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Short communication

Synthesis and biological evaluation of new securinine analogues as potential anticancer agents

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ABSTRACT

A series of new securinine analogues was prepared by Heck reaction from readily accessible securinine and commercially available iodoarenes. The *in vitro* cytotoxicity of the prepared compounds was assayed against a panel of four cancer cell lines: A375, A549, HCT-116 and HL-60 showing promising growth inhibition with excellent IC_{50} values in the nanomolar range. The plasmatic stability of the most potent analogue was also investigated demonstrating that they might serve as valuable leads for the development of anticancer drugs.

1. Introduction

Nature constitutes a wide reservoir of potential drugs that has been overlooked during the last decades, despite the fact that about half of the marketed agents in today's arsenal of drugs are either natural products or derivatives thereof [1]. The 2015 Nobel Prize [2] attributed for the discoveries of ivermectin and artemisinin, pleads for a closer look at the natural-product based drug discovery approach. Consequently and in the context of a strong demand for new anticancer drugs, this approach is highly relevant owing to the success of numerous semisynthetic drugs launched on the market such as docetaxel (Taxotere®) [3], vinflunine (Javlor®) [4] or everolimus (Afinitor®) [5].

Securinega suffruticosa is a shrub originating from Asia and Russia that is known to produce numerous alkaloids [6]. Among them, securinine **1** that possesses a four fused-rings structure along with a butenolide moiety, has been found to exhibit a wide spectrum of biological activities making it a promising candidate for the development of future therapies (Fig. 1). For example, compound **1** displays benefic effects on various affections such as amyotrophic lateral sclerosis or poliomyelitis [7–9].

Securinine **1** has also been found to be active as a gamma amino butyric acid (GABA) receptor antagonist [10]. In 2010, Wald et al. have demonstrated that securinine can impair the growth of colon cancer cells (HCT-116) [11]. The observed anticancer activity is proposed to happen through the modulation of the protein p73 expression. Later on, the same team assayed the activity of securinine on other cancer cell lines such as HL-60 (colon), OCI-AML3 (leukemia) and THP-1 (leukemia) [12–14]. More recently, Stockwell et al. have proven that securinine acts as a covalent inhibitor of protein disulfide isomerase (PDI), an enzyme that holds potential as a target for treatment of cancer [15].

However, to the best of our knowledge and despite the obvious biological properties displayed by securinine **1**, its use as a source of novel leads for the development of new drugs and other valuable bioactive agents remains scarce [16]. Therefore and in connection with our research program dedicated to discover new anticancer drugs from natural sources, we became interested in the use of

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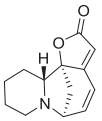


Securinine (**1**)

Fig. 1. Structure of securinine.

securinine for the synthesis of novel compounds with diversified structural arrangements possessing interesting biological activities. Considering the various chemical functions present on the securinine scaffold, we pursued our efforts towards the modification of the α,β,γ,δ-unsaturated system to define its structure-activity relationship [17]. Herein, we report the synthesis and biological evaluation of an original series of securinine derivatives.

2. Results and discussion

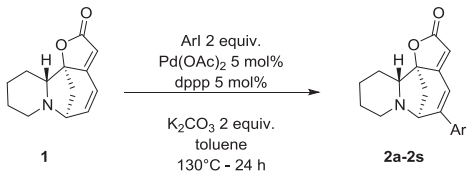
2.1. Chemistry

Some preliminary experiments led us to consider that Heck reaction [18] between securinine **1** and aryl halides could be feasible leading to a wide variety of C_{15} arylated securinine analogues. Toward this end, securinine **1** was reacted with a set of diversely functionalized aryl iodides, in the presence of 5 mol% of palladium acetate associated with dppp (1,3-bis(diphenyl phosphino) propane) as the ligand, and potassium carbonate as the base in toluene at 130 °C for 24 h. As outlined in Scheme 1, under our optimized conditions, the reaction proceeded with high regioselectivity in all cases, giving the desired derivatives **2a**–**2s** in good yields. Indeed, reaction conducted with electron rich aryl iodides as partner resulted in the formation of the corresponding C_{15} arylated products **2a**–**2h** in the range of 36%–97% yields. Similar results were obtained with electron poor aryl iodides, giving the corresponding compounds **2i**–**2q** in the range of 52%–85% yields. Finally, it should also be mentioned that no reaction takes place with *ortho*-substituted iodobenzene derivatives, probably due to an increased steric hindrance between the catalyst and the *ortho*-substituted group of the substrate during the course of the reaction.

