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Dihydroisoindolo[2,1-*a*]quinazoline-5,11-dione derivatives as potent and selective inhibitors targeting hepatitis B virus

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Abstract

The construction of dihydroisoindolo[2,1-*a*]quinazoline-5,11-dione derivatives (**4a–4m**), by the condensation isatoic anhydride, appropriate amines and 2-formylbenzoic acid by using silica sulfuric acid as catalyst was reported. These dihydroisoindolo[2,1-*a*]quinazoline-5,11-dione derivatives (DIQ) were identified as potent inhibitors of HBV capsid assembly. The newly synthesized dihydroisoindolo[2,1-*a*]quinazoline-5,11-dione derivatives **4a-4m** were characterized by ^{1}H NMR, ^{13}C NMR, and Mass spectrum and evaluated for their anti-HBV activity. Majority of the synthesized compounds inhibited the expression of viral antigens at low concentration. But five compounds, **4a**, **4b**, **4c**, **4f**, and **4m** were shown potent inhibition of HBV DNA replication at submicromolar range. Of these compounds, compound **4a** was the most active when compared with lamivudine.

Introduction

Worldwide hepatitis B virus (HBV) becomes serious problem which is causing disease for more than 2 billion people. More than 360 million individuals were chronically infected from liver cirrhosis and hepatocellular carcinoma (Dény and Zoulim, 2010). The current therapies including vaccines, immunomodulators, interferon-α, polyethylene glycol interferon-α and nucleoside drugs for treating HBV are still unsatisfactory, due to high recurrence, drug resistance and inevitable side effects including influenza-like illness, myalgia, headache, reduction of neutrophilic granulocyte and blood platelet, etc (Sato and Mori, 2010; Locarnini and Mason, 2006; Wong et al., 1993; Fattovich et al., 1998). Therefore, it is important to explore novel classes of drugs with different antiviral targets and mechanisms for anti-HBV purposes.

On the other hand Hybrid molecules which can form by combining two heterocyclic cores of different nature often possess improved biological activities (Hyodo et al., 1995; Furumi et al., 1998). There are several literature precedence for the biologically active hybrids in the literature such as steroid-antibiotic (Oaksmith and Ganem, 2009), steroid-nucleoside, (Kortylewicz et al., 2009) triterpenoid-peptide (Vasilevsky et al., 2011) and DNA-cleaving agent- amino acid (Breiner et al., 2007; Breiner et al., 2006; Kovalenko and Alabugin, 2005; Yang et al., 2011) etc.

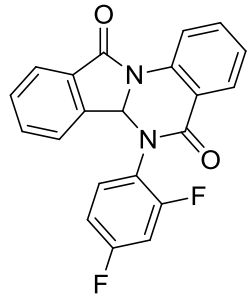
Multicomponent reactions (MCRs), are the most powerful technique to access complex molecules in a single synthetic operation from easily available building blocks. (Foye, 1991; Bonsignore et al., 1993) MCRs have led to interesting heterocyclic scaffolds, and are very useful in
the construction of diverse chemical libraries of 'drug-like' molecules (Ellis, 1977; Arnesto et al., 1989). One of the hybrid molecule, 6,6a-dihydroisoindolo[2,1-*a*]quinazoline-5,11-diones which are synthesized from isatoic anhydride, appropriate amines and 2-formylbenzoic acid. These compounds are less focused heterocyclic compounds which are having biological importance (Siva Kumar et al., 2011).

Materials and Methods

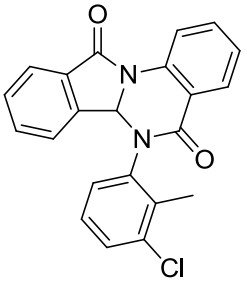
Chemistry: Chemicals and reagents were purchased

either from sigma or Merck, and all reagents were of analytical reagent grade. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates and visualized under UV light. ^{1}H NMR spectra were recorded with Varian Mercury Plus 400 MHz instrument.^{13}C NMR spectra were recorded with a Varian Gemini 100 MHz instrument. All the chemical shifts are reported in δ (ppm) using TMS as an internal standard. Multiplicity is indicated by one or more of the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); the coupling constants (*J*) correspond to the order of the multiplicity assignment. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under nitrogen atmosphere.

