Tetrahedron 69 (2013) 2550–2554

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of the new oligopeptide pyrrole derivative isonetropsin and its one pyrrole unit analogue

Alessandra Montalbano, Barbara Parrino, Patrizia Diana, Paola Barraja, Anna Carbone, Virginia Spanò, Girolamo Cirrincione *

Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari, STEMBIO, via Archirafi 32, 90123-Palermo, Italy

ARTICLE INFO

Article history:
Received 29 October 2012
Received in revised form 30 December 2012
Accepted 21 January 2013
Available online 1 February 2013

Keywords:
Netropsin
DNA minor groove binders
Isonetropsin
Oligopeptide pyrrole

ABSTRACT

We have designed and synthesized isonetropsin and its one pyrrole unit analogue in which the amine and carbonyl groups have been switched in positions 2 and 4, respectively instead of 4 and 2 positions of the natural antibiotic netropsin.

1. Introduction

Netropsin (**1**), formerly named congocidine, is a natural occurring oligopeptide antibiotic isolated in 1951 from *Streptomyces netropsis*.[1] Its first total synthesis was reported in 1963 by Julia and Préau-Joseph;[2] a new and more efficient synthesis was described in 1985 by Lown and Krowicki,[3] who obtained netropsin through a seven step procedure in 11% overall yield.

Netropsin strongly binds to the DNA minor groove, showing high A/T selectivity.[4] Sequence specificity and high affinity are due to a combination of interactions including hydrogen bonding between the amines and carbonyls of A/T bases and the amides of netropsin, van der Waals contacts and electrostatic interactions with the DNA phosphate backbone.[5]

Netropsin is able to inhibit some DNA-binding enzymes as bacterial RNA and DNA polymerases, DNAase I, gyrase and mammalian topoisomerase I and II,[6] and shows antiviral properties. The antiviral activity is strictly connected to the position of the peptidic bond. In fact, derivatives bearing the carboxamide groups in positions 2 and 5 of the pyrrole ring were revealed to be less active than compounds in which the peptidic bonds were in positions 2 and 4 of the pyrrole ring.[7]

A great number of analogues of the natural antibiotic were synthesized and their biological properties were investigated.[8]

On this basis, we have designed and synthesized netropsin isomers in which the amine and carbonyl groups are switched in position 2 and 4, respectively instead of the 4 and 2 positions of the natural antibiotic. In order to evaluate the influence of the position of these two functional groups, as well as the number of pyrrole units, we planned the synthesis of the one pyrrole unit derivative **2**, 'isonetropsin analogue', and of the two pyrrole units derivative, 'isonetropsin' **3** (Fig.1).

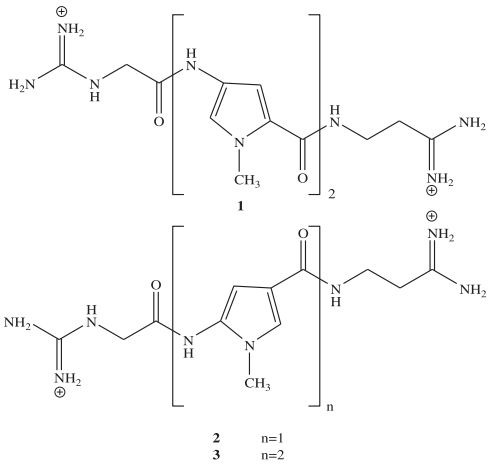


Fig. 1. Netropsin **1**, isonetropsin analogue **2** and isonetropsin **3**.

* Corresponding author. Tel.: +39 09123896810; fax: +39 09123860854; e-mail address: girolamo.cirrincione@unipa.it (G. Cirrincione).

0040-4020/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.tet.2013.01.076

2. Docking

In order to investigate the potential interaction of netropsin analogues with the DNA minor groove, docking modelling studies were performed. The X-ray structure with code 261D[9] was selected from the Protein Data Bank; the DNA fragment was prepared using Protein Preparation Wizard. Docking was carried out using Glide software XP mode default parameters.[10]

Both analogues overlap well with netropsin but analogue **3** seems to be more efficient at interacting with the DNA minor groove (Fig. 2). Comparing docking score results, isonetropsin analogue **2** shows a lower affinity towards the DNA fragment than Netropsin whereas isonetropsin **3** shows a docking score value comparable with that of Netropsin.

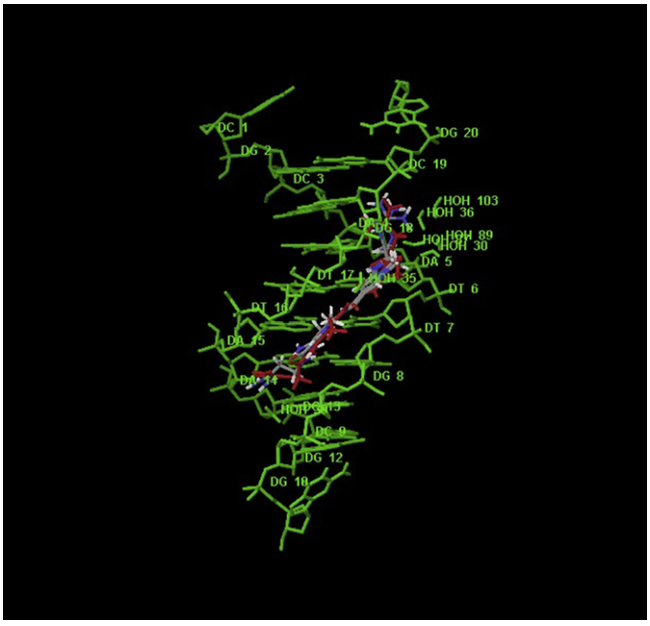
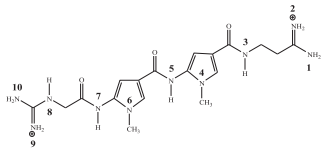


Fig. 2. Superimposition of analogues **2**, **3** and Netropsin (red) in the DNA minor groove fragment.

