Toxicon 75 (2013) 35–43

Contents lists available at SciVerse ScienceDirect

Toxicon

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

High-throughput receptor-based assay for the detection of spirolides by chemiluminescence

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ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form 13 June 2013
Accepted 21 June 2013
Available online 1 July 2013

Keywords:
Cyclic imines
Nicotinic acetylcholine receptors
α-bungarotoxin
Competition assay
Single-laboratory pre-validation

ABSTRACT

The spirolides are marine toxins that belong to a new class of macrocyclic imines produced by dinoflagellates. In this study a previously described solid-phase receptor-based assay for the detection of spirolides was optimized for high-throughput screening and prevalidated. This method is based on the competition between 13-desmethyl spirolide C and biotin-α-bungarotoxin immobilized on a streptavidin-coated surface, for binding to nicotinic acetylcholine receptors. In this inhibition assay the amount of nAChR bound to the well surface is quantified using a specific antibody, followed by a second anti-mouse IgG antibody labeled with horseradish peroxidase (HRP). The assay protocol was optimized for 384-well microplates, which allowed a reduction of the amount of reagents per sample and an increase of the number of samples per plate versus previously published receptor-based assays. The sensitivity of the assay for 13-desmethyl spirolide C ranged from 5 to 150 ng mL^{-1}. The performance of the assay in scallop extracts was adequate, with an estimated detection limit for 13-desmethyl spirolide C of 50 μg kg^{-1} of shellfish meat. The recovery rate of 13-desmethyl spirolide C for spiked samples with this assay was 80% and the inter-assay coefficient of variation was 8%. This 384-well microplate, chemiluminescence method can be used as a high-throughput screening assay to detect 13-desmethyl spirolide C in shellfish meat in order to reduce the number of samples to be processed through bioassays or analytical methods.

1. Introduction

In the early 1990s a new class of macrocyclic imines, known as spirolides (Fig. 1), was identified in toxic extracts of the digestive glands of mussels and scallops from the Atlantic coast of Nova Scotia, Canada (Hu et al., 1995). The marine dinoflagellates *Alexandrium ostenfeldii* and *Alexandrium peruvianum* have been described as the causative organisms producing spirolides (Cembella et al., 1999, 2000; Touzet et al., 2008), and have been found at multiple locations worldwide, including Canada, Scotland, Norway, USA (Gulf of Maine), Denmark, Adriatic Sea, Spain, France and Chile (Hu et al., 1995; John et al., 2003; Aasen et al., 2005; Anderson et al., 2005; Ciminiello et al., 2006; MacKinnon et al., 2006; Villar Gonzalez et al., 2006; Amzil et al., 2007; Álvarez et al., 2009). In spite of the worldwide appearance of spirolide producing algal blooms, no episodes of human intoxication have been reported,

Abbreviations: BSA, Bovine serum albumin; α-BTX, α-bungarotoxin; CR, cross-reactivity; CV, coefficient of variation; HRP, horseradish peroxidase; i.p., intraperitoneal; LC–MS, liquid chromatography–mass spectrometry; LoD, limit of detection; MBA, mouse bioassay; MWCO, molecular-weight-cutoff; nAChR, nicotinic acetylcholine receptor; PBS, phosphate-buffered saline; SD, standard deviation; SEM, standard error of the mean.

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0041-0101/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxicon.2013.06.015