2.2. Biological evaluation

2.2.1. Cytotoxicity

The *in vitro* cytotoxicity of the new securinine analogues **2a**–**2s** was first evaluated against colon cancer cell line HCT-116 at concentrations of 20 μM, 10 μM and 1 μM. The results of these



2a Ar = Ph, 70%
2b Ar = *m*Me-C_6H_4, 67%
2c Ar = *p*Me-C_6H_4, 70%
2d Ar = *m*OMe-C_6H_4, 74%
2e Ar = *p*OMe-C_6H_4, 67%
2f Ar = *m,p*OCH_2O-C_6H_3, 57%
2g Ar = *m*$N(Me_2)$-C_6H_4, 97%
2h Ar = *p*$N(Me_2)$-C_6H_4, 36%
2i Ar = *m*F-C_6H_4, 59%
2j Ar = *p*F-C_6H_4, 75%
2k Ar = *m*Cl-C_6H_4, 85%
2l Ar = *m,p*Cl-C_6H_3, 70%
2m Ar = *m*CF_3-C_6H_4, 52%
2n Ar = *m*CO_2Me-C_6H_4, 67%
2o Ar = *p*CO_2Me-C_6H_4, 60%
2p Ar = *m*C(O)Me-C_6H_4, 69%
2q Ar = *p*C(O)Me-C_6H_4, 64%
2r Ar = 2-naphtyl, 63%
2s Ar = 2-fluorene, 48%

Scheme 1. Synthesis of C-15 arylated securinine analogues.

Table 1
In vitro cell growth inhibitory effect of compounds **2b**, **2d**, **2k** on HCT-116, HL-60, A549 and A375.

Entry	Compound	IC_{50} (μM)			
		HCT-116	HL-60	A549	A375
1	**2b**	0.28	0.13	0.14	0.07
2	**2d**	0.26	0.14	0.12	0.08
3	**2k**	0.23	0.14	0.11	0.08
4	Securinine **1**	>10	>10	>10	6.1
5	Etoposide	>10	0.86	0.59	0.24

experiments are reported in Table 2 as percentage of growth inhibition after a 72 h exposure to the compound compared to non-treated cells. Under our experimental conditions, securinine **1** was found weakly active, inducing only approximately 50% growth inhibition at 10 μM. This value is in agreement with the previously reported data in which IC_{50} on two cell lines (A549 and MCF-7) is around 25 μM [19]. At first, the presence of the phenyl appendage on securinine **1** seemed to have a negligible impact on cytotoxic activity since compound **2a** displayed a growth inhibition similar to that obtained for **1**. However, it was found that the substitution pattern on the aromatic ring had a strong influence on the cytotoxic potency. Indeed, while compounds bearing a substituent in *para* position were poorly active compared to securinine **1**, those bearing a substituent in *meta* position showed equivalent or even superior cytotoxicity compared to the parent securinine. In particular, compounds **2b**, **2d** and **2k** exhibited an increased potency towards HCT-116 cancer cells with at least 75% growth inhibition at 1 μM.

Based on these encouraging results, the most potent C_{15} arylated securinine analogues **2b**, **2d** and **2k** were subjected to additional biological evaluation aiming at measuring the IC_{50} values on HCT-116 cell line, as well as against three additional cancer cell lines, namely, A375 (melanoma), A549 (lung) and HL-60 (leukemia). Etoposide, a widely used anticancer drug deriving from naturally occurring podophyllotoxine, was employed as a standard compound in this study. To our delight, the results depicted in Table 1 showed that compounds **2b**, **2d** and **2k** exhibited significant higher activities than both the parent compound **1** and etoposide against all four tested cancer cell lines. Accordingly, the IC_{50} values of all the three above mentioned compounds appeared to be lower than 300 nM on HCT-116 while both securinine and etoposide proved to be inactive. Significantly better IC_{50} values of 130 nM and 140 nM, respectively, were obtained for compounds **2b**, **2d** and **2k** against HL-60 cell line, whereas etoposide had an IC_{50} of 860 nM and securinine was still found to be inactive. Comparable results were obtained on cancer cell line A549. Finally, while cell line A375 appeared to be slightly more sensitive to securinine and etoposide with IC_{50} values of 6100 nM and 240 nM, respectively, we were pleased to find that those values are by far significantly inferior to those obtained for the securinine analogues **2b**, **2d** and **2k**. Indeed, those compounds possess an IC_{50} of 70 nM and 80 nM respectively, which correspond to a 80-fold potency increase from the original compound.

2.2.2. Plasmatic stability assay

Compound **2d** that proved to be the most potent securinine analogue, was then engaged in plasmatic stability assay in comparison with the parent securinine **1** in order to anticipate further *in vivo* experiments. For instance, **2d** displayed a similar profile to that of securinine and indicating that both compounds are stable after 120 min incubation in rat, mouse or human plasma Fig. 2.