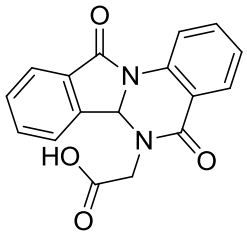
6-(2, 4-difluorophenyl)-6, 6a-dihydroisoindolo [2, 1-a] quinazoline-5, 11-dione (4a): Off-white solid; mp 201-203 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.15 (dd, *J* = 7.04, 5.14 Hz, 2H), 7.96 (d, *J* = 7.66 Hz, 1H), 7.77-7.69 (m, 1H), 7.59 (m, 1H), 7.43-7.35 (m, 2H), 7.17-7.09 (m, 1H), 6.92-6.69 (m, 2H), 6.56 (s, 1H) 6.43 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$); δ 166.1, 165.1, 139.1, 138.1, 135.2, 134.7, 134.4, 134.1, 133.1, 133.3, 133.2, 131.4, 129.9, 129.4, 126..3, 125.7, 121.3, 113.1, 113.0, 105.8, 105.0, 104.9, 71.8; Mass ESI calcd. $C_{21}H_{12}F_2N_2O_2$; ([M+H] +), 362.09; found: ([M+H] $^+$), 362.5.



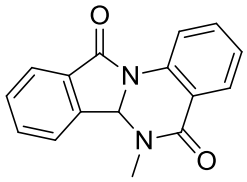
6-(3-chloro-2-methylphenyl)-6, 6a-dihydroisoindolo [2, 1-a] quinazoline-5, 11-dione (4b): Pale yellow solid; mp 188-190 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.23 (d, *J* = 7.9 Hz, 2H), 7.96 (d, *J* = 7.6 Hz, 1H), 7.74 (dd, *J* = 11.2, 4.4 Hz, 1H), 7.63-7.49 (m, 2H), 7.49-7.39 (m, 4H), 6.56 (s, 1H), 6.19 (d, *J* = 7.7 Hz, 1H), 1.67 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$); δ166.2, 164.4, 139.3, 138.9, 138.3, 137.9, 136.9, 135.0, 131.1, 129.9, 128.6, 127.9, 126.4, 125.5, 125.4, 121.4, 121.1, 72.8, 14.9; Mass ESI calcd. $C_{22}H_{15}ClN_2O_2$; ([M+H] +), 374.08; found: ([M+H] $^+$), 374.5.



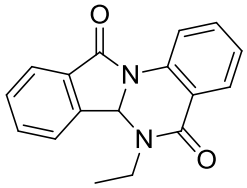
2-(5, 11-dioxoisoindolo [2, 1-a] quinazolin-6(5H, 6aH, 11H)-yl) acetic acid (4c): White solid; mp 243-247 ^{0}C; ^{1}H NMR (400 MHz, DMSO-d_6); δ 8.08 (dd, *J* = 14.5, 7.9 Hz, 2H), 7.97 (d, *J* = 7.4 Hz, 1H), 7.88-7.73 (m, 4H), 7.42 (t, *J* = 7.5 Hz, 1H), 6.59 (s, 1H), 4.64 (d, *J* = 18.1 Hz, 1H), 4.34 (d, *J* = 18.1 Hz, 1H); ^{13}C NMR (400 MHz, DMSO-d_6); δ 171.0, 164.7, 163.6, 139.3, 137.2, 134.1, 133.6, 132.4, 131.1, 129.0, 125.8, 125.4, 120.3, 119.9, 70.6, 44.7; Mass ESI calcd. $C_{17}H_{12}N_2O_4$; ([M+H] +), 308.08; found: ([M-H]) $^-$, 306.5.



6-methyl-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (4d): White solid, Mp = 188-189 °C 1 H NMR (400 MHz, $CDCl_3$): 8.16 (dd, *J*=7.8, 1.0 Hz, 1H), 8.11 (dd, J=7.8, 1.0 Hz, 1H), 8.03 (dd, J=7.8, 1.0 Hz, 1H), 7.78-7.61 (m, 4H), 7.35-7.31 (m, 1H), 6.12 (s, 1H), 3.32 (s, 3H, CH3); ^{13}C NMR (CDCl3, 50 MHz):164.8, 163.8, 137.9, 136.5, 133.3, 132.6, 132.4, 130.4, 128.9, 125.5, 125.0, 124.9, 120.0 (2C), 71.1, 29.9; Mass ESI calcd. $C_{16}H_{13}N_2O_2$; ([M+H] +), 265.0; found: ([M-H]) $^-$, 265.6.

