ChemInform Abstract: Isoxazoline Containing Natural Products as Anticancer Agents: A Review

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · MARCH 2014

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2014.02.063

European Journal of Medicinal Chemistry 77 (2014) 121–133

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Invited review

Isoxazoline containing natural products as anticancer agents: A review

Kamalneet Kaur [a], Vinod Kumar [a,*], Anil Kumar Sharma [b], Girish Kumar Gupta [c]

[a] Department of Chemistry, Maharishi Markandeshwar University, Mullana, Ambala 133207, India
[b] Department of Biotechnology, Maharishi Markandeshwar University, Mullana, Ambala 133207, India
[c] Department of Pharmaceutical Chemistry, Maharishi Markandeshwar University, Mullana, Ambala 133207, India

ARTICLE INFO

Article history:
Received 27 October 2013
Received in revised form
25 February 2014
Accepted 28 February 2014
Available online 1 March 2014

Keywords:
Isoxazolines
Anticancer
Cytotoxic
Stereochemistry
Natural products

ABSTRACT

Isoxazolines are an important class of nitrogen and oxygen containing heterocycles that belong to the azoles family which have gained much importance in the field of medicinal chemistry as the anticancer agents. Moreover, natural products are always expectedly regarded as an important hoard of a large number of potential chemotherapeutic candidates. Therefore, this review mainly focuses on the existence of isoxazoline derivatives in natural sources, their isolation and uses there of as anticancer agents besides highlighting the synthetic pathways to achieve these compounds. Structural–activity relationship and the influence of stereochemical aspects on anticancer activity of such compounds have also been discussed. It covers the literature upto 2014 and would certainly provide a great insight to scientific community to accelerate further research for the development of some novel anticancer drugs.

1. Introduction

Nowadays, cancer has gradually become the leading cause of death worldwide and seriously endangering the health and life of humans for a long period [1]. It has been reported that cancer can be caused by one of the three ways namely, incorrect diet, genetic predisposition and environmental contaminants [2]. Consistent efforts have been made to fight against this disease in the past few years as a result of advancements in cellular and molecular biology leading to the development of potent anticancer agents capable of targeting the cancerous tissues with minimal side effects. Natural products have appreciably contributed to the development of a large number of anticancer drugs [3–10]. About 50% of all anticancer drugs approved internationally are either natural products or natural product mimics and were developed on the basis of the knowledge obtained from small or macromolecules existing in nature [11].

Recently, various azole derivatives have attracted considerable attention in the field of anticancer research [12–18]. Among them, Δ^2-isoxazoline derivatives are an important class of five membered nitrogen-oxygen containing heterocyclic compounds that exhibited promising antineoplastic properties. The general chemical structure of Δ^2-isoxazoline is shown in Fig. 1.

Some important examples of synthetic Δ^2-isoxazoline scaffolds are 3,5-diaryl-isoxazoline linked 2,3-dihydroquinazolinone hybrids **1** [19], arylisoxazoline containing anthranilic diamides **2** [1], 3,5-diaryl-isoxazoline linked pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) conjugates **3** [20] and dibenzo[*b*,*f*]azepinetethered isoxazoline derivatives **4** [21] that act as potent anticancer agents with an improved pharmacokinetics profile Fig. 2. Anticancer properties associated with isoxazole compounds are summarized in Fig. 3.Viewing the importance of natural products as well as Δ^2-isoxazoline containing pharmacores in the field of cancer research, the present review is mainly focused on those natural products which bear Δ^2-isoxazoline moiety exhibiting anticancer potential. Furthermore, we discussed about various pathways and influence of stereochemical aspects particularly on anticancer activity of such compounds.

