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Synthesis and Estrogen Receptor Binding Affinities of Novel Pyrrolo[2,1,5-*cd*]indolizine Derivatives

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Abstract—A series of pyrrolo[2,1,5-*cd*]indolizine derivatives has been synthesized and evaluated as ligands for the estrogen receptor. Properly substituted mono- and di-hydroxy derivatives showed binding in the low nanomolar range in accordance with their structural resemblance to estrogen. © 2000 Elsevier Science Ltd. All rights reserved.

The steroid hormone estrogen has a powerful influence on the development and maintenance of the female reproductive organs and other sexual characteristics. In recent years estrogen's involvement in the biological function and sustenance of other tissues, such as the skeleton, the cardiovascular system, and the central nervous system, in both males and females has also been recognised.[1–5] Subsequently, the decreased production of ovarian steroids which occurs after the climacteric has been linked to a number of post-menopausal degenerative changes, particularly osteoporosis and coronary heart disease.[6] Among the various treatment modalities available, estrogen replacement therapy has proven effective in reducing the risks associated with these degenerative changes. Even though the beneficial effects of estrogen replacement on a wide variety of organ systems and tissues appear indisputable, these benefits are achieved at the expense of an increase in the risk of endometrial hyperplasia and breast cancer which, in turn, has reduced patient compliance.[7,8] This realization has created the need to develop non-steroidal compounds, which interact with the estrogen receptor (ER) and antagonise the effects of estrogen on uterine and mammary tissue, whilst mimicking the effects of estrogen on other tissues such as bone.[9–11] Recently we reported on the synthesis and pharmacological activity of a novel non-steroidal tissue selective antagonist 1-ethyl-2-(4-hydroxyphenyl)pyrrolo[2,1,5-*cd*]indolizine (NNC 45-0095, **6c**), which binds with high affinity ($IC_{50} = 9.5$ nM) to the estrogen receptor and exhibits full protection against bone loss in the ovariectomized mouse model for post-menopausal osteoporosis.[12] Herein, we report on the synthesis and estrogen receptor binding properties of a series of novel pyrrolo[2,1,5-*cd*]indolizine derivatives, which were prepared with the aim of further exploring the ER binding activity relationship found when varying the pattern of hydroxy-group substitution seen in NNC 45-0095 and highlight, by means of molecular modeling, the structural resemblance of the pyrrolo[2,1,5-*cd*]indolizine compounds to estrogen.

Chemistry

Preparation of the non-commercially available starting materials **2a**–**c** is outlined in Schemes 1–3. The synthesis of 4-methoxy-2-*n*-propylpyridine **2a** (Scheme 1) proceeded via the intermediate 4-nitro-2-propylpyridine-*N*-oxide, which was reacted with methanolic sodium methoxide followed by reduction with zinc in aqueous sulphuric acid. Attempts were made to prepare 4-benzyloxy-2-*n*-propylpyridine in a similar fashion[13,14] but the reaction of 4-nitro-2-*n*-propylpyridine-*N*-oxide with sodium benzyl alcoholate in benzyl alcohol failed. The synthesis of 5-benzyloxy-2-*n*-propylpyridine **2b** was performed by a modified literature procedure[15] and is outlined in Scheme 2. 2-Chloro-5-nitropyridine was reacted with the sodium salt of diethyl ethylmalonate followed by hydrolysis and decarboxylation to give the corresponding 2-*n*-propylpyridine. The latter was reduced, diazotized and hydroxylated to give the corresponding 5-hydroxypyridine, which was alkylated to afford **2b**. The synthesis of 3-hydroxymethyl-2-*n*-propylpyridine **2c** (Scheme 3) was performed by analogy to the method

