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Synthesis of novel cytotoxic 3-azolylsteroids *via* Cu-catalyzed C–N coupling

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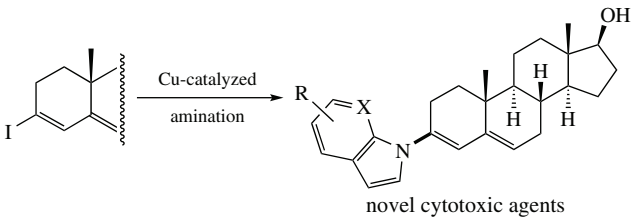
A series of novel 3-azolylandrosta-3,5-dienes was prepared *via* Cu-catalyzed Ullmann C–N coupling between a readily available steroidal vinyliodide and a variety of NH-heterocycles. The cytotoxic activity of the target compounds was evaluated against selected cancer cell lines (MCF-7, SKOV-3, DU-145, PC-3). Compound bearing gramine fragment showed the highest antiproliferative effect with IC_{50} values in the range of 2.0–10.1 μM.



Keywords: steroids, copper catalysis, nitrogen heterocycles, iodoalkenes, antiproliferative activity, MTT assay.

Steroids are well-recognized as ubiquitous natural compounds governing a plethora of crucial biochemical processes. For decades polycyclic steroidal core represented a privileged scaffold for design of new drugs with a broad spectrum of clinical utility,[1] including anticancer therapy.[2] The introduction of heterocyclic substituents deserved much interest in recent years as a tool for modulating activity of steroid derivatives.[3] Thus, a number of heteroarylated steroids have shown remarkable cytotoxic effect against hormone-dependent cancer cell lines.[4] In 2011, abiraterone acetate, a pyridine-substituted androstane, was approved by the FDA for the treatment of castrate-resistant prostate cancer.[5]

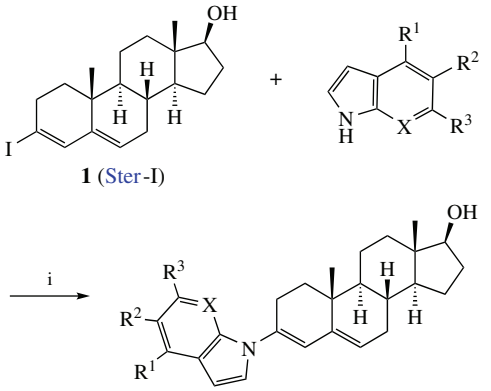
Despite a significant progress in the synthesis of complex structures, the preparation of functionalized steroids in selective and efficient fashion remains a challenging task. One of the possible solutions is the use of up to date methods based on transition metal catalysis, which have markedly advanced the chemistry of steroids and related compounds.[6] Our research group has developed miscellaneous approaches to modification of steroid molecules employing palladium[7] and copper[8] catalyzed transformations. In particular, we have elaborated an efficient protocol for the preparation of azolylandrostanes *via* Cu-catalyzed Ullmann C–N coupling.[8(b)] In continuation of this work, we decided to explore further the scope of this method and demonstrate its applicability to reaction with various functionalized indoles. Inspired by a promising antiproliferative activity of compounds whose structure combines steroidal and heterocyclic moieties, we performed biological assays of the obtained library. Herein, we report results of this study establishing 3-azolylandrosta-3,5-dienes as a novel type of cytotoxic agents.

The attachment of heterocyclic moieties to steroidal backbone was performed by the Cu-catalyzed Ullmann C–N coupling. The starting iododiene **1** was readily prepared from testosterone according to a described procedure.[8(b)] Due to a high importance of Cu-catalyzed amination, a variety of protocols were reported for this transformation.[9] Nevertheless, a careful optimization of reaction conditions is still needed to achieve satisfactory results with new substrates. Thus, in our previous report we have shown that iodosteroids can be efficiently coupled with NH-heterocycles using 10% CuI and 20% dipivaloylmethane in DMSO at 100 °C in the presence of K_2CO_3 as a base.[8(b)] In the present work, this protocol was applied to carry out the reaction between steroidal iododiene **1** (Ster-I) and a number of azoles (Scheme 1). Under optimized conditions, almost all reactions reached completion in 24 h furnishing the target 3-azolyl-substituted steroids **2a**–**j** in good to excellent yields.

The proposed approach allowed us to perform the C–N coupling with indoles bearing various functionalities, such as ester (**2a**,**b**), nitrile (**2d**,**e**), and nitro group (**2g**–**i**). Although azoles bearing strong electron-withdrawing groups and possesing increased NH-acidity are known to demonstrate low reactivity in Ullmann coupling, in our case, the reaction of 4- and 5-nitroindoles **2g**,**h** with iodosteroid proceeded efficiently providing yields of ~95%. However, 7-nitroindole reacted sluggishly affording product **2i** in only 28% yield (by ^{1}H NMR), due to combined negative effect of high NH-acidity and steric hindrance. The cross-coupling of iododiene **1** with 7-azaindole also occurred more slowly in comparison with most studied indoles; therefore, the corresponding product **2j** was isolated in moderate 53% yield.

