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OBJECTIVES

The aim of our research was to design and synthesized new SBH analogs derived from 5-bromo salicylaldehyde and different acid hydrazides.

METHODS

In the present study we report synthesis and physicochemical characterization of two new hydrazones - 5-bromo-salicylaldehyde 4- hydroxy-benzoylhydrazone and 5-bromo-salicylaldehyde isonicotinoyl hydrazone. The compounds were prepared by the Schiff base condensation between 5-bromo salicylaldehyde and 4- hydroxy-benzhydrazide or isonicotinoyl hydrazide in ethanol. The structures of the new hydrazones were confirmed by elemental analyses, IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The cytotoxic activity of the new compounds was assessed using the MTT dye reduction assay.

RESULTS AND CONCLUSIONS

Two new potential anticancer compounds were synthesized and were tested for *in vitro* cytotoxic activity on a panel of human leukemic and tumor cell lines.

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AN UNEXPECTED PRODUCT FROM THE REACTION OF ACENOCOUMAROL AND PHOSPHORYL CHLORIDE

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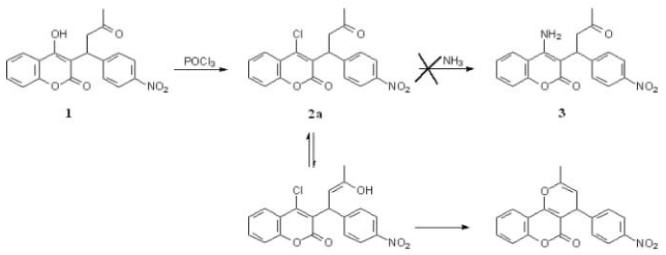
INTRODUCTION

Coumarins are large class of compounds with the common structure of benzo-α-pyrone (2H-1-benzopiran-2-one). The family of coumarins is characterized by wide structural diversity. According to the substitution in benzene and pyrone rings they can be generally classified in the following categories: (i) simple coumarins, (ii) furano-coumarins,

Scheme 1



(iii), pyranocoumarins, (iv) biscoumarins and triscoumarins, and (v) coumarinolignans.

Simple coumarins and their analogues, such as bis and tris-cumarins continually attract research interest due to their diverse biological activities, such as anticoagulant, anti-inflammatory, anti-tumoral, antimicrobial and antioxidant activity. Also, they have been reported to be CNS active and possess enzyme inhibition properties. These properties of coumarins made them very attractive for re-design, organic synthesis and development [1].

Acenocoumarol (**1**) [(*RS*)-4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2*H*-chromen-2-one, logP 1,98; logS (predicted) –4,53] is an anticoagulant that functions as a vitamin K antagonist. Molecule of acenocoumarol has a single chiral center that gives rise to two different enantiomeric forms. (*R*)-(+)-Acenocoumarol has a longer plasma elimination half-life and slower plasma clearance, compared to the (*S*)-(–)-enantiomer. Related to these pharmacokinetic characteristics, (*R*)-(+)-acenocoumarol is more potent *in vivo* as an anti-coagulant than the (*S*)-(–)-enantiomer and is largely responsible for the overall anticoagulant response [2].

Acenocumarol is metabolized predominantly by CYP2C9, while its target on the enzyme vitamin K epoxidase is VKORC1. Genetic variants in CYP2C9 and VKORC1 are associated with variability in coumarin anticoagulant dose requirement, over-anticoagulation, bleeding risk and stabilization [3, 4].

Starting from acenocoumarol as a leading compound our aim was to synthesize a novel 4-amino derivate of acenocoumarol. Due to the electronic similarity of oxygen (hydroxyl group) and nitrogen (amino group) we supposed that (*RS*)-4-amino-3-[1-(4-nitrophenyl)-3-oxobutyl]-2*H*-chromen-2-one (**3**) could be a potential bioisostere of acenocoumarol.

MATERIALS AND METHODS

Synthesis of (*RS*)-2-methyl-4-(4-nitrophenyl)pyrano(-)[3,2-c]chromen-5-one (4)

The mixture of (*RS*)-Acenocoumarol (Alkaloida Chemical Co) and $POCl_3$ (Merck Chemicals) was heated at 70 °C for 4 hours. Afterwards, the reaction mixture was cooled to room temperature then slowly poured onto crushed ice and stirred for 45 min. Crystals of **4** were formed, which were separated by filtration and washed with cold water.

The obtained compound was characterized by FTIR, ^{1}H-NMR (**Fig. 1**) and ^{13}C-NMR (**Fig. 2**) spectra.

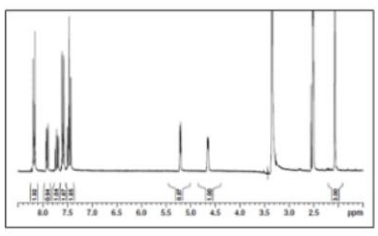


Fig. 1. ^{1}H-NMR spectra of 4

Македонски фармацевтски билтен 57 (додаток), 2011

RESULTS AND DISCUSSION

First, the reaction of **1** with phosphoryl chloride was performed to obtain 4-chloro derivative **2a**. Subsequently, target compound **3** was planned to be obtained in nucleophiic reaction of ammonia with intermediate **2a** (**Scheme 1**). However, after the first step and replacement of the hydroxyl group in position 4 at coumarin moiety, unplanned cyclization occurred. With intramolecular nucleophilic attack, oxygen from hydroxyl group at enolate form **2b** replace chlorine atom resulting in formation of compound **4**. The reaction was performed at three different temperatures (70 °C, 90 °C and boiling temperature of phosphoryl chloride) in order **2a** to be obtained and isolated. However, in all the reaction conditions, as a product of reaction, the compound **4** was obtained. Thus, the future research will be aimed to synthesize and isolate compound **2a** as intermediate for obtaining novel compounds with potential anticoagulant activity.