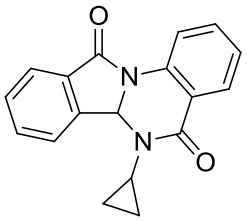


6-ethyl-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (4e): White solid, Mp = 156-159 °C 1 H NMR (DMSO-d6, 400 MHz): 8.01 (d, J=7.4 Hz, 1H), 7.99-7.95 (m, 3H), 7.87-7.84 (m, 1H), 7.77 (d, J=7.4 Hz, 1H), 7.74-7.69 (m, 1H), 7.40-7.36 (m, 1H), 6.60 (s, 1H), 3.90-3.83 (m, 1H), 3.72-3.67 (m, 1H), 1.03 (t, J=7.4 Hz, 3H, CH3) 13C NMR (DMSO-d6, 100 MHz): 164.3, 162.6, 138.4. 136.4, 133.2, 133.1, 131.8, 130.6, 128.3, 126.0, 125.0, 124.3, 120.3, 119.9, 69.7, 37.2, 13.4 IR (KBr): 3403, 1711, 1668, 1603, 1490, 1469 cm-1 HRMS (ESI): calcd for C17H15N2O2 (M+H)+ 279.1134, found 279.1135

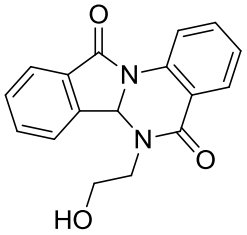


6-cyclopropyl-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (4f): White solid, Mp = 155-158 °C 1 H NMR (CDCl3, 400 MHz): 8.19-8.14 (m, 2H), 8.05 (d, J-6.9Hz,

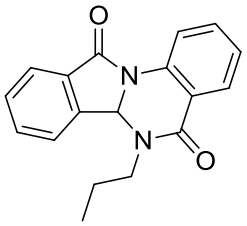
1H), 7.92 (d, J-6.9Hz, 1H), 7.70-7.60 (m, 3H), 7.32-7.28 (m, 1H), 6.18 (s, 1H), 2.73-2.68 (m, 1H), 1.19-1.11 (m, 1H), 0.90- 0.84 (m, 1H), 0.71-0.64 (m, 1H), 0.08-0.02 (m, 1H) 13C NMR (DMSO-d6, 100 MHz):164.4, 164.3, 138.8, 136.7, 133.3, 132.3, 131.6, 130.2, 128.4, 127.3, 124.6, 123.7, 120.4, 119.2, 71.2, 25.9, 11.1, 9.1 IR (KBr):3427, 1726, 1663, 1601, 1487, 1466, cm-1 HRMS (ESI): calcd for C18H15N2O2 (M+H)+ 291.1134, found 291.1132.



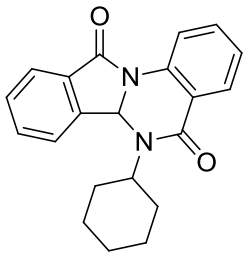
6-(2-hydroxyethyl)-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (4g): White solid, Mp = 215-217 °C ^{1}H NMR (400 MHz, DMSO-d^{6}); δ 8.22 (d, *J* = 7.66 Hz, 1H), 8.03-7.98 (m, 2H), 7.94 (d, *J* = 7.21 Hz, 1H), 7.87-7.82 (m, 1H), 7.78-7.69 (m, 2H), 7.41-7.35 (m, 1H), 6.62 (s, 1H), 4.11-3.86 (m, 2H), 3.60 (m, 3H); ^{13}C NMR (400 MHz, DMSO-d^{6}); δ 164.7, 163.3, 138.9, 136.9, 133.6, 133.3, 132.4, 130.9, 128.7, 127.7, 125.3, 124.5, 120.7, 120.2, 71.4, 59.3,45.3; Mass ESI calcd. $C_{17}H_{14}N_2O_3$; ([M+H] $^{+}$), 294.10; found: ([M+H] +), 294.6.