We performed biological assays of the prepared azolyl steroids **2a**–**h** as well as a series of their analogues **3**–**9** synthesized previously in our group. The antiproliferative activity of the obtained library comprising 22 compounds was evaluated against the human breast (MCF-7), ovarian (SKOV-3), and prostate (DU-145, PC-3) cancer cell lines using the MTT assay (Table 1).

Almost all studied compounds, with the exception of **2c** and **8**, showed anticancer activity against at least one of selected cell lines (IC_{50} < 50 μM). The cytotoxic effect was highly dependent upon the nature of azaheterocycle and position of functional



a $R^1 = R^2 = H$, $R^3 = CO_2Me$, X = CH; 70%
b $R^1 = R^3 = H$, $R^2 = CO_2Me$, X = CH; 79%
c $R^1 = R^3 = H$, $R^2 = F$, X = CH; 90%
d $R^1 = CN$, $R^2 = R^3 = H$, X = CH; 95%
e $R^1 = R^3 = H$, $R^2 = CN$, X = CH; 88%
f $R^1 = R^3 = H$, $R^2 = OMe$, X = CH; 62%
g $R^1 = NO_2$, $R^2 = R^3 = H$, X = CH; 94%
h $R^1 = R^3 = H$, $R^2 = NO_2$, X = CH; 96%
i $R^1 = R^2 = R^3 = H$, $X = C(NO_2)$; (28%)
j $R^1 = R^2 = R^3 = H$, X = N; 53%

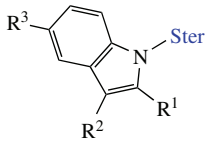
Scheme 1 *Reagents and conditions*: i, CuI (10 mol%), $(Bu^tCO)_2CH_2$ (20 mol%), K_2CO_3, DMSO, 100 °C, 24 h.

Table 1 Cytotoxicity of the synthesized steroids against the human cancer cell lines.

Compound	IC_{50}/μM			
	MCF-7	SKOV-3	DU-145	PC-3
2a	>50	>50	>50	44.7
2b	42.1	>50	>50	44.2
2c	>50	>50	>50	>50
2d	29.6	42.7	>50	>50
2e	37.5	>50	>50	>50
2f	–	–	>50	31.2
2g	>50	>50	48.7	>50
2h	36.2	32.3	44.4	41.9
3a	37.5	>50	>50	>50
3b	9.0	22.5	>50	>50
3c	33.5	40.3	>50	47.4
3d	34.5	36.3	48.2	>50
3e	2.0	3.0	9.1	10.1
4	38.7	39.2	>50	>50
5a	4.5	25	>50	>50
5b	>50	8.5	>50	>50
6a	31	47	>50	>50
6b	17.7	18.8	42.8	35.1
7a	18.9	>50	⩾50	⩾50
7b	16.5	20.5	>50	>50
8	>50	>50	>50	>50
9	10.5	16.5	–	–

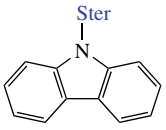
groups. For instance, among regioisomeric cyano-substituted indolylandrostanes **2d**,**e** and **3b**, the most remarkable antiproliferative effect was observed for **3b** bearing 3-positioned nitrile moiety (IC_{50} value of 9.0 μM against MCF-7). Rather high activity against MCF-7 was also found for imidazole derivative **5a** with IC_{50} value of 4.5 μM. Steroid **3e** bearing gramine fragment proved to be the most active and inhibited growth of all cancer cell lines with IC_{50} values in the range of 2.0–10.1 μM. Interestingly, compound **9** containing acetylene instead of indole as a linker between steroidal scaffold and dialkylamino group also demonstrated remarkable antiproliferative activity against MCF-7 and SKOV-3 with IC_{50} values of 10.5 and 16.5 μM, respectively.

In conclusion, Cu-catalyzed C–N coupling was utilized as a convenient tool for the preparation of 3-azolyl steroids in generally high yields up to 96%. A number of NH-indoles bearing both electron-donating (OMe) and electron-withdrawing (F, CN, CO_2Me, NO_2) groups were efficiently coupled with testosterone-derived iododiene. The cytotoxicity assays of the obtained compounds against hormone-dependent cancer cell lines were accomplished. Compound **3e** bearing gramine moiety exhibited the most promising cytotoxic profile.



3a–e

a $R^1 = R^2 = R^3 = H$
b $R^1 = R^3 = H$, $R^2 = CN$
c $R^1 = Me$, $R^2 = H$, $R^3 = Cl$
d $R^1 = Ph$, $R^2 = R^3 = H$
e $R^1 = R^3 = H$, $R^2 = CH_2NMe_2$



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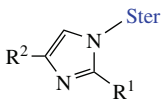
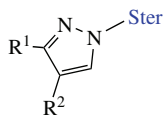


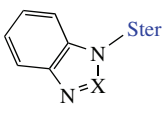
5a,b

a $R^1 = R^2 = H$
b $R^1 = Me$, $R^2 = NO_2$



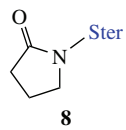
6a,b

a $R^1 = NO_2$, $R^2 = H$
b $R^1 = H$, $R^2 = Br$



7a,b

a X = CH
b X = N



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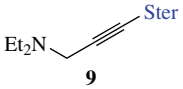

9

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.05.025.

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