Naturally Occurring and Synthetic Bioactive Molecules as Novel Non-Nucleoside HBV Inhibitors

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Abstract: Hepatitis B virus (HBV) infection is a severe health problem all over the world. However, there is still no satisfactory anti-HBV therapeutic strategy. Currently, promising alternative approaches toward the control of HBV infection include the development of structurally novel and more potent inhibitors obtained from natural products and structural modifications of synthetic molecules as seen in many cases. In this review, we will focus our interest on representative naturally occurring and synthetic small molecule non-nucleoside inhibitors with high anti-HBV potency and potential for future therapeutic regimens to combat HBV infection.

Keywords: HBV, Inhibitor, Non-nucleoside inhibitor, Chemotherapeutics, Natural products, Synthetic molecules, Heterocyclic compounds.

INTRODUCTION

Hepatitis B virus (HBV) is a major public health issue worldwide. An estimated 400 million people are chronically infected. In addition, chronic HBV infection is also one of the major risk factors for liver cancer. Current treatments for chronic HBV infection include the usage of interferon-α and nucleoside drug lamivudine (3TC), adefovir, entecavir and telbivudine. However, the use of interferon-α can lead to lower response rate in patients (only no more than 30 percent chronic Hepatitis B patients respond to interferon therapy) while that of nucleoside inhibitors can provoke severe side-effects and the emergence of resistant viruses [1-3]. Hence, new drugs for the treatment of HBV infection are still highly desired. Under these circumstances, the development of effective non-nucleoside anti-HBV agents is an alternative choice.

Currently, many classes of naturally occurring and synthetic non-nucleoside HBV inhibitors have been identified and appear in the recent literature or patents. This progress holds a considerable promise to the development of alternative, highly effective and specific therapeutics agents and ultimately the eradication of HBV infection [4-5].

This review focuses on the recent advances in discovery, biological activities studies and/or structural modifications of several distinct classes of new natural products and synthetic molecules as potent non-nucleoside anti-HBV agents.

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1. NATURALLY OCCURRING ANTI-HBV MOLECULES

As well known, natural products, as the most consistently successful source of lead drug discovery, continue to offer more opportunities to find lead compounds or drugs. For instance, traditional Chinese medicinal herbs have been widely used to treat chronic liver diseases in China, and the results of several control experiments to assess the efficacy and safety for chronic HBV infection are promising.

In this section, new natural products with anti-HBV activity are categorized according to their chemical structures, such as terpenes, alkaloids, flavanoids, and phenylalanyl. Many natural product-derived compounds can be used with existing anti-HBV agents to reduce the possibility of drug-resistance and show a synergetic effect.

Because hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) and HBV DNA are markers whose level is indicative of viral replication, the EC_{50} values (against the secretion of HBsAg and/or HbeAg) and/or selective indices (SI), and the HBV DNA expression levels after administration, are the basic evaluation indices of bioactive molecules *in vitro* (cell culture models) or *in vivo* (duck) experiment, and will be presented in this article.

1.1. Terpenes

In 2006, it was reported that some protostane-type triterpenes exhibit significant antiviral activity against HBV *in vitro* [6]. Recently, it has been demonstrated that several derivatives of Alisol A (**1**), a protostane-type triterpene, extracted from the rhizomes of *Alisma orientalis* Juzep, possesses potent anti-HBV activity *in vitro* [7]. Especially, compound **2-6** showed high activities against the secretion of HBsAg, HBeAg and remarkable SI values (Table **1**) [8-10].

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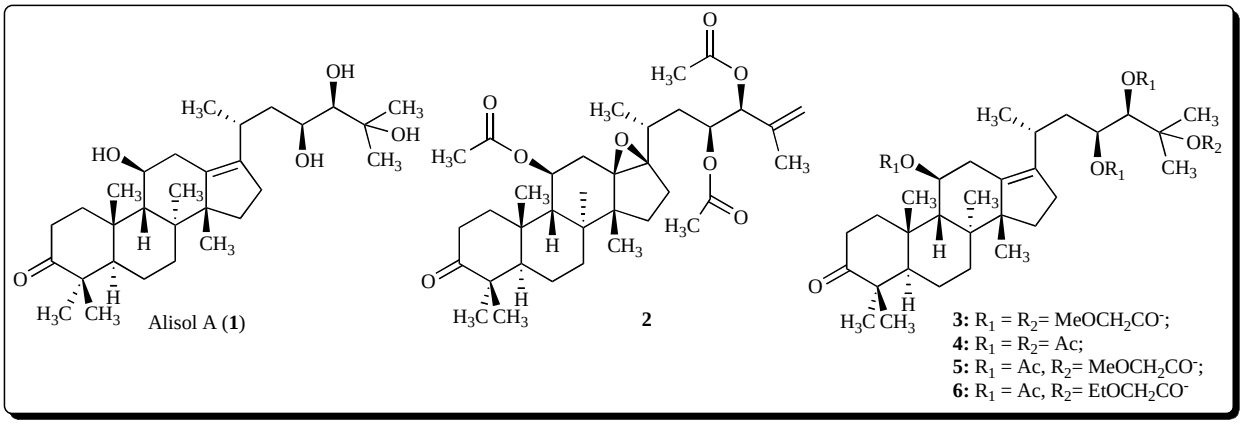


