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UK-73,093 : A NON-PEPTIDE NEUROTENSIN RECEPTOR ANTAGONIST

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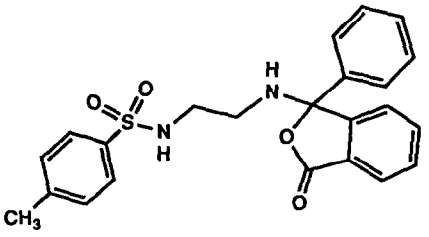
Abstract: UK-73,093 was identified in a screening program as a compound able to displace [^{3}H]-neurotensin from its bovine brain receptor. We describe the discovery of this compound, species differences in receptor affinity and its characterization as a functional neurotensin antagonist *in vitro* and *in vivo*.

Neurotensin (NT) is a thirteen amino acid peptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH), isolated from bovine hypothalamus[1]. High concentrations of NT are found in discrete regions of the mammalian central nervous system[2,3], including a coexistence with dopamine in the mesocortical pathway[3-7]. Centrally administered NT produces neurophysiological and behavioral actions mediated through NT receptors on midbrain dopamine neurons[7-11]. Thus, the actions of exogenously administered NT on dopaminergic pathways are well documented. However, the role of endogenous NT in modulating dopaminergic functions remains to be tested, due to the lack of a nonpeptide receptor antagonist.

In the course of screening to discover nonpeptide NT receptor antagonists, we identified a compound UK-73,093 (Fig. 1) by its ability to displace [^{3}H]-NT from its receptor at low micromolar concentrations. In this paper we describe the synthesis, binding properties, and antagonist activity of UK-73,093 in *in vitro* biochemical and *in vivo* behavioral paradigms.

Figure 1

Structure of UK-73,093



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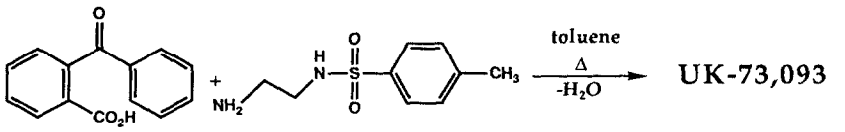
Synthesis:

Preparation of UK-73,093 (N-(2-(3-oxo-1-phenyl-benzo(c)furan-1-ylamino)ethyl)-p-toluenesulfonamide (Scheme 1):

This compound is prepared in one step from o-benzoyl benzoic acid and N-(2-aminoethyl)-4-methylbenzene sulfonamide by the methodology of Houlihan[12,13].

Scheme 1



Biological Methods : Ligand Binding

Brain tissue consisting of frontal cortex from cows (obtained from a local slaughterhouse), adult male Sprague-Dawley rats, or post-mortem human (obtained from the National Disease Research Interchange) was homogenized (Polytron) in cold 50 mM Tris·HCl buffer, pH 7.4, and diluted to 1 g wet tissue/100 mL. Intact N1E-115 cells (3 x 10^5 cells/tube) were handled as described below. Following centrifugation and resuspension twice (30,000g, 20 min), the pellet was resuspended at 10 mg (original wet tissue weight)/mL in 50 mM Tris.HCl buffer, pH 7.4, containing 1 mg/ml BSA, 0.04 mg/ml bacitracin, and 0.34 mg/ml EDTA. [^{3}H]-NT (40 Ci/mmol, Amersham) was diluted to give a final assay concentration of 2 nM. Assays were carried out in 1 ml polystyrene test tubes, with each tube containing 100 µL radioligand, 100 µL test compound, and 800 µL tissue suspension. Following a 10 min incubation (25° with gentle orbital shaking), unbound ligand was removed with a Brandell harvester (0.2% polyethyleneimine presoak, 50 mM Tris pH 7.4, 0° wash) and the bound ligand, on fiberglass mats (Whatman GF/B), was counted in a RackBeta scintillation counter (LKB). Non-specific binding was estimated by the addition of NT (Sigma; 1 µM final concentration) to control wells.

