99 (m, 8H), 6.32 (d, 1H, J=8.0 Hz), 6.64 (d, 1H, J=8.0 Hz), 7.47 (d, 1H, J=8.0 Hz), 9.04 (s, 1H). HRMS (ESI^+): found 221.09209 (Calcd. for $C_{11}H_{13}O_3N_2$ $[M+H]^+$: 221.09207).

Methyl 7-amino-4-ethoxy-1*H*-indole-2-carboxylate (**17f**): pale yellow solid, yield, 75%. m.p. 138–140 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44-1.47 (t, J=7.0 Hz, 3H), 3.94 (s, 3H), 4.10 (q, 2H, J=7.0 Hz), 6.33 (d, 1H, J=8 Hz), 6.59 (d, 1H, J=8.0 Hz), 7.35 (s, 1H), 8.92 (s, 1H). HRMS (ESI^+): found 235.10787 (Calcd. for $C_{12}H_{15}O_3N_2$ $[M+H]^+$: 235.10772).

Methyl 7-amino-4-propoxy-1*H*-indole-2-carboxylate (**18f**): pale yellow solid, yield, 76%. m.p. 130–132 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05–1.09 (t, 3H, J=7.0 Hz), 1.82-1.89 (m, 2H), 3.95 (s, 3H), 3.98–4.01 (t, 2H, J=6.5 Hz), 6.34 (d, 1H, J=8.0 Hz), 6.59 (d, 1H, J=7.5 Hz), 7.34 (s, 1H), 8.96 (s, 1H). HRMS (ESI^+): found 249.12349 (Calcd. for $C_{13}H_{17}O_3N_2$ $[M+H]^+$: 249.12337).

4.2. *Antiviral assays*

Madin-Darby Canine Kidney (MDCK) cells and Coxsackie viruses (Cox B3 Nancy strain) were purchased from ATCC. Influenza A strains were all obtained from the Institute of Virology, Chinese Academy of Preventive Medicine.

4.2.1. *Anti-Coxsackie B3 activity assays*

Confluent Vero cells grown in 96-well plates were infected with a median tissue culture infective dose of 100 (100TCID50) Cox B3 viruses. After 1 h of viral adsorption at 37 °C, the monolayers were washed with phosphate buffered saline (PBS) and incubated at 37 °C in the maintenance medium (MEM+2% fetal bovine serum (FBS)) with or without different concentrations of test compounds. Viral cytopathic effect (CPE) was measured when the viral control group reached a level of 4 and the antiviral activity of test compounds was determined by the Reed and Muench analyses.

4.2.2. *Anti-influenza assays*

Confluent MDCK cells grown in 96-well microplates were infected with influenza A at a median tissue culture infective dose $TCID_{50}$ of 100. After 2 h of viral adsorption at 37 °C, the monolayers were washed with PBS and incubated at 37 °C in the maintenance medium with or without different concentrations of test compounds. Viral cytopathic effect (CPE) was measured when the viral control group reached a value of 4 and the antiviral activities of the synthesized compounds were determined by Reed and Muench analyses.

4.2.3. *Anti-HSV assays in vitro*

Confluent Vero cells grown in 96-well microplates were infected with $100TCID_{50}$ HSV-1 virus respectively. HEL cells were infected with HCMV. Tests were performed as follows: After 1hr adsorption at 37 °C, the monolayer cells were washed by PBS

and incubated at 37 °C in the maintenance medium (MEM+2% FBS) with or without different concentrations of test compounds or positive control drug. CPE was observed when viral control group reached 4+ and the antiviral activity of compound was determined by the Reed & Muench analyses.

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