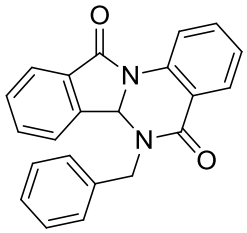
6-propyl-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (4h): White solid, Mp = 127-130 °C 1 H NMR (DMSO-d6, 500 MHz): 8.01-7.95 (m, 4H), 7.87-7.84 (m, 1H), 7.77-7.69 (m, 2H), 7.39- 7.36 (m, 1H), 6.57 (s, 1H), 3.73-3.67 (m, 2H), 1.46-1.29 (m, 1H), 1.27-1.22 (m, 1H), 0.76 (t, J-7.3Hz, 3H, CH3) 13C NMR (DMSO-d6, 100 MHz):164.3, 162.7, 138.5, 136.4, 133.1, 133.2, 131.8, 130.7, 128.4, 126.0, 125.0, 124.3, 120.3, 119.9, 70.0, 43.7, 21.3, 10.8 IR (KBr):3423, 2960, 1728, 1653, 1489, 1470 cm-1 HRMS (ESI): calcd for C18H17N2O2 (M+H)+ 293.1290, found 293.1286.



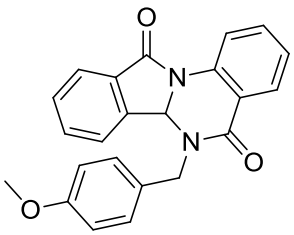
6-cyclohexyl-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (4i): White solid, Mp = 148-150 °C 1 H NMR (DMSO-d6, 500 MHz): 8.01 (d, J=7.9 Hz, 1H), 8.00-7.95 (m, 2H), 7.87-7.86 (m, 2H), 7.80-7.68 (m, 2H), 7.38-7.34 (m, 1H), 6.58 (s, 1H), 3.58-3.53 (m, 1H), 2.51-2.39 (m, 2H), 2.15- 2.05 (m, 1H), 1.91-1.01 (m, 7H) 13C NMR (DMSO-d6,100 MHz): 164.0, 163.4, 138.5, 136.3, 133.2, 133.0, 132.2, 130.7, 128.2, 126.9, 124.8, 124.4, 121.4, 119.3, 71.1, 57.6, 29.9, 28.1, 26.0, 25.6, 25.0 IR (KBr): 3428, 2965, 1735, 1659, 1495, 1478 cm-1 HRMS (ESI): calcd for C21H21N2O2 (M+H)+ 333.1603, found 333.1602.



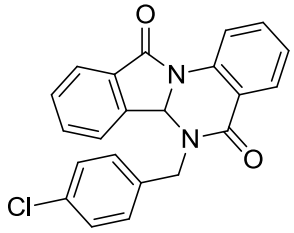
6-benzyl-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (4j): White solid, Mp = 148-150 °C 1 H NMR (DMSO-d6, 400 MHz): 8.06 (d, J-7.9 Hz, 2H), 7.87-7.84 (m, 1H), 7.77-7.72 (m, 2H), 7.65-7.59 (m, 2H), 7.44-7.40 (m, 1H), 7.22-7.05 (m, 5H), 6.72 (s, 1H), 5.11-5.00 (m, 2H) 13C NMR (DMSO-d6, 100 MHz):164.3, 163.3, 138.0, 137.3, 136.7, 133.5, 132.9, 131.7, 130.5, 128.6, 128.4 (2C), 126.6, 126.0, 125.9 (2C), 125.1, 124.1, 120.0, 119.9, 70.2, 45.7 IR (KBr):3422, 3055, 1723, 1660, 1486, 1470 cm-1 HRMS (ESI): calcd for C22H17N2O2 (M+H)+ 341.1290, found 341.1282.



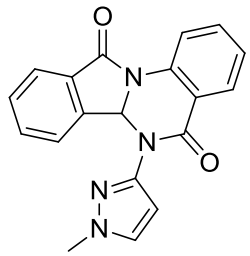
6-(4-methoxybenzyl)-6,6a-dihydroisoindolo[2,1-a] quinazoline-5,11-dione (4k): White solid, Mp = 137-139 °C 1 H NMR (DMSO-d6, 400 MHz): 8.06-8.04 (m, 2H), 7.88-7.86 (m, 1H), 7.77-7.62 (m, 4H), 7.43- 7.39 (m, 1H), 6.95 (d, J-8.8 Hz, 2H), 6.77 (d, J-8.8 Hz, 2H), 6.68 (s, 1H), 5.04-4.89 (m, 2H), 3.66 (s, 3H, CH3) 13C NMR (DMSO-d6, 100 MHz):164.3, 163.2, 157.9, 138.1, 136.6, 133.4, 132.9, 131.7, 130.5, 129.0, 128.6, 127.3 (2C), 126.1, 125.1, 124.1, 120.1, 119.8, 113.8 (2C), 70.2, 54.9, 45.1 IR (KBr):3428, 2833, 1724, 1660, 1488, 1466 cm-1 HRMS (ESI): calcd for C23H19N2O3 (M+H)+ 371.1396, found 371.1398.