Table 1. Anti-HBV Activity of Alisol A Derivatives

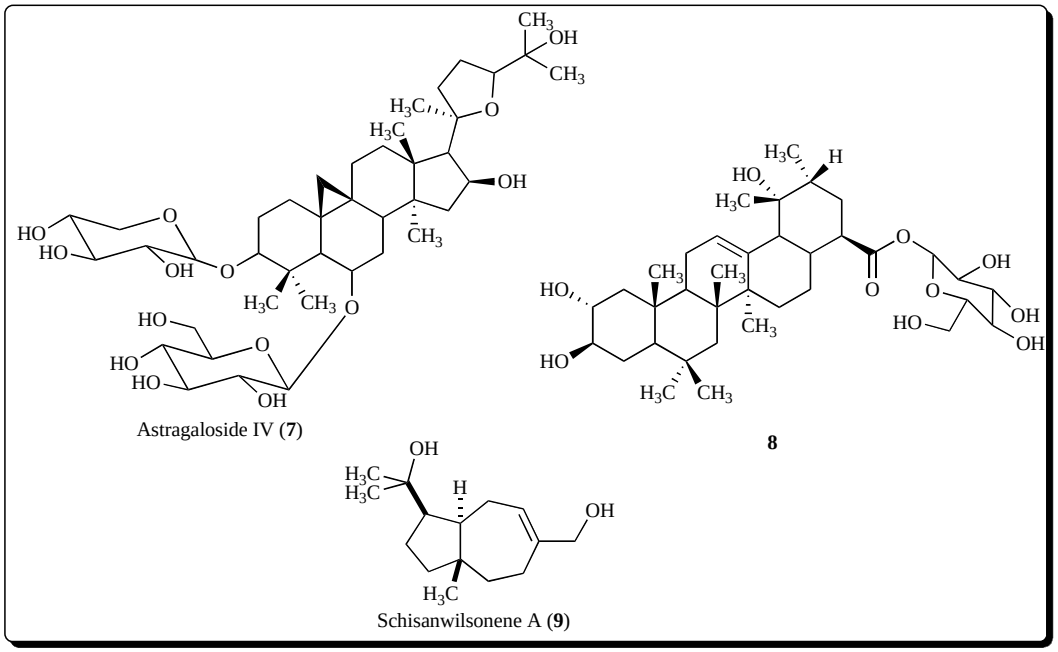
Compounds	EC_{50}[a]		SI[b]		Ref
	HBsAg	HBeAg	HBsAg	HBeAg	
2	0.024 mM	0.028 mM	> 108	> 93	8
3	0.028 mM	0.027 mM	> 90	> 93	9
4	0.0048 mM	0.011 mM	>333	>145	10
5	0.0044 mM	0.012 mM	= 209	= 77	10
6	0.014 mM	0.018 mM	>200	>156	10

[a]Means 50% inhibitory concentration in HepG2.2.15 cells.
[b]Selectivity index (SI: CC_{50}/EC_{50}); –means no antiviral activity at the concentration lower than its CC_{50}. CC_{50}: Means 50% cytotoxic concentration.

Additional studies in rats showed that compound **4** has favorable pharmacokinetic profiles with elimination half-time ($t_{1/2}$) of 1.63 h and oral bioavailability of 40.9%, which was selected for further evaluation as a novel HBV inhibitor [10].

Triterpenoid saponin astragaloside IV (**7**), extracted from Chinese herb *Radix Astragali* (Huangqi), can suppress secretion of HBV antigens effectively in the human HBV-transfected liver cell line HepG2 2.2.15 (inhibition rates: 23.6% for the secretion of HBsAg; 22.9% for that of HBeAg at 127.4 mM after 9 d of treatment). And its inhibitory activity on secretion of HBV antigens is more potent than that of 3TC without significant cytotoxicity [11].

A triterpenoid saponin, 2*α*, 3*β*, 19*α*-trihydroxyurs-12-en-28-oic acid *β*-d-glucopyranosyl ester (**8**), isolated from the



rhizomes of the Tibetan herb *Potentilla anserina* L, could decrease the expression levels of HBsAg, HBeAg and HBV DNA in the HepG2 2.2.15 cell evaluation *in vitro* and the inhibitory effect was not due to the cytotoxicity of the triterpenoid saponin. Moreover, this compound exhibits inhibitory activities against duck HBV DNA replication in Peking ducklings *in vivo* [12].

A carotane-type sesquiterpenoid Schisanwilsonene A (**9**), extracted from the fruits of *Schisandra wilsoniana*, exhibited remarkable anti-HBV activity with inhibition rates of 76.5% and 28.9% for HBsAg and HBeAg secretion at 0.21 mM, respectively [13].