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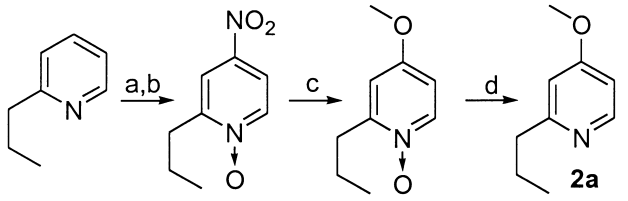
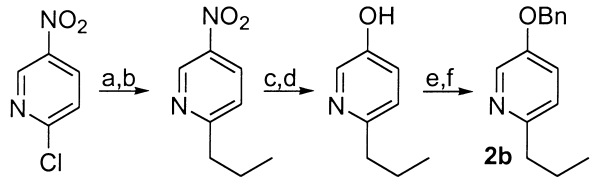
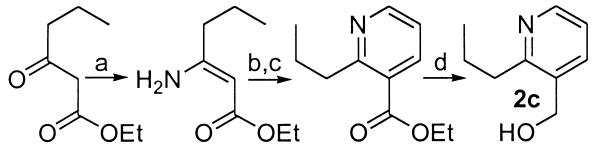


Scheme 1. Synthesis of 4-methoxy-2-*n*-propylpyridine (**2a**). Reagents and conditions: (a) 35% H_2O_2/AcOH, 75 °C, 13 h; (b) H_2SO_4/HNO_3, 70 °C, 22 h; (c) NaOMe/MeOH, 25 °C; (d) Zn/1 M H_2SO_4, 75 °C, 12 h.



Scheme 2. Synthesis of 5-benzyloxy-2-*n*-propylpyridine (**2b**). Reagents and conditions: (a) Diethyl ethylmalonate/Na; (b) H_2SO_4 (aq), reflux 18 h; (c) Pd/C, EtOH; (d) 1. HCl (aq)/$NaNO_2$, 0 °C, 0.5 h, 2. 70 °C, 2 h, 3. $NaHCO_3$; (e) NaH/THF 0 °C; (f) benzyl bromide/DMSO, 25 °C, 3 h.



Scheme 3. Synthesis of 3-hydroxymethyl-2-*n*-propylpyridine (**2c**). Reagents and conditions: (a) NH_3/ether, 25 °C, 8 h; (b) acrolein/piperidin/EtOH reflux, 3 h; (c) sulphur, 150 °C, 3 h; (d) $LiAlH_4$/THF, 25 °C, 3 h.

described for the corresponding 3-hydroxymethylpicoline.[16] The pyrrolo[2,1,5-*cd*]indolizines **6** were prepared in a straightforward fashion as outlined in Scheme 4. A halo-ketone **1** and the 2-alkylpyridine **2** were reacted under Tschitschibabin conditions[17] to afford the indolizine **3**. Reaction of the latter[18] with dimethyl acetylenedicarboxylate (DMAD), followed by oxidation with 2,3-dichloro-5,6-dicyanoquinone (DDQ) gave the diester **4**, which was hydrolyzed and decarboxylated to afford compound **5**. Removal of the *O*-protection of **5** gave the final product **6** (see Table 1).

Results and Discussion

Estrogen receptor (ER) binding data of the pyrrolo[2,1,5-*cd*]indolizines **6** are summarized in Table 1. The receptor binding was determined in ER-rich cytosol from rabbit uterine tissue.[19] The IC_{50}-value for a particular compound represents the concentration at which [^{3}H]-17β-estradiol is displaced by 50% of the maximal binding. We systematically modified various regions of the parent compound, 2-phenylpyrrolo[2,1,5-*cd*]indolizine, to elucidate important features for activity. The first approach was to synthesize the mono-hydroxy compounds **6a**–**d**, which illustrated that *para*-hydroxy substitution on the benzene ring (**6c**, 9.5 nM) was preferential to meta-hydroxy substitution (**6d**, 370 nM). Despite a high degree of symmetry of the parent molecule, it was shown from **6a** (1300 nM) and **6b** (180 nM) that hydroxy substitution in the pyridine ring was less favored compared to *para*-hydroxylation of the pendant phenyl group of **6c**. Introduction of an additional hydroxy group on the pyrroloindolizine moiety represented by compounds **6f** and **6g**, further improved the ER affinities, and led to binding affinities (both 0.9 nM) close to that of 17β-estradiol itself (0.7 nM). Variation of the alkyl-substitution pattern at the R_2 position revealed that optimum ER binding was obtained when small alkyl chains were introduced. When R_2 is ethyl (**6c**), *n*-propyl (**6j**) or *i*-propyl (**6k**) sub-nanomolar binding affinities were obtained; whereas binding affinities for the corresponding *n*-butyl (**6n**) and 2-pentyl (**6p**) were much poorer, 200 nM and 950 nM, respectively. Introduction of a hydroxymethyl substituent in the R_3 position of **6c** revealed that this modification (**6e**, 12 nM) had no effect on ER binding. The above results clearly demonstrate that the phenolic group of **6c** should best be aligned with the phenolic moiety of 17β-estradiol and the most favoured 3D-structures of the R_2 substituents correspond well to the volume defined by the B-ring in 17β-estradiol, as shown in Figure 1. The missing mimetic of the B-ring in **6h** can explain the poor activity of this compound.