6-(4-chlorobenzyl)-6,6a-dihydroisoindolo[2,1-a]quinazoline-5,11-dione (4l): White solid, Mp = 178-181 °C 1 H NMR (DMSO-d6, 400 MHz): 8.06-8.04 (m, 2H), 7.86-7.84 (m, 1H), 7.78-7.73 (m, 2H), 7.65- 7.61 (m, 2H), 7.44-7.40 (m, 1H), 7.24 (d, J-8.8 Hz, 2H), 7.07 (d, J-8.8 Hz, 2H), 6.71 (s, 1H), 5.12-4.99 (m, 2H) 13C NMR (DMSO-d6, 100 MHz): 164.3, 163.4, 138.0, 136.7, 136.6, 133.6, 133.0, 131.7, 131.1, 130.6, 128.7, 128.3 (2C), 127.9 (2C), 126.1, 125.2, 124.1, 120.0 (2C), 70.2, 45.1 IR (KBr): 3429, 2932, 1726, 1664, 1490, 1470 cm-1.



6-(1-methyl-1H-pyrazol-3-yl)-6, 6a-dihydroisoindolo [2, 1-a] quinazoline-5, 11-dione (4m): White solid; mp 139-152.5 ^{0}C; ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.19 (d, *J* = 8.0 Hz, 2H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.69 (t, *J* = 15.6 Hz, 1H), 7.55 (t, *J* = 14.8 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 1H), 7.39 (m, 2H), 6.65 (s, 1H), 6.59 (d, *J* = 7.6 Hz, 1H), 6.01 (s, 1H), 4.00 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$); δ 165.3,164.5,145.6,138.8,137.0,133.9,132.2,132.1,130.1,129.4 ,125.19,125.14, 124.4, 120.3, 119.9,105.8, 71.8, 39.6; Mass ESI calcd. $C_{19}H_{14}N_4O_2$; ([M+H] +), 330.11; found: ([M+H] $^+$), 330.6.



Biological evaluation

Cell culture: HepG2 2.2.15 cells, derived from HepG2 human hepatocellular carcinoma cells, were stably transfected with a head-to-tail HBV DNA dimer and were maintained in MEM with heat-inactivated 10% fetal bovine serum (FBS) and 1% antibiotics. In parallel experiments, human Huh7 hepatoma cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with heat-inactivated 10% FBS and 1% antibiotics. HepG2 2.2.15 and Huh7 cells were both grown at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Cell viability assay: The cytotoxic effects of synthesized compounds were determined using a Cell Titer 96 cell proliferation assay kit (MTS) (Promega, Madison, WI, USA). In order to pinpoint the toxicity limits in HepG2 2.2.15 and Huh7 cells, they were plated into 96-well plates at a density of 4 x 10^4 cells/mL for 24 hours. Cells were then treated with serial dilutions of compounds ranging 2.5-160 mg/mL, and the mixture was incubated for 3 days. Cell toxicity was calculated according to the maker's protocol. All the results were performed in triplicates, and results are presented as relative percentages over that of the control group.

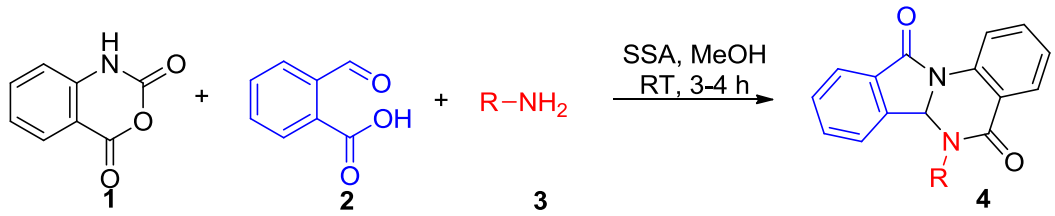
Determination of HBV expression levels: After treating HepG2 2.2.15 cells or HBV-transfected Huh7 cells, levels of the HBsAg and HBeAg proteins were measured in culture media using an EIA kit (Johnson and Johnson, Skillman, NJ, USA) according to the manufacturer's instructions.