1.2. Alkaloids

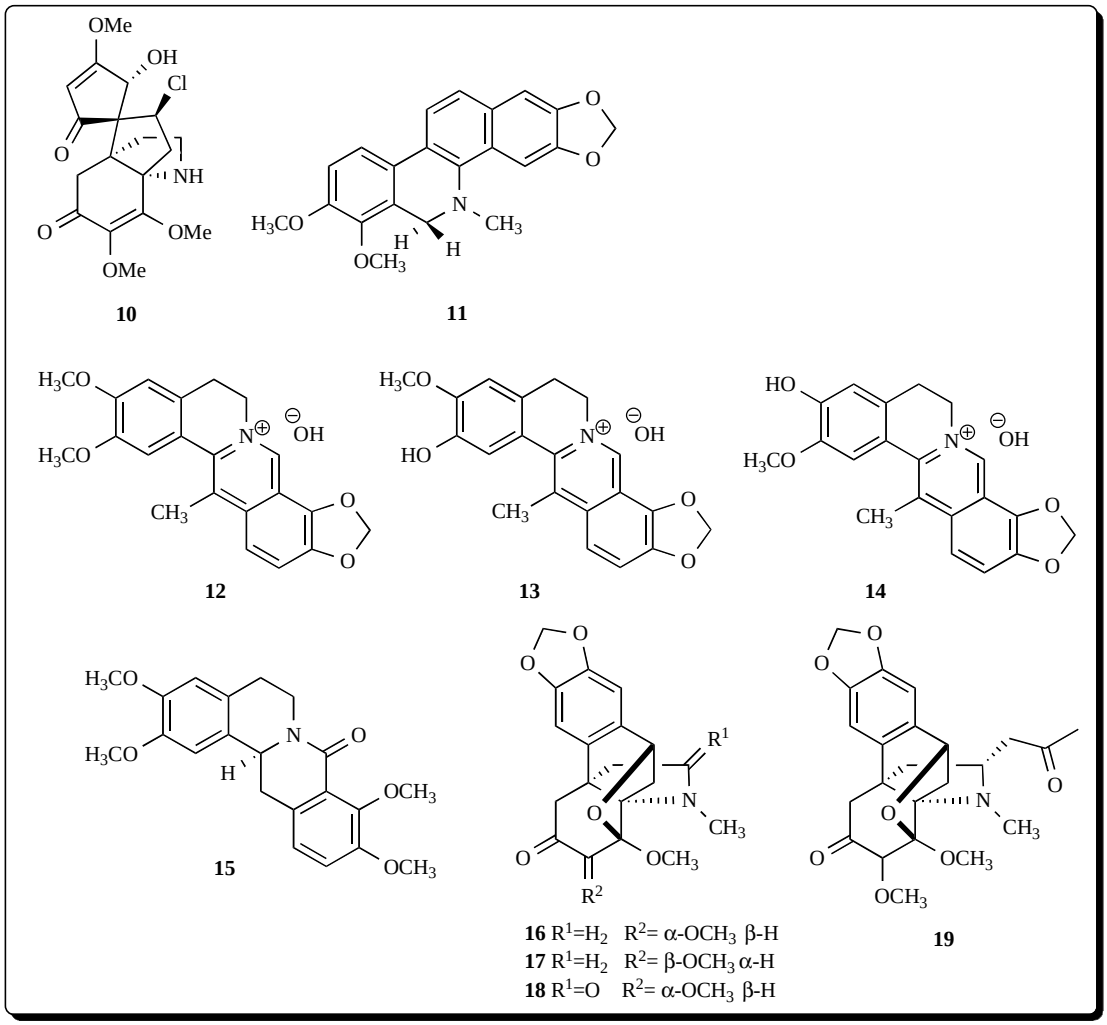
In 2007, Chen, *et al*. reported that dauricumidine (**10**), isolated from *Hypserpa nitida* Miers (Menispermaceae), exhibited an EC_{50} value of 0.450 mM (SI = 4.13) on HBsAg secretion of the Hep G2.2.15 cells line [14]. The same authors also disclosed that dihydrochelerythrine (**11**), isolated from Corydalis saxicola Bunting, exhibited higher activity against HBsAg and HBeAg secretions with EC_{50} < 0.05 $\times 10^{-3}$ mM, SI > 3.5, respectively [15].

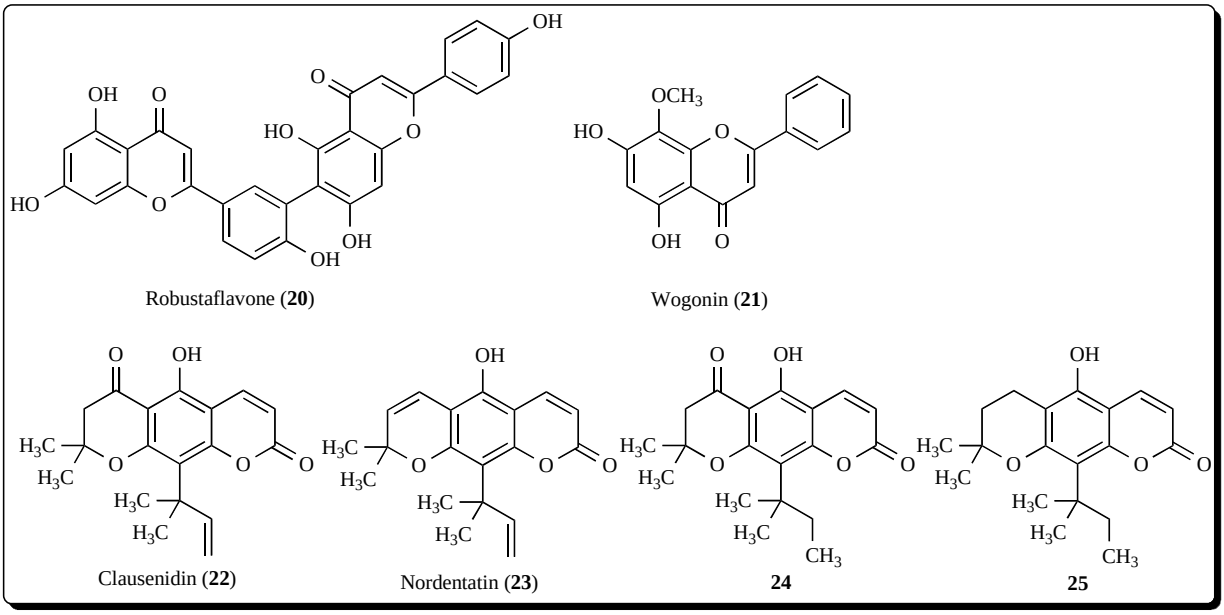
Protoberberine-type alkaloids dehydrocavidine (**12**), dehydroapocavidine (**13**), and dehydroisoapocavidine (**14**), isolated from the herb *Corydalis saxicola* Bunting (Papaveraceae), exhibited high inhibitory activity against HBsAg and HBeAg, but no cytotoxicity against the Hep G2.2.15 cell line, and the inhibitory effect of them was dose-dependent [16].

