Bioorganic & Medicinal Chemistry 21 (2013) 4432–4441

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel artemisinin derivatives with potential usefulness against liver/colon cancer and viral hepatitis

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ARTICLE INFO

Article history:
Received 27 January 2013
Revised 12 April 2013
Accepted 18 April 2013
Available online 29 April 2013

Keywords:
Chemotherapy
HBV
HCV
Hepatoblastoma
Hepatocarcinoma

ABSTRACT

Antitumor and antiviral properties of the antimalaria drug artemisinin from *Artemisia annua* have been reported. Novel artemisinin derivatives (AD1–AD8) have been synthesized and evaluated using in vitro models of liver/colon cancer and viral hepatitis B and C. Cell viability assays after treating human cell lines from hepatoblastoma (HepG2), hepatocarcinoma (SK-HEP-1), and colon adenocarcinoma (LS174T) with AD1–AD8 for a short (6 h) and long (72 h) period revealed that AD5 combined low acute toxicity together with high antiproliferative effect (IC_{50} = 1–5 μM). Since iron-mediated activation of peroxide bond is involved in artemisinin antimalarial activity, the effect of iron(II)-glycine sulfate (ferrosanol) and iron(III)-containing protoporphyrin IX (hemin) was investigated. Ferrosanol, but not hemin, enhanced antiproliferative activity of AD5 if the cells were preloaded with AD5, but not if both compouds were added together. Five derivatives (AD1 > AD2 > AD7 > AD3 > AD8) were able to inhibit the cytopathic effect of bovine viral diarrhoea virus (BVDV), a surrogate in vitro model of hepatitis C virus (HCV), used here to evaluate the anti-*Flaviviridae* activity. Moreover, AD1 and AD2 inhibited the release of BVDV-RNA to the culture medium. Co-treatment with hemin or ferrosanol resulted in enhanced anti-*Flaviviridae* activity of AD1. In HepG2 cells permanently infected with hepatitis B virus (HBV), AD1 and AD4, at non-toxic concentrations for the host cells were able to reduce the release of HBV-DNA to the medium. In conclusion, high pharmacological interest deserving further evaluation in animal models has been identified for novel artemisinin-related drugs potentially useful for the treatment of liver cancer and viral hepatitis B and C.

1. Introduction

Primary and secondary liver cancers, whose most frequent origin is colon cancer, are among the main causes of death due to cancer worldwide.[1] Advances in surgery and radiotherapy permit to cure a certain number of these patients nowadays, however these approaches cannot always be applied, and pharmacological regimens are of limited usefulness because drugs available may elicit undesirable side effects, initial chemoresistance or the development of drug refractoriness during treatment.[2,3]

Hepatitis B virus (HBV), which belongs to the genus *Orthohepadnavirus* of the family *Hepadnaviridae*, causes chronic infection in the host liver that may result in cirrhosis and eventually hepatocellular carcinoma.[4] The WHO estimates that, in spite of the availability of a safe vaccine, there are 300 million people infected with HBV.[5] Currently the most commonly used drugs to treat chronic hepatitis B are pegylated interferon and nucleoside analogues, such as lamivudine and adefovir, which are not fully effective in many cases, owing to a wide range of adverse effects[6] and the appearance of viral mutant strains that are resistant to the drug.[7]

Infection by hepatitis C virus (HCV), which belongs to the genus *Hepacivirus* of the family *Flaviviridae*, is another important health problem with 130 million people affected worldwide. The probability of this people to become chronically infected is high (50–85%).[8] Unfortunately, in approximately 20% of these patients this condition may evolve to cirrhosis and hepatocellular carcinoma.[9]

Abbreviations: ART, artemisinin; ARS, artesunate; AD, artemisinin derivative; DHA, dihydroartemisinin.

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0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.04.059

Moreover, no safe vaccine against HCV has yet been developed. Treatment of chronic hepatitis C with pegylated interferon and ribavirin lack complete efficacy and good tolerability, which may be further complicated by the emergence of strains resistant to currently available drugs. Together these factors account for a frequent failure of the pharmacological treatments of these patients.[10]