SEC general procedure: Capsid assembly was initiated by mixing Cp149 with test compounds in 2 buffer incubation 24 hours, respectively. Assembly reactions were examined on a Superose column (Biosep-SEC-S3000) mounted on HPLC system equipped with an auto injection module. The column was equilibrated with 100 mM HEPES pH 7.5, 300 mM NaCl at 0.6 mL/min.

Results and Discussion

In the initial studies we investigated the cylclization reaction of 2-formylbenzoic acid (**3**) with isatoic anhydride (**1**) and 2,4-difluoro aniline (**2a**) using SSA as catalyst in ethanol under reflux (**Scheme-1**) for 2 hours which yielded the required compound **4a** in 89% (Table I, entry 1). Several amines were used for checking the compatibility of the method. All the amines including aliphatic, aromatic and also heterocyclic amines were well tolerated with the current methodology to yield the desired products in good to better yields. Thus, the structures of the synthesized target compounds are listed in Table I.

Inhibition activities for HBV replication of the compounds **4a-4m** were determined in the HepG2.2.15 cells, which constitutively produces HBV genomes, and secretes virus-like particles (Korba and Gerin, 1992). Lamivudine (3TC) was used as positive control. To ascertain the cytotoxic effects of the tested compounds, the cell viability was determined after the cells exposed to the compounds for 48 hours (Table I).



Scheme 1: Synthesis of 5-(aminomethylene)thiazolidine-2,4-diones (a) Ac2O, HC(OEt)3, reflux (b) acetonitrile, 45°C, 30 min

Table I

Synthesis of a novel series of substituted 5-(aminomethylene)thiazolidine-2,4-diones[a]

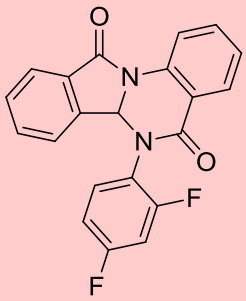
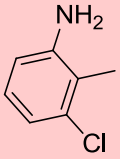
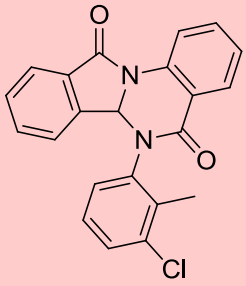
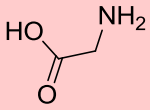
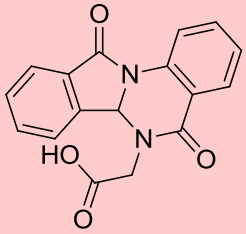
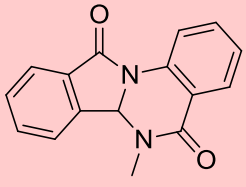
Entry	2° Amine (3)	Product	Yield (%)[b]
1		 **4a**	89
2		 **4b**	93
3		 **4c**	81
4		 **4d**	78

Table I

Synthesis of a novel series of substituted 5-(aminomethylene)thiazolidine-2,4-diones[a] (Cont.)