In 2008, Chen, *et al*. reported that the alkaloid (-)-8-oxotetrahydropalmatine (**15**), isolated from the aerial parts of *Pericampylus glaucus*, showed an EC_{50} value of 0.14 mM in inhibiting HBsAg secretion with a high SI value of 22.4 in Hep G2.2.15 cells and is deserving of further anti-HBV evaluation. Moreover, the hasubanane-type alkaloids **16-19** were found to possess weak to moderate activity against HBsAg secretion, with EC_{50} values of 0.47–1.72 mM and CC_{50} values of 0.64–2.96 mM, respectively, which led to SI values of 1.36–3.07 [17].

1.3. Flavanoids

Robustaflavone (**20**), a naturally occurring biflavanoid isolated from *Rhus succedanea*, was found to be a potent HBV inhibitor in 2.2.15 cells, with an EC_{50} of 0.25 $\times 10^{-3}$ mM, and a SI value of 153. Robustaflavone hexaacetate inhibited HBV replication with an EC_{50} of 0.73 $\times 10^{-3}$ mM, but exhibited no cytotoxicity at concentrations up to 1 mM. Robustaflavone also exhibited pronounced synergistic anti-





HBV activity with both penciclovir and 3TC [18]. Moreover, a total synthesis method of robustaflavone was developed to provide a general route for the preparation of structural analogues [19].

Recently, Wogonin (**21**), the major active constituent isolated from the traditional Chinese medicine plant *Scutellaria radix*, mainly used for the treatment of inflammatory conditions including hepatitis, has attracted much attention in its potent biological activities. Wogonin possesses potent anti-HBV activity both *in vitro* and *in vivo*, which effectively suppressed the secretion of the HBV antigens with an EC_{50} of 0.014 mM at day 9 for both HBsAg and HBeAg in the HepG2.2.15 cells line [20].

Natural pyranocoumarins clausenidin (**22**), nordentatin (**23**), isolated from the medicinal plant *Clausena excavate*, suppressed HBsAg in HepA2 cells. Among their synthesized analogues, **24** and **25** were the most potent HBV inhibitors with EC_{50} values of 1.14 $\times 10^{-3}$ and 1.34 $\times 10^{-3}$ mM, respectively [21].

1.4. Helioxathin and its Synthetic Derivatives

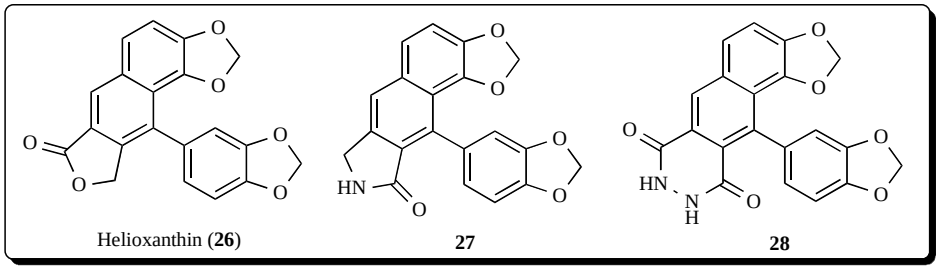
Helioxathin (**26**), an arylnaphthalene lignan lactone, isolated from the root of *Heliopsis scabra* Dunal (Compositae) [22] and the whole plant of *Taiwania cryptomerioides* Hayata (Taxodiaceae) [23], inhibits the replication of a number of viruses. Thus, a series of helioxanthin analogues were synthesized and evaluated for their antiviral activities.