2. Naturally occurring anticancer isoxazoline derivatives

2.1. Subereamolline A

It has been reported that methanol extract of sponge *Suberea mollis*, collected from Hurghada at the Egyptian Red Sea coast yielded a bioactive dibrominated metabolite, (+)-subereamolline A **5**. It potently inhibits the migration and invasion of metastatic human breast cancer cells i.e. MDA-MB-231 at the nanomolar dose level Fig. 4 [22,23]. From this study, it was found that the presence of terminal ethyl carbamate moiety is an important factor for the

* Corresponding author.
E-mail address: vinodbatan@gmail.com (V. Kumar).

http://dx.doi.org/10.1016/j.ejmech.2014.02.063
0223-5234/

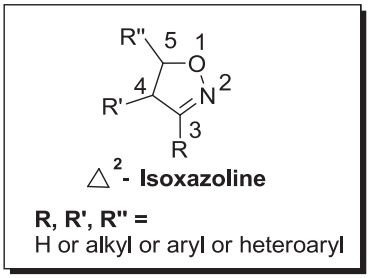


Fig. 1. General structure of Δ^2 – Isoxazoline.

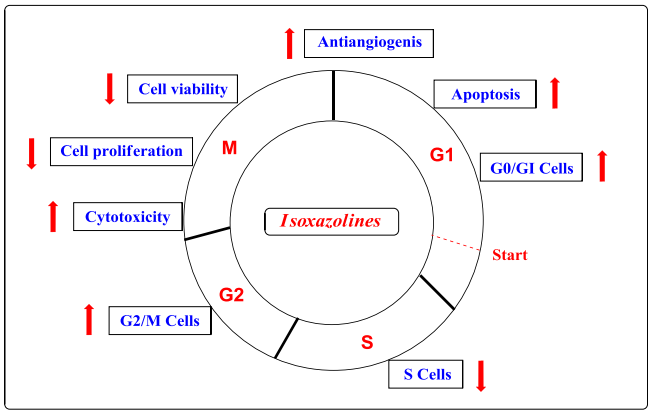


Fig. 3. Proposed anticancer properties of isoxazoline compounds.

antimigratory activity [23]. The compound (+)-**5**, having S-configuration at the spirocentre displayed high potency against cancer cells even at nanomolar dose level while anticancer effect of (−)-**5**, a non natural isoxazoline derivative having R-configuration at the same spirocentre obtained during the total synthesis has not been reported till date. Therefore, compound (+)-**5** may act as a novel scaffold for the design of more efficient breast cancer migration and invasion inhibitor to control malignant form of cancer.

Shearman et al. reported the first total synthesis of (+)-subereamolline A **5** and (−)-subereamolline A **5** by using preparative chiral HPLC separation of the corresponding racemates [24]. In this approach, 2-hydroxy-4-methoxybenzaldehyde **6** reacted with *N*-bromosuccinimide (NBS) followed by benzyl protection of the phenolic oxygen to obtain aldehyde **7** in 92% yield. The compound **7** so obtained was further converted into the azlactone **8** by treating **7** with *N*-acetylglycine and sodium acetate in the presence of acetic anhydride. Azlactone **8** on further saponification with barium hydroxide and subsequent condensation with *O*-benzylhydroxylamine yielded carboxylic acid **9** with 49% yield along with an oxime **10** (22% yield) as a side product. The treatment of **9** with trimethylsilyldiazomethane gave corresponding methyl ester which on subsequent hydrogenolysis over palladium black gave oxime methyl ester **11** as a cyclization precursor. Oxidative cyclization of **11** with iodobenzene diacetate using acetonitrile as a solvent gave (±)-**12** which underwent diasteroselective reduction with $Zn(BH_4)_2$ to produce trans isomer (±)-**13** as the major product along with cis isomer (±)-**15** as the minor one. Alkaline hydrolysis of methyl ester (±)-**13** with lithium hydroxide gave spiroacid (±)-**15** in an overall yield of 11% starting from aldehyde **6**. The coupling of spiroacid (±)-**14** with amine **16** in the presence of *N,N′*-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) afforded (±)-subereamolline A **5** with 91% yield. However, improved yield (96%) of (±)-subereamolline A **5** was obtained by using propylphosphonic anhydride (T3P) as a coupling agent. The resolution of (±)-subereamolline A **5** was carried out by using preparative chiral HPLC which gave (+)-**5** having absolute configuration R and S at C-1 and C-6 chiral centre, respectively and (−)-**5**, having S and R configuration at C-1 and C-6 chiral centre, respectively (Scheme 1).