Biological Methods : In Vitro Functional Characterization

UK-73,093 was tested for functional activity vs. NT 8-13-stimulated cyclic [^{3}H]GMP formation in murine N1E-115 neuroblastoma cells as described[14]. N1E-115 cells (obtained from Dr. Elliott Richelson) were cultured in high glucose DMEM supplemented with 10% fetal bovine serum. Cells (passage 10-12, 12-18 days from subculture) were harvested for assay by removing the growth medium and suspending the cells with gentle shaking in Ca^{2+}/Mg^{2+}-free Dulbecco's Phosphate Buffered Saline (pH = 7.4, D-PBS), followed by centrifugation (250 x g, 5 min) and washing 2X in complete D-PBS supplemented with 1 mg/ml glucose. Cellular labeling was achieved by adding [^{3}H]guanosine (ICN Radiochemicals; 10 µCi/ml) and shaking at 37° C for 45 min at 80 rpm. Cells were centrifuged as above, the radioactive supernatant discarded, cells suspended in D-PBS at a concentration of 3 x 10^5 cells/ 240 µl and distributed in 240 µl aliquots into each well of 24 well multiwell trays (Linbro

Plate, Flow). After equilibration of cells at 37°C for 15 min at 80 rpm, UK-73,093 was added (30 µl) and allowed to equilibrate an additional 5 min, followed by agonist (30 µl NT 8-13) addition for 45 sec. The reaction was terminated by the addition of 30 µl of 50% (wt/vol) trichloroacetic acid. Cyclic [^{3}H]GMP was isolated by ion exchange chromatography[14] and radioactivity quantified in a RackBeta counter (LKB).

Biological Methods : Behavioral Studies

Male Sprague-Dawley rats, 300-350 grams, were anesthetized with chloral hydrate and prepared for stereotaxic surgery according to NIH Guidelines for the Care and Use of Laboratory Animals. Bilateral guide cannulae, 24 gauge hypodermic stainless steel, were implanted into the ventral tegmental area[15]. At least one week after surgery, animals were administered saline or UK-73,093 suspended in saline vehicle, intraperitoneally in a volume of 2 ml/kg, five minutes before intracerebral microinjection. NT (Bachem Bioscience Inc., Philadephia, PA) was microinjected bilaterally through a 31 gauge microinjection tube, inserted through the guide cannula to extend 2 mm ventral to the ventral tip of the guide cannula, 3 nmoles/0.5 µl in saline vehicle, infused over a one minute period.

Behavioral Testing: Exploratory locomotor activity was measured using a Digiscan Optical Activity Meter (Omnitech, Columbus, OH). Each animal was placed individually in the open field chamber for 120 minutes of habituation. The rat was then replaced in a carrying cage and administered the intraperitoneal injection, followed five minutes later by the intracerebral injection. Rats given the highest dose of UK-73,093 showed twisting body movements for several minutes after the intraperitoneal injection. Immediately after the intracerebral microinjection, the rat was returned to the Digiscan activity meter. Horizontal locomotor activity was electronically recorded at 30 minute intervals for 120 minutes after the injection.

Statistical analysis: Behavioral scores were analyzed by One Way Analysis of Variance, followed by a Newman-Keuls test for significant differences between individual means. Data were analyzed for treatment effects over time after injection, and for drug dose as compared to vehicle at the first 30 minute interval time point.

Histological analysis: Each animal was used twice, with at least one week between uses. After the end of each experiment, animals were sacrificed and brains sectioned and stained for histological localization of the cannula placements. Data from animals with cannula outside of the ventral tegmental area were eliminated from the statistical analysis.

Results and Discussion

UK-73,093 specifically displaced radiolabelled NT from its bovine brain receptors with an IC_{50} value of 5.5 ± 1.3 µM (n = 6, Fig. 2A). Measurement of NT-stimulated cyclic [^{3}H]GMP formation in murine N1E-115 neuroblastoma cells showed that UK-73,093 behaved as a competitive antagonist with an K_i value of 4.4 ± 2.6 µM (Figure 2B). In the absence of NT, this compound (up to 32 µM) did not significantly elevate cyclic [^{3}H]GMP production, indicating that it has no agonist activity.

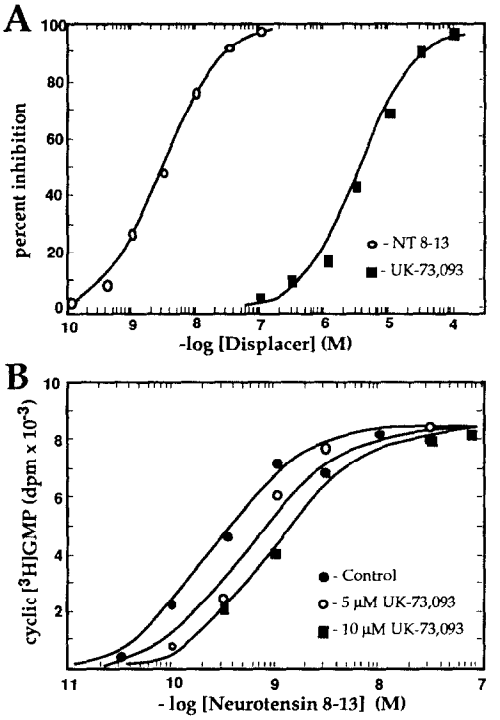


Figure 2

(A) [^{3}H]Neurotensin Displacement
(B) In Vitro Functional Activity of UK-73,093