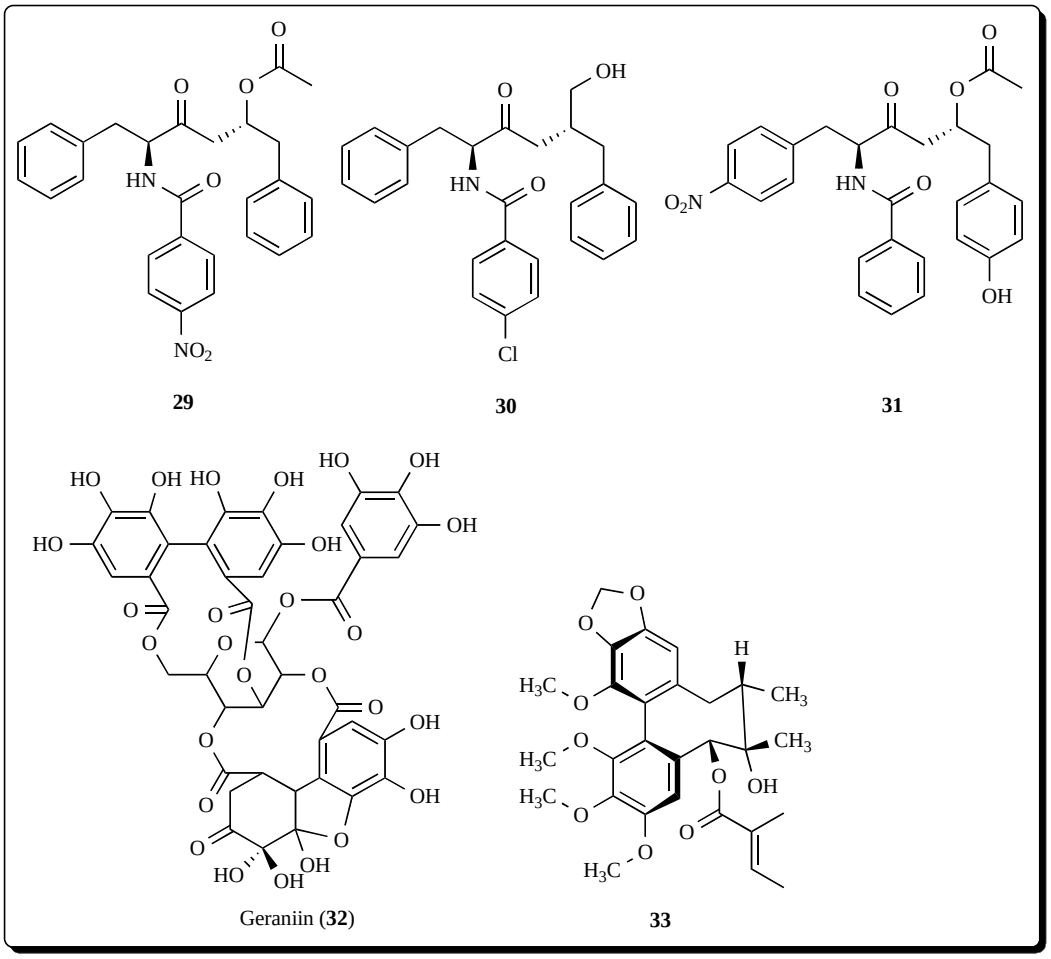
In 2001, a patent literature disclosed that helioxanthin and its analogues exhibit significant antiviral activity against HBV and flavivirus *in vitro*. It was found that they decreased cellular RNA levels of HBV and antigen expression as well as selective inhibition of HBV replication in a cell culture model [24]. Especially, lactam derivative **27** and helioxanthin cyclic hydrazide **28** exhibited significant anti-HBV activity *in vitro* (EC_{50} = 0.08 $\times 10^{-3}$ and 0.03 $\times 10^{-3}$ mM, respectively) [25].

Compound **28** suppressed both HBV RNA and protein expression, as well as DNA replication of both wild-type and 3TC-resistant virual strains, by post-transcriptional down-regulation of critical transcription factors in HBV-producing cells, thus diminishing HBV promoter activity. This action mechanism is unique and different from other anti-HBV compounds previously described [26].

1.5. Other Anti-HBV Agents from Natural Product

The EC_{50} of Matijing-Su (MTS, *N*-(*N*-benzoyl-l-phenylalanyl)-*O*-acetyl-l-phenylalanol) derivatives **29** (1.40 $\times 10^{-3}$ mM), **30** (2.33$\times 10^{-3}$ mM) and **31** (2.36 $\times 10^{-3}$ mM), etc. and the SI value of **31** (45.9) of the inhibition on the replication of HBV DNA were better than those of the positive control 3TC (EC_{50} = 82.4 $\times 10^{-3}$ mM, SI = 41.6) in the evaluation for anti-HBV activities in 2.2.15 cells line [27].





Geraniin (**32**), isolated from the ethyl acetate extract of Chinese *G. carolinianum* L, inhibited HBsAg and HBeAg secretion by more than 85.8% and 63.7%, respectively, at the non-cytotoxic concentration of 0.209 mM. The inhibition effects against HBsAg and HBeAg secretion by geraniin were higher than those by the positive control 3TC, with inhibitory rate 33.5% and 32.2% respectively at the same dosage. Because HBeAg is involved in immune tolerance during HBV infection, geraniin might be a candidate of anti-HBV agent to overcome the immune tolerance in HBV-infected patients [28].

Saikosaponins, the main active constituents of *Bupleurum spp.*, have been shown to possess hepatoprotective, immunomodulatory, anti-tumor and anti-viral activities. Especially, saikosaponin c showed a significantly lower level of HBeAg in Hep G2.2.15 cells. It also possessed activity in inhibiting HBV DNA replication; this inhibitory effect was not due to the cytotoxicity of saikosaponin c or its effect on Hep G2.2.15 cell proliferation [29].

