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Synthesis and Pharmacology of a Novel Pyrrolo[2,1,5-*cd*] Indolizine (NNC 45-0095), a High Affinity Non-Steroidal Agonist for the Estrogen Receptor

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Abstract—1-Ethyl-2-(4-hydroxyphenyl)pyrrolo[2,1,5-*cd*]indolizine (NNC 45-0095) is a novel compound which represents the parent pharmacophore structure of a series of pyrrolo[2,1,5-*cd*]indolizine derivatives with mixed estrogen agonist/antagonist properties. NNC 45-0095 binds with high affinity to the estrogen receptor (IC_{50} = 9.5 nM) and exhibits full protection of bone loss in the ovariectomized mouse model for post-menopausal osteoporosis.

The central role of endogenous estrogen in the development and maintenance of the female reproductive organs and other sexual characteristics has long been acknowledged. More recently 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 a real or perceived increase in risk of endometrial hyperplasia and breast cancer which, in turn, has reduced patient compliance.[7,8] This realisation 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] Here we report for the first time on the synthesis of the novel non-steroidal full estrogen agonist, 1-ethyl-2-(4-hydroxyphenyl)pyrrolo[2,1,5-*cd*]indolizine (NNC 45-0095), a compound which represents the parent pharmacophore structure of a series of pyrrolo[2,1,5-*cd*]indolizine derivatives with mixed estrogen agonist/antagonist properties for the prevention or treatment of estrogen related diseases or symptoms (manuscript in preparation). Herein, we describe the compound's synthesis, the in vitro effects in receptor binding and cell proliferation assays, and the effects on bone and uterine tissue in an ovariectomized (OVX) mouse model for post-menopausal disorders.

Synthesis

The synthesis of NNC 45-0095 was performed as outlined in Scheme 1. 2-Propylpyridine **1** and the haloketone **2** were reacted under Tschitschibabin conditions[12] to afford the indolizine **3**. Reaction of the latter with dimethyl acetylenedicarboxylate (DMAD), followed by oxidation with 2,3-dichloro-5,6-dicyanoquinone (DDQ) gave the diester **4**, which was hydrolysed to the diacid **5.** Decarboxylation of **5** afforded the pyrroloindolizine **6**, which was debenzylated to the final product NNC 45-0095.[13]

Pharmacological Evaluation

The estrogen receptor (ER) is traditionally regarded as a ligand-inducible transcription factor that regulates the

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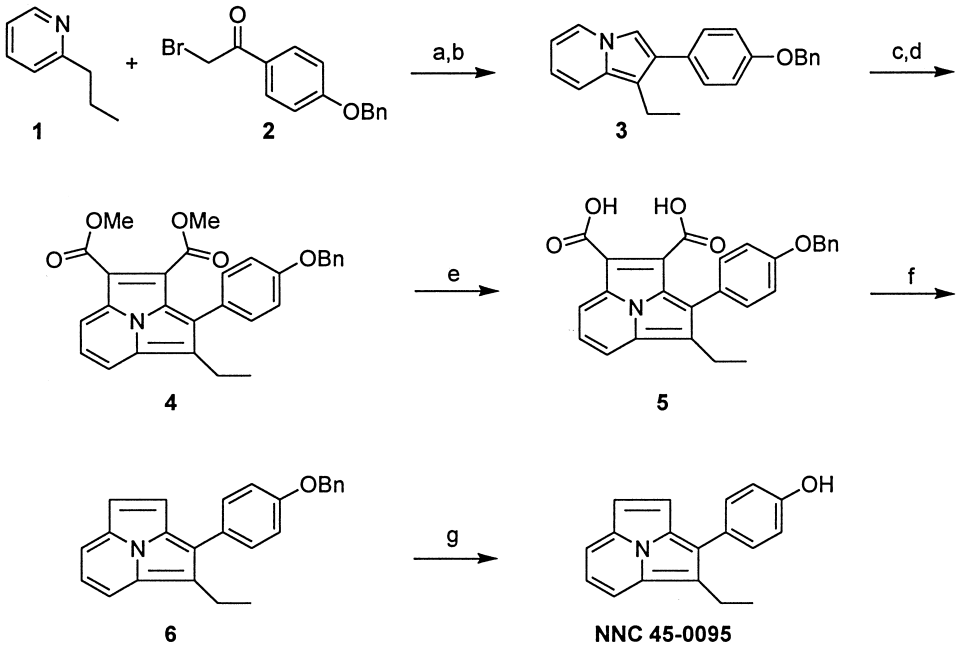

Scheme 1. Synthesis of 45-0095: (a) acetone, reflux and isolate intermediate quaternary pyridinium salt; (b) $NaHCO_3$, H_2O, reflux; (c) DMAD, toluene, 0 °C; (d) DDQ, rt; (e) KOH, H_2O, MeOH, reflux then dilute HCl; (f) Cu, quinolene, 170 °C; (g) H_2, Pd/C, EtOH.

transcription of estrogen responsive genes by virtue of its binding as a homodimer to imperfect palindromic sequences, estrogen responsive elements, on DNA. Consequently, the biological activity of ER-bound ligand is considered genomic and the initial identification of estrogen-like compounds is often based on ER affinity for the ligand in question. Hence, the ability of NNC 45-0095 to compete with [^{3}H]-17β-estradiol for receptor binding was determined in ER-rich cytosol from rabbit uterine skeletal muscle tissue.[14] As shown in Table 1, the concentrations of NNC 45-0095 and 17β-estradiol, which displaced 50% of the maximal binding of [^{3}H]-17β-estradiol to ER (IC_{50}), were 9.5 and 0.7 nM, respectively.