Analyzing the binding mode of the two analogues, the amide groups of analogue **3** form bifurcated hydrogen bonds to bases on opposite strands of two adjacent base pairs in a similar way to netropsin (Table 1).

Table 1
Schematic view of analogue **3** interactions with DNA

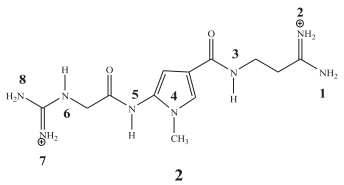


Analogue 3	DNA bases
N1	N3 Adenine 14
N3	O2 Thymine 7
	N3 Adenine 15
N5	O2 Thymine 6
	O2 Thymine 16
N9	O2 Thymine 17
	N3 Guanosine 18
N10	N3 Guanosine 18

Analogue **2** interacts through hydrogen bonds with the DNA bases, and due to its reduced length, it is not able to fit well along the minor groove (Table 2).

On the basis of these docking virtual screening results we planned the synthesis of both derivatives to test them as new DNA-interactive compounds.

Table 2
Schematic view of analogue **2** interactions with DNA



Analogue 2	DNA bases
N1	N3 Adenine 15
N3	O2 Thymine 6
N6	O2 Thymine 17
	N3 Guanosine 18
N8	N3 Guanosine 18

3. Results and discussion

The crucial step of the synthetic pathway was the isolation of the 2-aminopyrrole derivatives. In fact in comparison with the 3-aminopyrroles used for the total synthesis of netropsin, 2-aminopyrroles are much more unstable and their isolation and handling are very difficult. They are liable to oxidation and often decompose in acid medium, and even during purification processes on silica gel.[11a,b]

The key intermediate in the synthesis of the netropsin analogue is 1-methyl-2-nitropyrrole-4-carboxamidopropionamidine hydrobromide (**12**) (Scheme 1). This synthetic pathway takes place from 1,1,1-trichloro-4-[(2,2-dimethoxyethyl)-amino]but-3-en-2-one (**6**) obtained in quantitative yields by reacting aminoacetaldehyde dimethyl acetal (**4**) with 4-ethoxy-1,1,1-trichloro-3-buten-2-one (**5**) prepared according to the procedure previously reported.[12]

Derivative **6** was refluxed in acetic acid for 2 h, affording 1*H*-3-trichloroacetylpyrrole (**7**) that was nitrated at −50 °C with a mixture of nitric acid and acetic anhydride allowing the isolation of compound **8** in good yield.

Methylation of the pyrrole nitrogen was achieved through reaction with potassium *tert*-butoxide, tris[2-(2-methoxyethoxy) ethyl]amine (TDA-1) and iodomethane; the *N*-methyl derivative **9** was then condensed with the amidine side chain synthesized as previously reported;[13] after an appropriate work-up of the reaction mixture it was possible to isolate derivative **12** in good yield.

Derivative **12** was dissolved in ethanol and hydrogenated over 10% Pd on charcoal. The work-up of this reaction was carried out under argon atmosphere in a glove box. The amine thus obtained was very unstable turning into a black tar very quickly, so it was treated with ethanol saturated with gaseous HCl to obtain the corresponding hydrochloride. Nevertheless, the hydrochloride was also quite unstable and it decomposed during NMR acquisition.

Therefore the amine **13** was used as formed immediately, without further purifications and it was condensed with guanidineacetic acid hydrochloride, previously activated with carbonyldiimidazole (CDI), affording the one pyrrole unit isonetropsin analogue **2**.

The key intermediate in the synthesis of isonetropsin **3** is compound **14**, which derives from condensation of the amine **13** with another pyrrole unit.

First of all we reduced **12**, as previously mentioned and we carried out the reaction between the amine with trichloro derivative **9** but the reaction was not successful; so we tried the condensation with 1-methyl-2-nitro-1*H*-pyrrole-4-carboxylic acid (**10**) properly activated. This latter was synthesized through alkaline hydrolysis of derivative **9**. Unfortunately the attempt to condense **10**, activated with CDI, and the amine **13** was not successful. Instead carboxylic acid **10**, reacted with $SOCl_2$ in refluxing toluene, to give the acid chloride **11**, which was directly condensed with amine **13** affording the dimer **14** in good yield.

Derivative **14** was hydrogenated over 10% Pd on charcoal to give the amine **15**, which, without further purifications, was reacted with guanidineacetic acid hydrochloride as previously described for derivative **2**, affording isonetropsin **3**.