Total phenolics from traditional Chinese medicine Oenanthe javanica (OJTP) can efficiently inhibit HBV replication in Hep G2.2.15 cells line and inhibit duck HBV replication *in vivo* [30]. Traditional Chinese medicine *Rheum palmatum L.* ethanol extract (RPE) could inhibit HBV effectively. The combined anthraquinone chrysophanol 8-*O*-beta-D-glucoside has been identified as the major active compound in RPE [31].

Ganoderic acid, from *Ganoderma lucidum*, inhibited replication of HBV in HepG2215 cells over 8 days at 8 μg/ml, and significantly protected the mice from liver injury induced by *M. bovis* BCG plus lipopolysaccharide (from Escherichia coli 0127:B8) at the same dosage [32]. Schisantherin C (**33**), a nuturally occurring lignan, exhibited potent anti-HBV activity with potency against HBsAg and HBeAg secretion by 59.7% and 34.7%, respectively, at 0.097 mM [33].

2. SYNTHETIC BIOACTIVE MOLECULES

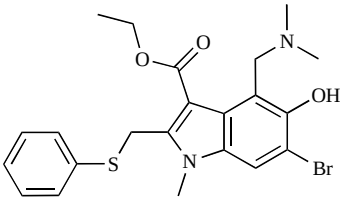
In this section, an overview of the recent work in the field of application of heterocyclic compounds such as indole, benzimidazole, thiazole, quinoline, oxadiazole, thiazole, pyrimidine, pyridine derivatives and some other related compounds as anti-HBV agents is presented. Many compounds have shown promising anti-HBV activity and further research in this area may lead to more efficacious and safer anti-HBV drugs.

2.1 Arbidol Based Derivatives

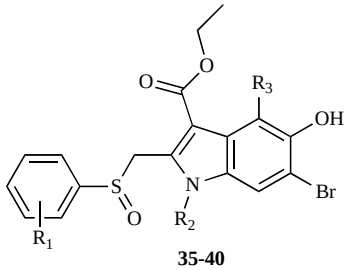
Arbidol (**34**) and its derivatives displayed a variety of biological activities, such as antiviral activities, immunostimulative and interferon-induced effects [34-36]. With

arbidol as the lead compound, Gong, P's group synthesized several series of new derivatives (Figs. **1-4**) and disclosed their favorable anti-HBV activities [37-43].

The SI values of inhibition on replication of HBV DNA in 2.2.15 cells of 6-bromo-5-hydroxy derivatives **35** (>8.7), **36** (10.8) [37], and 5-hydroxy derivatives **41** (9.38), **42** (8.85) [38] and **45** (8.41) [39] were greater than those of the other evaluated compounds including 3TC (7.0). 6-bromo-5-hydroxy derivatives **37-40** [37] and 5-hydroxy derivatives **43** and **44** [38] exhibited significant anti-HBV activities, and the EC_{50} values on replication of HBV DNA of these compounds were 6.2×10^{-3}, 11.25×10^{-3}, 9.43×10^{-3}, 10.58×10^{-3} and 52.14×10^{-3}, 31.67×10^{-3} mM, respectively, which were far more potent than the positive control 3TC (0.995 mM).



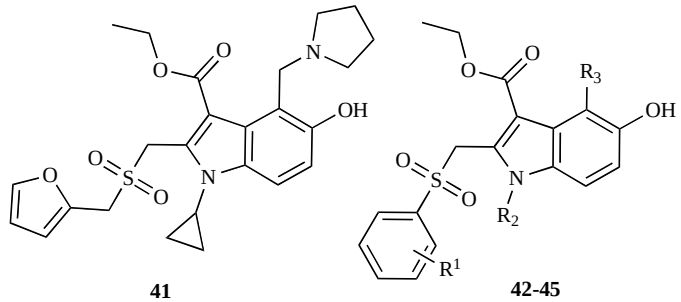
Arbidol (**34**)



35-40

35: R^1 = 4-fluoro; R^2 = methyl; R^3 = morpholinomethyl.
36: R^1 = 3-chloro; R^2 = methyl; R^3 = morpholinomethyl.
37: R^1 = 3,4-difluoro; R^2 = cyclopropyl; R^3 = 1*H*-imidazol-2-yl)methyl.
38: R^1 = 3,4-difluoro; R^2 = methyl; R^3 = (2-methyl-1*H*-imidazol-1-yl)methyl.
39: R^1 = 4-fluoro; R^2 = cyclopropyl; R^3 =guanidinomethyl.
40: R^1 = 3,4-difluoro; R^2 = cyclopropyl; R^3 = (4-methylpiperazin-1-yl)methyl.

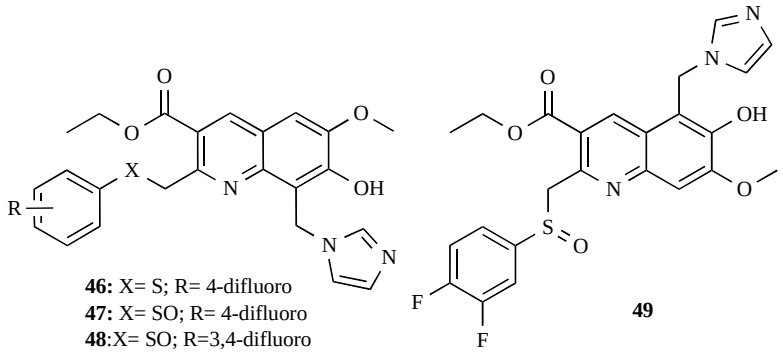
Fig. (1). Structures of ethyl 6-bromo-5-hydroxy-1H-indole-3-carboxylates.