Radioligand binding and cyclic GMP experiments were performed as described in the text.

UK-73,093 caused dose-dependent antagonism of NT-induced hyperlocomotion, during the first 30 minute interval after microinjection of 3 nmoles NT bilaterally into the ventral tegmental area (Fig. 3). Pretreatment with UK-73,093 significantly inhibited NT-induced hyperlocomotion at doses of 80 and 120 mg/kg i.p.. The dose of 40 mg/kg UK 73093 did not significantly inhibit NT-induced hyperlocomotion. (ANOVA $F_{3,16}$ = 6.88, $p<.01$, Newman-Keuls $p<.01$ for UK-73,093 80 and 120 mg/kg as compared to vehicle + NT). Treatment group sizes were N=7 for vehicle + NT, N=4 for UK-73,093 40 mg/kg + NT, N=5 for UK-73,093 80 mg/kg + NT, N=5 for UK-73,093 120 mg/kg + NT.

Behavioral analysis of UK-73,093 employed a well-established model[6], hyperlocomotion induced by NT microinjected into the ventral tegmental area (saline+saline = 1738 ± 580, N=9; vehicle+NT = 4211 ± 761, N=7), in which UK-73,093 showed efficacy in a dose-dependent manner. Importantly, over the dose range tested, up to 120 mg/kg i.p., UK 73093 had no effect alone on baseline exploratory locomotor activity(UK-73,093 120 mg/kg+saline = 2301 ± 339, N=7), indicating that the drug alone was not producing sedation or non-specific interference with motor ac-

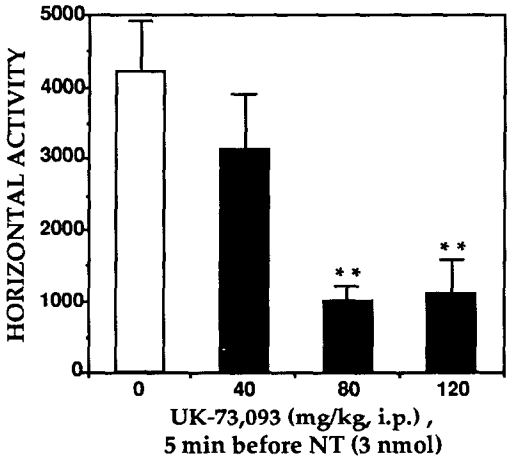
tivity. These data suggest that UK-73,093 is active in blocking the behavioral effects of NT.

This compound displayed no activity (at concentrations up to 100 μM) in displacing labelled NT from its receptors on human frontal cortex. Additionally, in contrast to the affinity of UK-73,093 in the cow and mouse (IC_{50} = 4-6 μM), it is approximately 10-fold weaker in the rat (IC_{50} = 49 μM). This considerable species selectivity is comparable to that which have been observed with nonpeptide Substance P[16,18] and bombesin[25] receptor antagonists. Relatively rigid nonpeptide antagonists may thus reveal receptor differences between species that are not apparent using peptides.

Figure 3

UK-73,093 dose-dependent antagonism of NT-induced hyperlocomotion

Experiments were performed as described in the text (* * $p < 0.01$).



Without knowledge of the bioactive conformation of NT, nor the structure of the receptor complex with either NT or UK-73,093, we cannot speculate about which functional groups of the peptides are mimicked by UK-73,093. Although we believe that UK-73,093 occupies some of the same physical space at the receptor as the natural ligands, this is not assured by radioligand displacement or functional antagonism.

In conclusion, neurotensin now joins the growing list of peptides which act at G-protein coupled receptors for which nonpeptides antagonists have been discovered[17]: substance P[16,18], neurokinin A[19], CCK-A and CCK-B[20], angiotensin II[21], vasopressins[22], oxytocin[23], C5a[24].and bombesin[25].

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