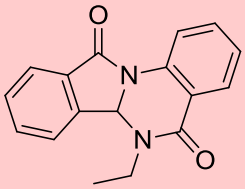
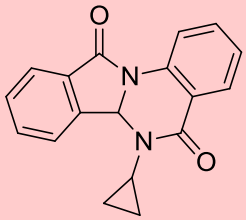
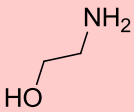
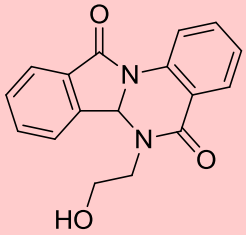
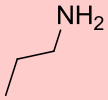
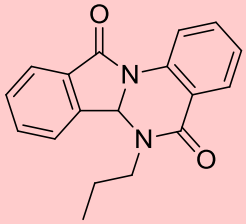
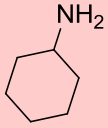
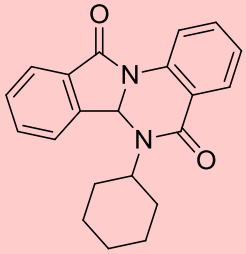
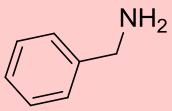
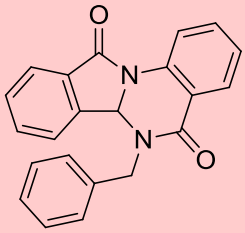
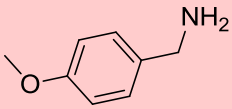
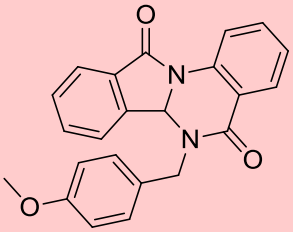
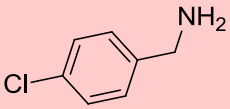
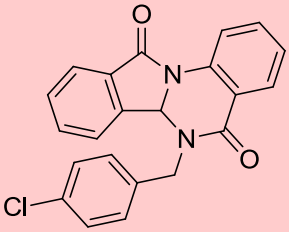
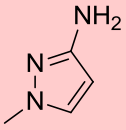
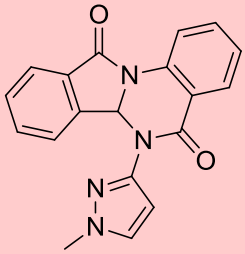
Entry	2° Amine (3)	Product	Yield (%)[b]
5		 **4e**	80
6		 **4f**	87
7		 **4g**	82
8		 **4h**	85
9		 **4i**	92

Table I

Synthesis of a novel series of substituted 5-(aminomethylene)thiazolidine-2,4-diones[a] (Cont.)

Entry	2° Amine (3)	Product	Yield (%)[b]
10		 **4j**	96
11		 **4k**	96
12		 **4l**	97
13		 **4m**	92

[a]All the reactions were carried out using isatoic anhydride **1a** (1.0 mmol), aniline **2a** (1.1 mmol), 2-formylbenzoic acid **3** (1.0 mmol) and SSA (15 mol%), ethanol (10 vol.), reflux, 2-3 hours; [b]Isolated yields

Hep G2.2.15 cell contained multiple copies of the HBV genome, which were stably integrated into the host cell genome and was widely used as a useful 'in vitro' model for evaluation of novel anti-HBV drugs. So, in the experiment, the Hep G2.2.15 cell line as in vitro cellular model was chosen.

All the synthesized derivatives were tested in vitro in HepG2.2.15 cells for cytotoxicity and anti-HBV activity. The properties of target compounds were summarized in Table II, in which they were compared to the drug lamivudine. Compounds **4a, 4b, 4c, 4f, 4n**, and **4m** displayed good to better anti HBV activity.

A comparison of the IC_{50} values of the derivatives **4a-4m** clearly indicated that the polarity of substituent and size of the substituent on the substitution had noticeable effect on its antiviral activities (**Table II**). **4a** (IC_{50} = 4.13 μM), bearing a 2,4-difluorophenyl substituent, was comparably potent to the known inhibitor of

Table II

Anti-HBV activity and cytotoxicity of target compounds in vitro

Compound	TC_{50} (μM)	HBsAg		HBeAg		HBV DNA Replication	
		IC_{50} (μM)	SI	IC_{50} (μM)	SI	IC_{50} (μM)	SI
4a	>1500	920	41	846	55.3	4.13	456
4b	147	203	5.8	594	42.5	13.6	245
4c	1339	458	4.6	215	39.7	12.6	265
4d	225	564	-	678	-	69.5	3.2
4e	171	-	-	177	-	36.6	4.7
4f	420	289	3.4	143	3.4	22.3	164
4g	126	880	-	12.3	-	60.6	14.2
4h	225	565	-	23.4	-	69.5	9.3
4i	1276	>1598	-	>974	-	58.3	7.6
4j	326	162	-	49.2	-	221	12.6
4k	122	92	-	77.5	-	49.3	18.7
4l	92	57	-	56.8	-	42.6	16.5
4m	157	768	41	296	123.4	8.4	386
3TC	>1634	1243	>1.6	1389	>1.8	0.95	>1289