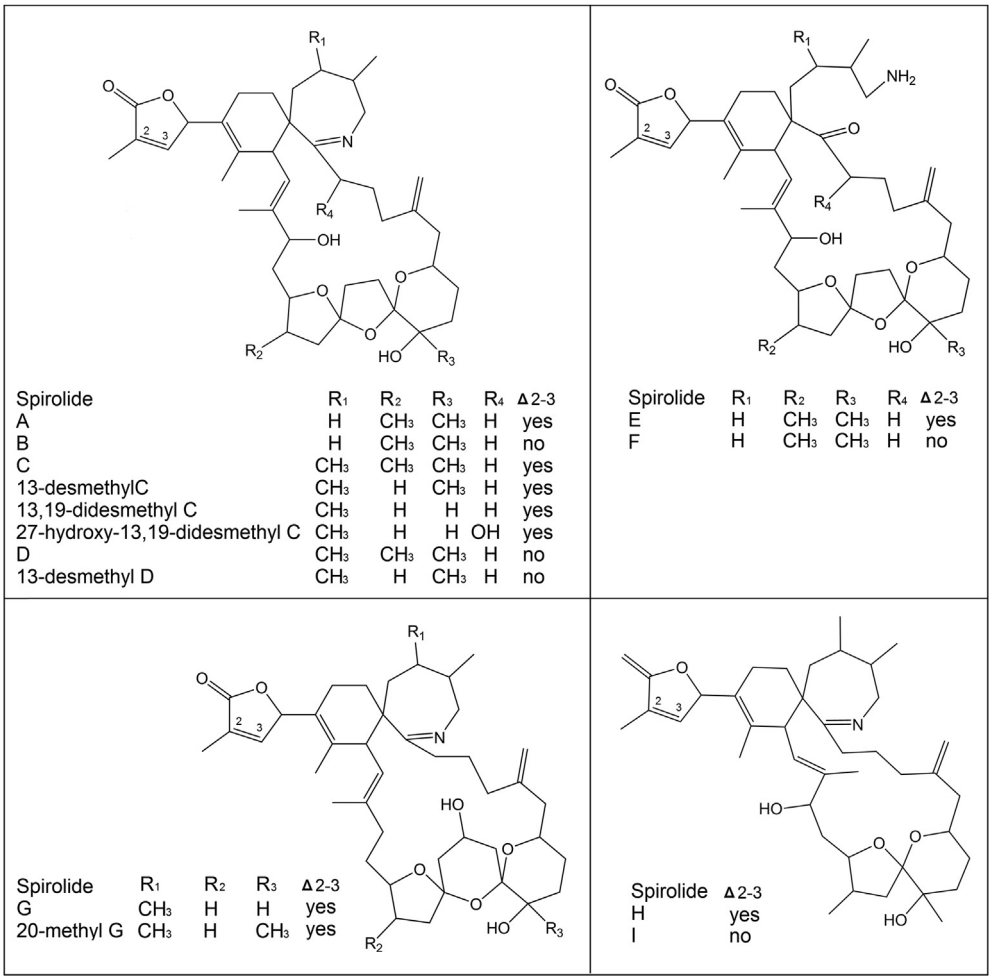

Fig. 1. Chemical structure of spirolides.

probably in part because the presence of spirolides is detected by the mouse bioassay (MBA) used for many years to protect humans from lipophilic toxins.

The cyclic imine moiety found in the structure of these compounds is believed to be responsible for the toxic effects in experimental animals (Hu et al., 1996). This moiety is also present in other toxins, like pinnatoxins, pteriatoxins, spiro-prorocentrimine, prorocentrolides and gymnodimine (Torigoe et al., 1988; Seki et al., 1995; Uemura et al., 1995; Lu et al., 2001; Takada et al., 2001a, 2001b), which belong together with the spirolides to the group of marine toxins known as cyclic imines. The mechanism of action of the spirolides is most likely related to the inhibition of the nicotinic acetylcholine receptor (nAChR), because representative molecules of the spirolides and gymnodimine have been shown to bind to the orthosteric site of the nAChR with high affinity and to block its activation (Kharrat et al., 2008; Bourne et al., 2010).

The methods more frequently used to detect cyclic imines have been the MBA and liquid chromatography-mass spectrometry (LC–MS) techniques (Hu et al., 2001; Richard et al., 2001; Stirling, 2001; Biré et al., 2002; Aasen et al., 2005; Sleno and Volmer, 2005; Ciminiello et al., 2006; Villar Gonzalez et al., 2006; Fux et al., 2007). The MBA consists of the intraperitoneal (i.p.) injection of shellfish extracts to mice. This technique has some disadvantages, among them the number of laboratory animals sacrificed every year for the purpose of marine toxin detection. The detection and quantification of marine toxins by LC–MS methods also has some drawbacks, it requires certified standards of these compounds, in many cases non-existent, and the detection of new analogues/toxins would be lost with these techniques. Furthermore this method is expensive and requires highly qualified personnel. The development of alternative techniques based on molecular interactions to detect marine toxins is necessary to provide high-throughput screening methods that allow a reduction of the number of samples to be tested by other, more expensive and/or ethically questionable methods. Recently, two receptor-based methods have been developed to detect gymnodimines and spirolides by fluorescence polarization and chemiluminescence using the competition between