An alternative alignment of the molecules in Figure 1 could be to place R_2 next to position 11β of 17β-estradiol, because this position generally is known to tolerate large substituents.[23] However, this alignment would reduce the common volume overlap of the two molecules and therefore this molecular fit is considered to be less favorable. Additionally, it can be observed that the 3D position of the second hydroxy group within 17β-estradiol corresponds to both the R_4 and R_5 substitution positions of the NNC 45-0095 series of compounds. This explains the very strong affinities observed for the di-hydroxy substituted pyrroindolizines **6f** and **6g**. If we further compare the best common molecular volume overlap between the series of compounds, the degree of molecular volumes overlap corresponds to the observed ER binding properties (data not shown).

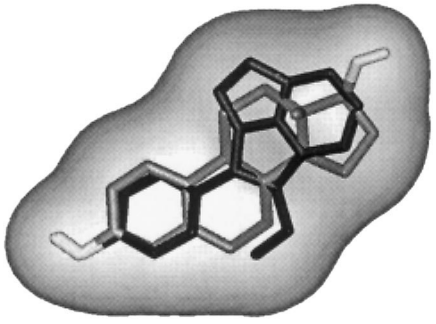
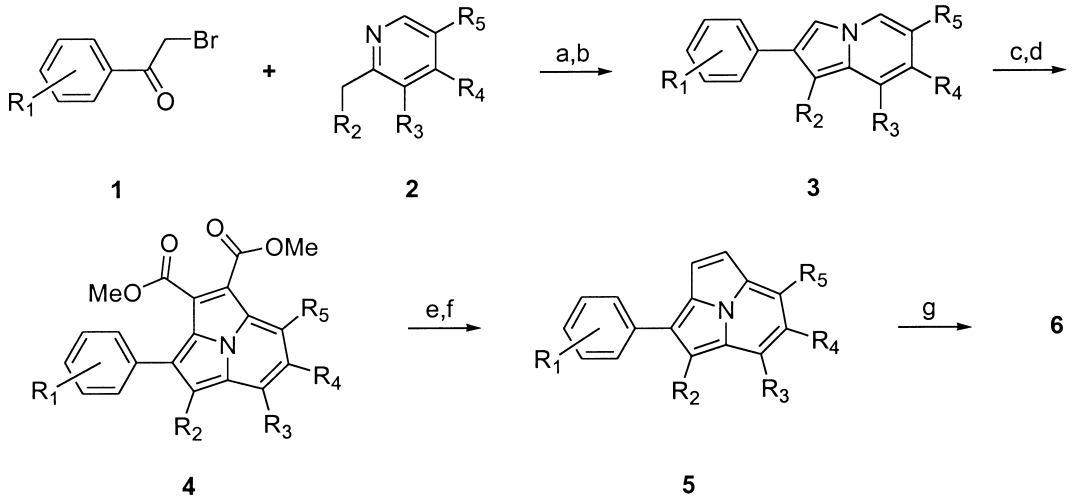
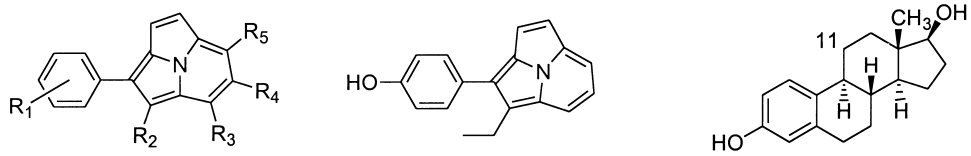