41 **42-45**

42: R^1= 4-methyl; R^2= cyclopropyl; R^3= (1*H*-imidazol-2-yl)methyl
43: R^1= H; R^2= cyclopropyl; R^3= (2-methyl-2*H*-imidazol-2-yl)methyl
44: R^1= 3-methoxy; R^2= methyl; R^3= pyrrolidin-1-ylmethyl
45: R^1= 3-methoxy; R^2= methyl; R^3=(4-methylpiperazin-1-yl)methyl

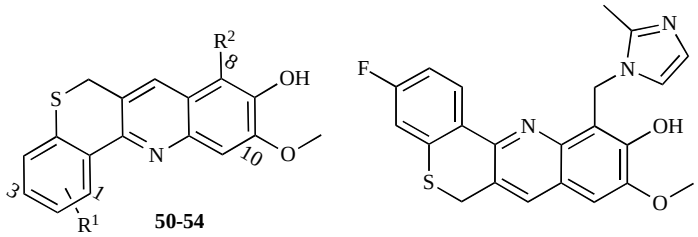
Fig. (2). Structures of ethyl 5-hydroxy-1H-indole-3-carboxylates.



46: X= S; R= 4-difluoro
47: X= SO; R= 4-difluoro
48:X= SO; R=3,4-difluoro

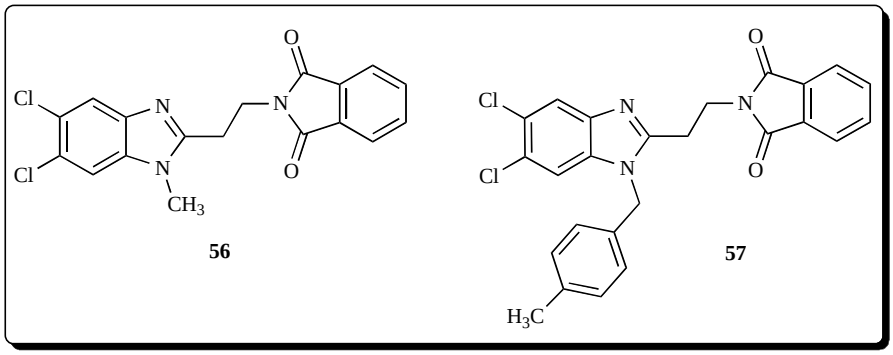
49

Fig. (3). Structures of ethyl 6(7)-hydroxyquinoline-3-carboxylate derivatives.



50: R^1= 2,4-dimethyl; R^2= piperidin-1-ylmethyl
51: R^1= 2-fluoro; R^2=pyrrolidin-1-ylmethyl
52: R^1= 2-methyl; R^2= (diethylamino)methyl
53: R^1= 2-methyl; R^2= morpholinomethyl
54: R^1= 3-fluoro; R^2= (diethylamino)methyl

Fig. (4). Structures of 6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9(10)-ols.



New ethyl 8-imidazolylmethyl-7-hydroxyquinoline-3-carboxylate derivatives **46** (EC_{50} = 12.6 $\times 10^{-3}$ mM, SI = 12.4), **47** (EC_{50} = 3.5 $\times 10^{-3}$ mM, SI = 37.9), and **48** (EC_{50} = 2.6 $\times 10^{-3}$ mM, SI = 61.6) showed more active abilities to inhibit HBV DNA replication than the positive control 3TC (EC_{50} = 0.343 mM, SI = 7.0) [40]. Ethyl 6-hydroxyquinoline-3-carboxylate derivative **49** possessed potent anti-HBV activity, with an EC_{50} of 4.7 $\times 10^{-3}$ mM, a 66-fold improvement over 3TC (EC_{50} = 0.311 mM) [41].

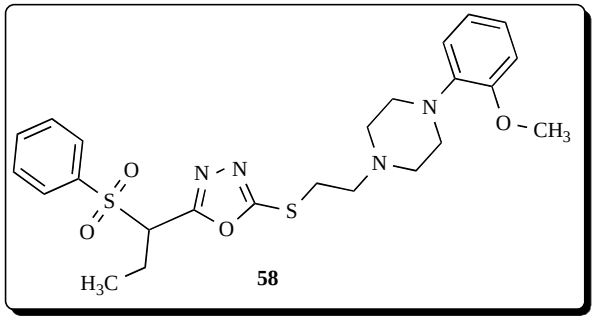
More recently, the same group reported the evaluation for anti- HBV activity and cytotoxicity of 6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9(10)-ols in Hep G2.2.15 cells. Compounds **50**-**55** were found to be potent anti-HBV compounds with EC_{50} values less than 50 $\times 10^{-3}$ mM [42,43]. Among them, compound **55** was the most effective anti-HBV agent (EC_{50} = 1.7 $\times 10^{-3}$ mM, SI = 60.3) [43].

2.2. Benzimidazole Derivatives

Benzimidazole derivatives **56** and **57** showed strong activity against HBV replication and low cytotoxicity in the evaluation for their anti-HBV activity and cytotoxicity *in vitro*. They have similar high antiviral potency (EC_{50}= 0.9 $\times 10^{-3}$ and 0.7 $\times 10^{-3}$ mM, respectively) and remarkable SI values (>1111 and 714, respectively), and could be promising leads for further development as novel HBV inhibitors [44].