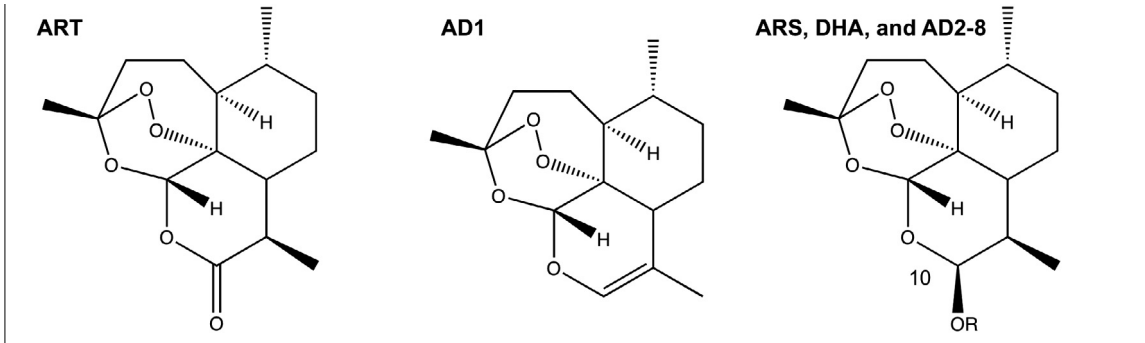
Artemisinin (ART) is a drug obtained from the plant *Artemisia annua* that has been recently recommended by the WHO in combination with other antimalaria drugs to treat drug-resistant *Plasmodium falciparum* strains, cerebral malaria and malaria in children.[11] In an attempt to improve ART bioavailability and efficacy, several derivatives have been synthesized such as dihydroartemisin (DHA), a reduced lactol that is more active but thermally less stable than ART; and artesunate (ARS), which is more active and less toxic than its parent drug. All these derivatives belong to a large family of compounds named artemisinins or artemisinin-like derivatives (ADs) that share the endoperoxide bridge and hence are expected to keep part of the pharmacological properties of ART.[12] Based on their cytotoxic activity against *Plasmodium falciparum*, ART and its semi-synthetic derivatives have shown promising results when they have been evaluated in vitro as anticancer and antiviral drugs.[13,14] More precisely, activity against viruses responsible for viral hepatitis B[15] and C[16] has been reported.

Recently several novel ADs with different bulky groups at position C10 of DHA have been synthesized (Fig. 1). The initial aim to synthesize this group of derivatives was to mimic the ability of ARS to be transformed into DHA through the cleavage of the ester moiety at different rates. The rational was that slower release of the active agent would improve the pharmacokinetic properties of the drug. Indeed these compounds have demonstrated in vitro cytotoxic effect against leukaemia cells, anti-angiogenic activity in vivo and ability to overcome chemoresistance mediated by multidrug resistance protein 1 (MDR1).[17] In the light of these remarkable characteristics further in vitro evaluation of eight of these novel drugs was recommended. The aim of the present work was therefore to investigate their antiproliferative effect against cells derived from primary liver cancer and colon adenocarcinoma as the most frequent origins of secondary liver cancer, and their antiviral activity versus the *Hepadnaviridae* and *Flaviviridae* families, accounting for viral hepatitis B and C. Since ART exerts its antimalarial activity through activation by iron, the activity of ADs in these in vitro models has been also investigated in the presence of iron(II)-glycine sulfate (ferrosanol) and iron(III)-containing protoporphyrin IX (hemin).

2. Materials and methods

2.1. Chemicals

ART, and ARS were obtained as previously described.[18] DHA and the ADs were synthesized following the methodology published elsewhere.[17] Dulbecco's modified Eagle's medium (DMEM), gentamicin, 3-amino-7-dimethylamino-2-methylphenazine (Neutral Red), $NaHCO_3$, L-glutamine, minimum essential medium (MEM), thiazolyl blue tetrazolium bromide (MTT), hemin and dimethylsulphoxide (DMSO) were provided by Sigma–Aldrich Quimica (Madrid, Spain). MEM GLUTAMAX™ was obtained from Invitrogen (Barcelona, Spain). Dodecyl sulphate sodium salt (SDS) was from Merck (Barcelona, Spain). Ciprofloxacine (Baycip®) was supplied by Bayer (Leverkusen, Germany). 4-(2-Hydroxyethyl)-1-piperazineethansulphonic acid (HEPES), trypsin and geneticin® (G418) were from Roche (Barcelona, Spain). Ferrosanol was purchased form UCB Pharma, S.A. (Madrid, Spain). Fetal calf serum (FCS) was obtained from TDI (Madrid, Spain).



Code	Artemisinin-like derivatives	MW	R	R
ART	Artemisinin	282.33	NO	-
ARS	Artesunate	384.42	YES	$COCH_2CH_2COOH$
DHA	Dihydroartemisinin	284.33	YES	H
AD1	Anhydrodihydroartemisinin	266.33	NO	-
AD2	10-dihydroartemisinyl acetate	326.38	YES	$COCH_3$
AD3	10-dihydroartemisinyl butyrate	354.44	YES	$COCH_2CH_2CH_3$
AD4	10-(2'-butyloxy) dihydroartemisinin	340.38	YES	$CH_2CH(CH_3)_2$
AD5	10-dihydroartemisinyl 2'- propylpentanoate	410.54	YES	$COCH(C_3H_7)_2$
AD6	10-dihydroartemisinyl 2', 2'-dimethylpropionate	368.46	YES	$COC(CH_3)_3$
AD7	10-dihydroartemisinyl perfluoropropionate	430.36	YES	COC_2F_5
AD8	10-dihydroartemisinyl dimethylcarbamate	355.43	YES	$CON(CH_3)_2$

Figure 1. Chemical structure of artemisinin (ART), artesunate (ARS), dihydroartemisinin (DHA) and novel ART derivatives (AD1–AD8).