Whether the high receptor binding affinity of NNC 45-0095 is reflected in intrinsic estrogenic activity was first assessed in vitro based on stimulation of alkaline phosphatase activity in the Ishikawa line of human endometrial adenocarcinoma cells.[15] As shown in Table 1, NNC 45-0095 was fully agonistic in this model with a maximal agonist activity of 105% relative to moxestrol and an EC_{50}-value of 13 nM. Also, this in vitro agonism of NNC 45-0095 correlated with observations of uterotrophic activity using changes in uterine weight in the immature mouse (Table 1) and in the mature ovariectomized (OVX) mouse (Fig. 1B) as a measure for estrogenicity.[16,17]

In addition to uterotrophic activity, estrogen also has positive therapeutic effects on various other tissues; for example in the skeleton, where estrogen restrains the bone resorption seen in post-menopausal women.[18,19] To determine if NNC 45-0095 retained estrogenic activity in bone remodelling, we investigated the anti-resorptive properties of this new pyrroloindolizine analogue in an OVX mouse model of post-menopausal osteoporosis.[20] Measures of total bone mineral density (BMD) were determined by the Archimedes principle[21] in the distal femur following 5 weeks of daily subcutaneous administration of compound and were compared to the effects of 17β-estradiol. Analysis showed

Table 1. Estrogen receptor binding affinity (ER-LBA), endometrial activity in vitro and uterotrophic activity in vivo for NNC 45-0095, moxestrol and 17β-estradiol

	ER-LBA[a]	Ishikawa		Immature mouse	
	IC_{50} (nM)	EC_{50} (nM)	E_{max}[b] (% max. stimulation relative to moxestrol)	EC_{50} (nmol/g)	% maximal relative agonism
NNC 45-0095	9.5	13	105	0.96	76
Moxestrol	2.5	0.1	100	—	—
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]E_{max} of moxestrol is 903% of basal proliferation. Values are presented as mean from 2–4 separate studies.

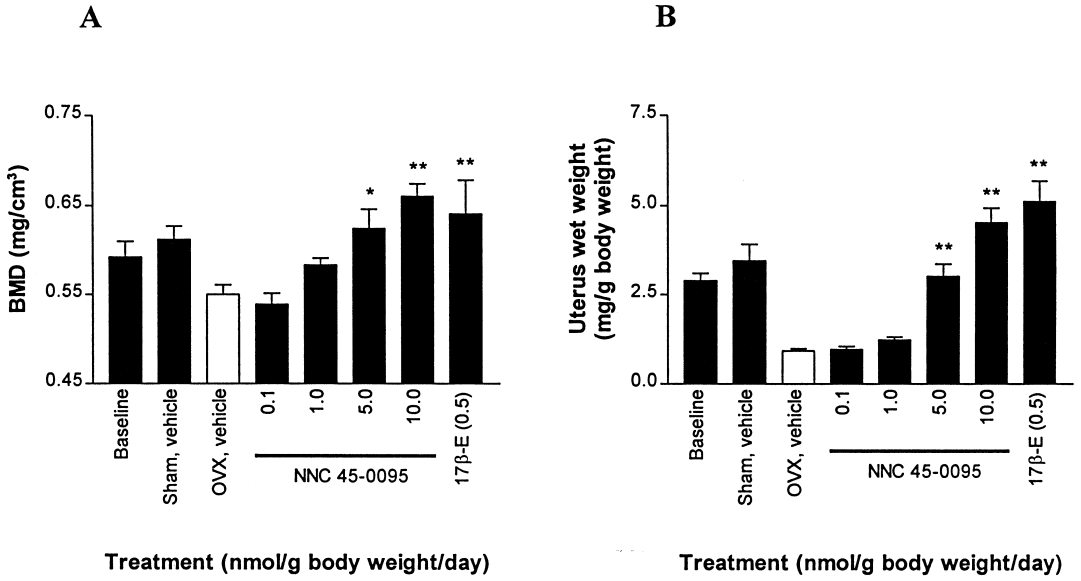

Figure 1. Effects of NNC 45-0095 and 17β-estradiol (17β-E) on total BMD in the distal femur of the OVX mouse (A) and uterine wet weight (B). NNC 45-0095 (0.1–10 nmol/g body weight) and 17β-estradiol (0.5 nmol/g body weight) were administered by the subcutaneous route five and three times weekly, respectively, for 5 weeks. Data represent mean and SEM for 6–8 animals per group. $*P < 0.05$ and $**P < 0.01$ versus OVX vehicle (ANOVA and Dunnett's multiple comparison test).

significant differences in mouse distal femur BMD (Fig. 1A). Vehicle treated mice OVXed for 5 weeks suffered significant decreases in BMD. Administration of NNC 45-0095 in the range of 0.1–10 nmol/g body weight at five times per week, completely prevented the loss of total BMD of the distal femur at 5 nmol/g. Likewise, 17β-estradiol at 0.5 nmol/g body weight administered three times weekly, significantly maintained total BMD at the level of sham-operated animals.

In conclusion, these data strongly suggest that this novel pyrroloindolizine, NNC 45-0095 possesses profound estrogenic activity. Moreover, this new high affinity non-steroidal ER ligand appeared as a full estrogen agonist when evaluated in classical estrogen responsive tissues such as the endometrium and the skeleton. The pharmacological profile of this parent pharmacophore may provide the basis for rational design of tissue selective ER ligands with mixed estrogen agonist/antagonist profile.

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