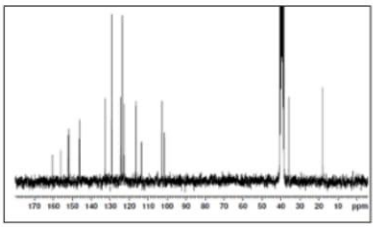


Fig. 2. ^{13}C-NMR spectra of 5

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НЕОЧЕКУВАН ПРОДУКТ ПРИ РЕАКЦИЈА НА АЦЕНОКУМАРОЛ И ФОСФОРИЛ ХЛОРИД

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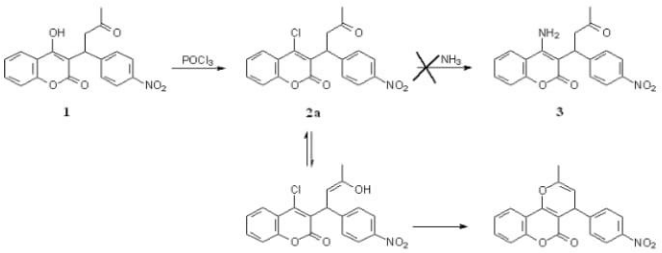
ВОВЕД

Кумарините се голема класа соединенија кои во својата струкура го содржат прстенот на бензо-α-пирон (2H-1-бензопиран-2-он). Фамилијата на кумарините се карактеризира со широка структурна различност и во зависност од супституентите на бензеновиот и пиронскиот прстен, кумарини-те се класифицирани во следните категории: (а) едноставни кумарини, (б) фурано кумарини, (в) пирано

Шема 1



кумарини (г) бис и трискумарини и (д) кумариноligани. Едноставните кумарини и нивните аналози, како бис и трис кумарините постојано го привлекуваат научниот интерес пред се заради нивната широка биолошка активноста и тоа како антикоагуланси, антиинфламаторни, антитуморни, антимикробни и антиоксидантни агенси. Докажано е дека овие соединенија се активни и на ниво на централниот нервен систем, а поседуваат и својства на ензимски инхибитори. Заради наведените особини кумарините се особено интересни од аспект на нивно ре-дизајнирање, синтеза и понатамошен развој [1].

Аценокумаролот (**1**) [(*RS*)-4-хидрокси-3-[1-(4-нитрофенил)-3-оксобутил]-2*H*-хромен-2-он, logP 1,98; logS (предвидена) –4,53] е антикоагуланс кој делува како антагонист на витаминот К. Молекулот на аценокумаролот има еден хирален центар кој укажува на постоење на две енантиомерни форми. (*R*)-(+)-аценокумаролот има подолго полувреме на плазма елиминација и побавен плазматски клиренс споредено со (*S*)-(−)-енантиомерот. (*R*)-(+)-енантиомерот е попотентен *in vivo* како антигоагуланс од (*S*)-(−)-енантиомерот, и воглавно е одговорен за севкупниот антигоагулантен одговор [2].

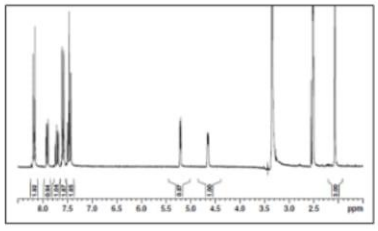
Аценокумаролот се метаболизира воглавно со CYP2C9, додека негова цел на ензимот витамин К епоксидаза е VKORC1. Генетските варијации во CYP2C9 и VKORC1 се асоцирани со варијабилноста во потребната доза на аценокумаролот, прекумерна антикоагулација, опасност од крварење и стабилиација [3,4].

Тргнувајки од аценокумаролот како водечко соединение, целта на овој труд беше да се синтетизира нов 4-аминодериват на аценокумаролот. Заради електронската сличност на кислородниот атом (хидроксилна група) и азотниот атом (амино група) претпоставивме дека (*RS*)-4-амино-3-[1-(4-нитрофенил)-3-оксобутил]-2*H*-хромен-2-он (**3**) може да биде потенцијален биоизостер на аценокумаролот.

МАТЕРИЈАЛИ И МЕТОДИ

Синтеза на (*RS*)-2-метил-4-(4-нитрофенил)пирано(-)[3,2-с]хромен-5-он (4)

Смесата од (*RS*)-Аценокумарол (Alkaloida Chemical Co.) и фосфорил хлорид ($POCl_3$) (Merck Chemicals) беше загревана на 70 °C во период од 4 часа. Понатаму, реакционата смеса беше оладена до собна температура а потоа беше истурена во ситно здробен мраз и постојано мешана за време од 45 мин. Притоа беа формирани кристали од **4** кои беа филтрирани и премиени со ладна вода. Добиеното соединение беше карактеризирано со FTIR, ^{1}H-NMR (**Сл. 1**) и ^{13}C-NMR (**Сл. 2**).



Сл. 1. 1H-NMR спектар на 4