Figure 1. NNC 45-0095 (**6c**, black) compared to 17β-estradiol (grey) and represented by the alignment derived from the pharmacophoric pattern recognition and superimposition program GASP.[20–22]



Scheme 4. Synthesis of mono- and di-hydroxy substituted pyrrolo[2,1,5-*cd*]indolizines (**6**). Reagents and conditions: (a) Acetone, reflux and isolate intermediate quaternary pyridinium salt; (b) $NaHCO_3/H_2O$, reflux; (c) DMAD/toluene, 0 °C. When R_2 is CH_2OH in **3** this substituent is protected by reaction with *t*-butyl-dimethylsilylchloride. Protecting group is removed in step (e); (d) DDQ, 25 °C, then reflux; (e) 1. KOH/H_2O/MeOH, reflux, 2. HCl (aq); (f) Cu/quinoline, 170 °C, (g) Deprotecting methods: **A**: H_2, Pd/C, EtOH/THF, 25 °C. **B**: BBr_3/CH_2Cl_2, −65–25 °C overnight. **C**: 1. $AlCl_3$/EtSH/CH_2Cl_2, 25 °C, 2. THF/6 M HCl, 0 °C, 3. Extraction with EtOAc. See Table 1

Table 1. Synthesis and estrogen receptor binding affinity (ER-LBA) of pyrrolo[2,1,5-*cd*]indolizines **6**



6 **6c (NNC 45-0095)** **17β-Estradiol**

	Substituents of **6**					O-Protecting of **5**			**5→6**	ER-LBA[a]
	R_2	R_3	R_4	R_5	R_1	R_1	R_4	R_5	Method[b]	IC_{50} (nM)
6a	Et	H	OH	H	H	—	OMe	—	B	1300
6b	Et	H	H	OH	H	—	—	OBn	A	180
6c	Et	H	H	H	p-OH	OBn	—	—	A	9.5
6d	Et	H	H	H	m-OH	OMe	—	—	B	370
6e	Et	CH_2OH	H	H	p-OH	OBn	—	—	A	12
6f	Et	H	H	OH	p-OH	OBn	—	OBn	A	0.9
6g	Et	H	OH	H	p-OH	OMe	OMe	—	B	0.9
6h	H	H	H	H	p-OH	OBn	—	—	A	700
6i	Me	H	H	H	p-OH	OMe	—	—	B	28
6j	*n*-Propyl	H	H	H	p-OH	OBn	—	—	A	5
6k	*i*-Propyl	H	H	H	p-OH	OMe	—	—	C	8
6l	$-(CH_2)_3-$		H	H	p-OH	OMe	—	—	B	30
6m	$-(CH_2)_4-$		H	H	p-OH	OMe	—	—	B	25
6n	*n*-Butyl	H	H	H	p-OH	OMe	—	—	C	200
6o	2-Phenylethyl	H	H	H	p-OH	OMe	—	—	B	180
6p	2-Pentyl	H	H	H	p-OH	OMe	—	—	B	950
17β-Estradiol		—	—	—	—	—	—	—	—	0.7

[a]Estrogen receptor binding affinity expressed as IC_{50}, i.e. the concentration of compound required to displace 50% of the maximal binding for [^{3}H]-17β-estradiol.
[b]For details see Scheme 4.

Conclusions

A novel series of pyrrolo[2,1,5-*cd*]indolizine derivatives has been synthesized and evaluated in vitro as ligands for the estrogen receptor. The di-hydroxy derivatives **6e** and **6f** are highly potent compounds, which exhibit binding and structural features closely related to 17β-estradiol. Further work is underway to apply the pyrrolo[2,1,5-*cd*]indolizine ring system as a scaffold for further derivatization in order to obtain compounds with mixed agonist/antagonist estrogen activity (anti-estrogens).

Acknowledgements

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