[a]TC_{50} is 50% cytotoxic concentration in HepG2.2.15 cells; [b]IC_{50} is 50% inhibitory concentration; [c]Selectivity index (SI: TC_{50}/IC_{50}); [d]No antiviral activity at the concentration lower than its TC_{50}; [e]3TC – Lamivudine

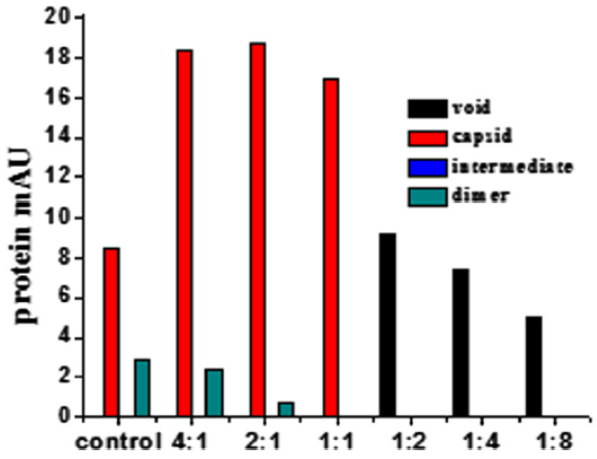


Figure 1: SEC showed the effect of **4a** on capsid assembly

HBV replication than 3TC (13.9 μM). When the attachments on dihydroisoindolo[2,1-*a*]quinazoline-5,11-dione was replaced by less rigid alkyl groups to give compounds **4c, 4d, 4e, 4f, 4g, 4h** (the IC_{50} values 12.6 μM, 69.5 μM, 36.6 μM, 22.3 μM, 60.6 μM and 69.5 μM respectively), their anti-HBV activities were reserved or even decreased. Replacing the alkyl attachment to benzylic groups **4j** to **4l** there is no improvement in anti-HBV activity. There is no effect on the anti HBV activity when the dihydroisoindolo[2,1-*a*]quinazoline-5,11-dione compounds was substituted with cyclohexane **4i**. Compounds, **4a** (IC_{50} = 4.13 μM), **4b** (IC_{50} = 13.6 μM), 4c (IC_{50} = 12.6 μM), **4f** (IC_{50} =22.3 μM) and **4m** (IC_{50} = 8.4 μM) showed high inhibition of HBV replication. It illuminated that the anti-HBV activity largely depends on the size, length and character of dihydroisoindolo[2,1-*a*]quinazoline-5,11-dione substituent.

Among all the compounds **4a, 4b**, and **4m** which are showing better anti-HBV activities, compounds containing the electron-withdrawing groups such as fluorine or chlorine and heterocyclic system.

To gain better understand into the mechanisms of our compounds, **4a** was investigated to examine the effect on preformed Hepatitis B virus (HBV) capsid and on HBV capsid assembly by size exclusion chromatography (SEC) (Stray et al., 2005). Recovered protein was assigned to the void (aberrant capsid induced by **4a**, 8.6 min), capsid (9.6 min) and dimer (12.6 min) based on the HPLC chromatogram.

By SEC, we had observed the effect of compounds **4a** on Cp149 (Figure 1). There was no change of the capsid morphology detected at low concentration of **4a** (Cp149:4a = 4:1 or 2:1). At higher concentration (Cp149:4a = 1:2 or 1:4), the increasing continuous spectrum of void and the decreasing dimers were observed. The results suggested that **4a** can break the equilibrium and change the product of HBV core protein self-assembly. By structural biology, we established a screening system for anti-HBV compounds that target on nucleocapsid. SEC would be a much better method to discover the strong antiviral compounds because of its objectivity, convenience and precision.

Conclusion

The present study describes the synthesis of dihydroisoindolo[2,1-*a*]quinazoline-5,11-dione derivatives (**4a–4m**). The newly synthesized analogues **4a-4m** were characterized by ^{1}H NMR, ^{13}C NMR, and MS and

evaluated for their anti-HBV activity, which provided 5 active derivatives inhibiting HBsAg secretion, 5 active derivatives suppressing HBeAg secretion and 5 active derivatives inhibiting HBV DNA replication. Interestingly, compound **4a** could inhibit not only HBsAg and HBeAg secretions but also HBV DNA replication with SI values of 41, 55.3 and 456. In view of their observed anti HBV activity, these compounds are seemed to have potential medicinal value.

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