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SYNTHESIS AND INVESTIGATION OF INHIBITOR PROPERTIES OF NEW ANALOGUE OF RIBAVIRIN AGAINST SOYBEAN LIPOXYGENASE

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Abstract: The aim of this work was to synthesize an analogue of ribavirin with potential inhibitor properties against soybean lipoxygenase (LOX). The newly synthesized inhibitor mimics the well-known natural inhibitor of different enzymatic systems ribavirin, but differ by the substitution of triazine base with the residue of thiourea, containing primary NH_2 group.

Different conditions of reactions of synthesis were studied, using a donor-acceptor mechanism in the presence of several Lewis acids.

INTRODUCTION

Lipoxygenases (LOX) are enzymes implicated in a broad range of inflammatory diseases as well as cancer, asthma and atherosclerosis proliferation. Participation of lipoxygenase in some process during these illness developments makes the metabolic pathway of LOX an interesting target for investigation of new inhibitors. The drugs available on the market against LOX reported to have various side effects. To develop potent and selective therapeutic agents against LOX, it is essential to have the knowledge of its active site (Boyington, Gaffney, Amzel, 1993, Prigge, Boyington et al., 1997).

Lipoxygenase applications

The lipoxygenase pathway has become an important therapeutic target for the prevention of different inflammatory diseases and in a cancer chemoprevention. So far, the Food and Drug Administration (FDA) approved drugs for diseases caused by LOX reported to have various side effects (Liu, Dube and Lancaster,

1996). Hence, it is essential to implement specific inhibitor, which will not affect other normal physiological functions.

The active site of animal LOX is similar to that of plant LOX with regards to its sequence homology and structure as seen by comparing rabbit LOX with soybean LOX (Boyington et al., 1993; Minor, Steczko et al. 1993; Gillmor, Villasenor A. and Fletterick, 1997; Minor, Steczko et al. 1996; Skrzypczak, Amzel et al. 2001).

Lipoxygenases are commonly found in the plant and animal kingdoms. Although the overall architecture of plant lipoxygenases such as soybean lipoxygenase is similar to mammalian lipoxygenases, they share little sequence similarity (about 25%) (Noguchi, Miyano, Matsumoto, 1996). In contrast, there are sequence similarities of about 60% among human 5-, 12- and 15-lipoxygenases. Even though these enzymes show a high sequence similarity, the regulatory mechanism of 5-lipoxygenase (5-LOX) is more complex compared to the other human lipoxygenases. In general, lipoxygenases are comprised of two domains; N-terminal and C-terminal domains. The N-terminal domain is a regulatory domain and consists mostly of β-barrels, while the C-terminal domain is a catalytic domain and consists mostly of α-helices (Sigal, 1991). The non-heme iron atom is located in the catalytic C-terminal domain, whereas the function of the N-terminal domain is not unambiguously characterized. For 5-LOX, it is clear that the N-terminal domain is essential for translocation to the nuclear membrane, whereas for the other LOXs, this is still under debate (Sigal, 1991; Chen, Funk, 2001).

The highest level of sequence identity between LOX from plants and mammals lies in the area of the catalytic domain containing the non-heme iron atom. Taking into account this fact, soybean LOX is a good alternative of a model design of inhibitors (Skrzypczak, Kanging et al. 2003).

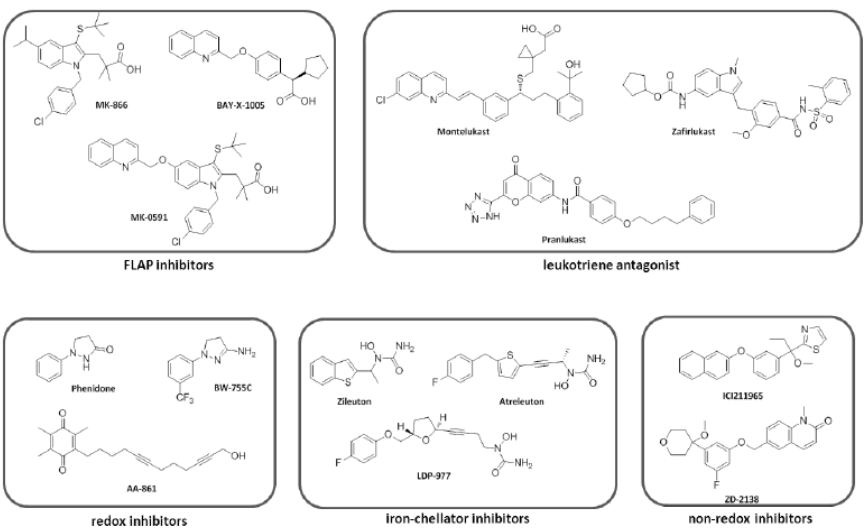

Figure 1. Structural variety in lipoxygenase inhibitors (Skrzypczak, Kanging et al. 2003).