2.3. 1,3,4-Oxadiazole Derivative

1,3,4-Oxadiazole derivative **58** inhibited the expression of HBsAg and HBeAg antigens in a concentration-dependent manner with no cytotoxicity and without any effects on the expression of HBV transcripts. The inhibition of virion production was comparable to that of 3TC, and EC_{50} values of 1.63 $\times 10^{-3}$ and 2.96 $\times 10^{-3}$ mM were obtained for compound **58** and 3TC, respectively [45].

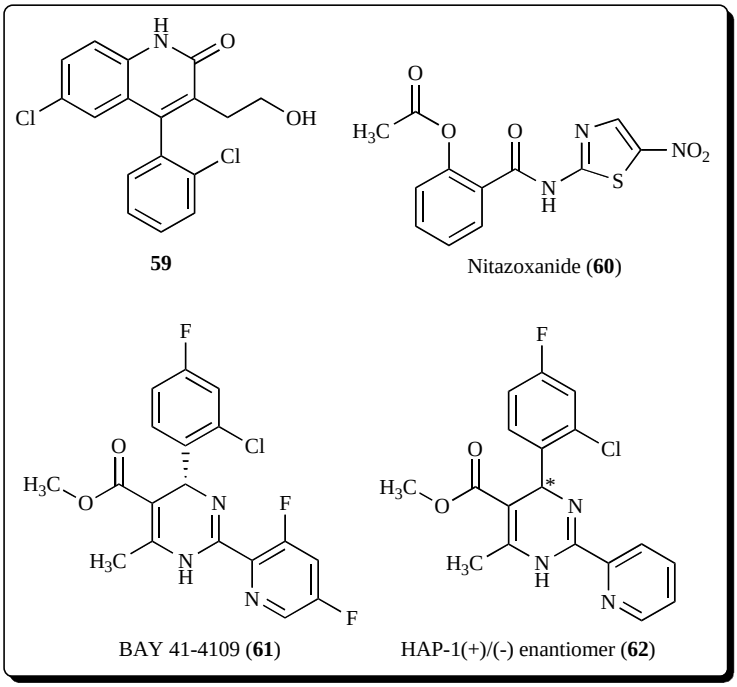


2.4. Quinolin-2-One Derivative

4-Aryl-6-chloro-quinolin-2-one **59** showed EC_{50} of 0.074 and 0.449 mM on HBsAg and HBeAg secretions, respectively, and led to higher SI values (SI = 23.2 and 3.4, respectively) [46].

2.5. Nitazoxanide (NTZ)

Nitazoxanide (NTZ, **60**), a thiazolide anti-infective agent, is active against anaerobic bacteria, protozoa, and a range of viruses in cell culture models. NTZ was equally effective at inhibiting replication of 3TC and adefovir dipovoxil-resistant HBV mutants. It also displayed synergistic interactions with 3TC or adefovir dipovoxil against HBV [47].

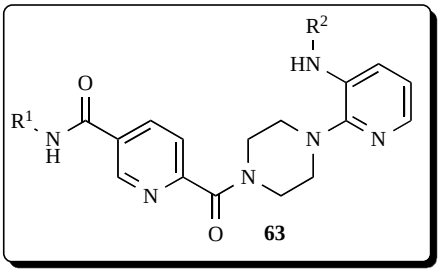


2.6. Heteroaryldihydropyrimidines (HAPs)

Heteroaryldihydropyrimidines (HAPs) derivatives BAY 41-4109 (**61**) [48] and HAP-1 (**62**, a racemic mix) [49] were identified as a novel class of HBV inhibitors in tissue culture and animal models with potency at nanomolar concentrations [48]. Further mechanism research has shown that HAPs may inhibit HBV replication by disturbing normal assembly process, which revealed a novel mechanism of action complementary to those of existing anti-HBV drugs [49-52].

2.7. Pyridinedicarboxylic Acid Derivatives

2,5-Pyridinedicarboxylic acid derivatives **63** were found to be the potent non-nucleoside HBV inhibitors targeting at the reverse transcriptase with $IC_{50} \leq 0.01$ μg/mL, and also they showed the low toxicity compared with the nucleoside analogues [53].



3. CONCLUSION

Currently, due to drug resistance, lower response rate, and adverse side-effects of current anti-HBV drugs, the search for novel drugs is mandatory.

Because of structural specificity, high activity and low toxicity of the natural products, they have been used as lead compounds for anti-HBV chemotherapeutic agents. Undoubtedly, natural products need to be investigated in more detail to obtain mechanistically and structurally novel and more potent derivatives and to explore their potential as novel adjuncts to established HBV therapy. On the other hand, the molecular design and synthetic strategy may also yield compounds with high potency to HBV wild and mutant stains and less untoward side effects. With the discovery and development of the 3D structures and functions of the targets which bound with the natural and synthetic HBV Inhibitors, the non-nucleoside typed anti-HBV drugs could be rationally designed through the known information of drug-target interaction.

In conclusion, the rapid pace of anti-HBV drug discovery, evidenced by the numerous inhibitors recently disclosed, will eventually yield novel, specific and highly efficacious anti-HBV therapies.

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