Role of arachidonic acid in metabolism

The identification of the role of arachidonic acid metabolites in several inflammatory diseases led to a significant drug discovery effort around arachidonic acid metabolizing enzymes. However, to date success in this area has been limited. This might be attributed to the lack of selectivity of the developed inhibitors and to a lack of detailed understanding of the functional roles of arachidonic acid metabolites in inflammatory responses and cancer. This calls for a more detailed investigation of the activity of arachidonic acid metabolizing enzymes and the development of more selective inhibitors (Solomon, Zhou et al., 1997).

MATERIALS AND METHODS

$Cu(OTf)_2$ (98%), AgOTf, ribose, trimethylchlorosilane (TMCS), pyridine, acetic anhydride, thiourea were obtained from Acros Organics. $BF_3.OEt_2$ was purchased from Fluka Chemika.

Synthesis of peracetylated ribose

1 eq. ribose was dissolved in acetic anhydride (10eq.) and pyridine was added (14 eq.). The reaction was stirred for 24 hours at room temperature and monitored by TLC.

Synthesis of 2,3,5-triacetyl-1-β-ribofuranosyl thiourea in presence of $Cu(OTf)_2$ Lewis acid

0.3 g peracetylated ribose was dissolved in 20 ml dry CH_2Cl_2. $Cu(OTf)_2$ (or AgOTf) and carbamide was added at 0˚C and the reaction mixture was stirred for 24 hours. The reaction was followed by TLC in CH_2Cl_2: MeOH (9:1). At the end of reaction time, the reaction mixture was neutralized with Et_3N to pH, 7-7.5. Several reaction conditions were studied and they are summarized in table 1. The solvent was evaporated under vacuum. The obtained product was purified by column flash chromatography, using silica gel (0.063-200 mesh) and CH_2Cl_2: MeOH 9:1, as eluting system.

2,3,5-triacetyl-1-β-ribofuranosyl thiourea by silylation with TMCS

Thiourea (1.5 eq.) was suspended in dry CH_2Cl_2 and Et_3N was added. TMCS (2 eq.) was added drop wise and stirred during 1 hour, at room temperature. Further 1 eq. peracetylated ribose was added. The reaction was monitored by TLC for 24 hours. H_2O was added to quench the reaction and the reaction mixture was stirred for 1 hour. The product was extracted with CH_2Cl_2, dried by Na_2SO_4 and the organic solvent evaporated. The aim product was purified by column flash chromatography using mobile phase CH_2Cl_2: MeOH 9:1.

RESULTS AND DISCUSSION

Ribavirin demonstrates well-expressed antiviral activities in vitro and in vivo: incorporation with mutagenic nucleoside by the viral RNA polymerase,

immunnomodulatory effects and inhibition of inosine monophosphate dehydrogenase (IMPDH). Unfortunately, its mechanism of action is still not well known (Hamamoto Y., Nakashima H.,Yamamoto N. et al., 1987).

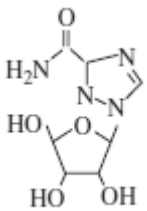

Figure 2. Chemical structure of ribavirin (Lin C. C., Philips L., Xu C. et al., 2004).

According to Steele et al. (Steele V. E., Holmes C. A., Hawk E. T et al. 1999) the presence of primary amino or hydroxyl functions are prerequisite for inhibition activity against lipoxygenase.

Taking into account these facts, we designed a new analogue of ribavirin, replacing triazine base in the first position of the ribose residue with thiourea (Fig. 3).

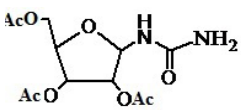

Figure 3. Chemical structure of ribavirin (Lin C. C., Philips L., Xu C. et al., 2004).

Different conditions for the synthesis of aim compound were investigated, which are summarized in table 1.

Table 1. Different condition for synthesis of 2,3,5-triacetyl-1-β-ribofuranosyl thiourea.

Donor	**eq.**	**Acceptor**	**eq.**	**Lewis acid**	**eq.**	**Conditions**	**Yield, %**
Peracetylated ribose	1	thiourea	2	$Cu(OTf)_2$	0.5	0˚C, 24 hours;	3.01
Peracetylated ribose	1	urea	2	AgOTf	0.5	1 hour, room temperature	23.84
Peracetylated ribose	1	thiourea	1.5	$Cu(OTf)_2$/TMCS	2	1 hour, room temperature	50.0
Peracetylated ribose	1 0.8 0.94	thiourea	2 1.6 1.89	$BF_3.OEt_2$	2 1.6 1.89	24 hours, room temperature	7.04 8.4 4.7

The approach used for the synthesis of new compound includes direct activation of the carbohydrate moiety by means of Lewis acids (table 1). As it can be seen by the obtained yields the most appropriate approach is using $Cu(OTf)_2$ as Lewis acid.

CONCLUSIONS

One new potential inhibitor of LOX, an analogue of ribavirin was synthesized. Different approaches for its synthesis were studied. It was revealed that direct activation in the presence of $Cu(OTf)_2$ as Lewis acid is the most appropriate method for synthesis.

The obtained inhibitor is an efficient synthetic analogue of ribavirin for soybean LOX inhibition.

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