2.2. Aerothionin and 11-oxoaerothionin

(+)-Aerothionin **17**, a tetra bromo compound having spirohexadienylisoxazoline pharmacore was first isolated from the acetone extract of marine sponges, *Aplysina aerophoba* and *Verongia thiona* Fig. 5 [25,26]. Kernan et al. have isolated (+)-**17** from the dichloromethane extract of verongid sponge, *Pseudoceratina durissima* collected from Bowl Reef and Great Barrier Reef Australia [27]. The compound (+)-**17** was also isolated from the methanol/dichloromethane extract of Carribbean sponge *Aplysina fistularis insularis* [28], methanol extract of the Red Sea sponge *Suberea mollis* [22], methanol extract of Great Barrier Reef sponge *Pseudoceratina* sp. (order Veronida, family Drinellidae) [29], dichloromethane/methanol extract of sponge *Psammaplysilla purpurea* [30], methanol/chloroform extract of Caribbean sea sponge *Aplysina lacunosa* [31], dichloromethane extract of *Aplysina gerardogreeni* [32],

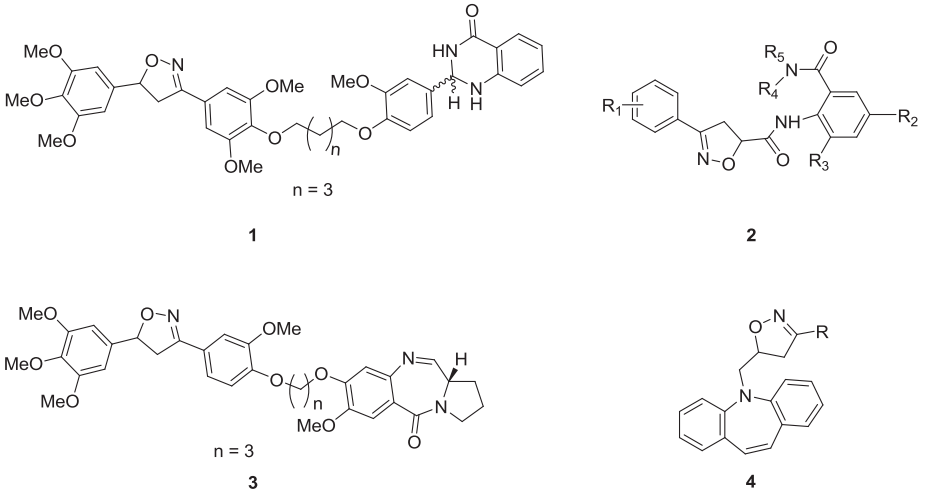


Fig. 2. Anticancer synthetic isoxazolines.

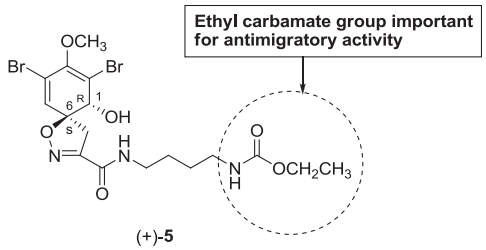


Fig. 4. Breast cancer inhibitor.

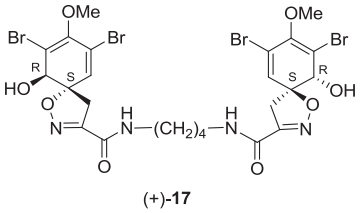
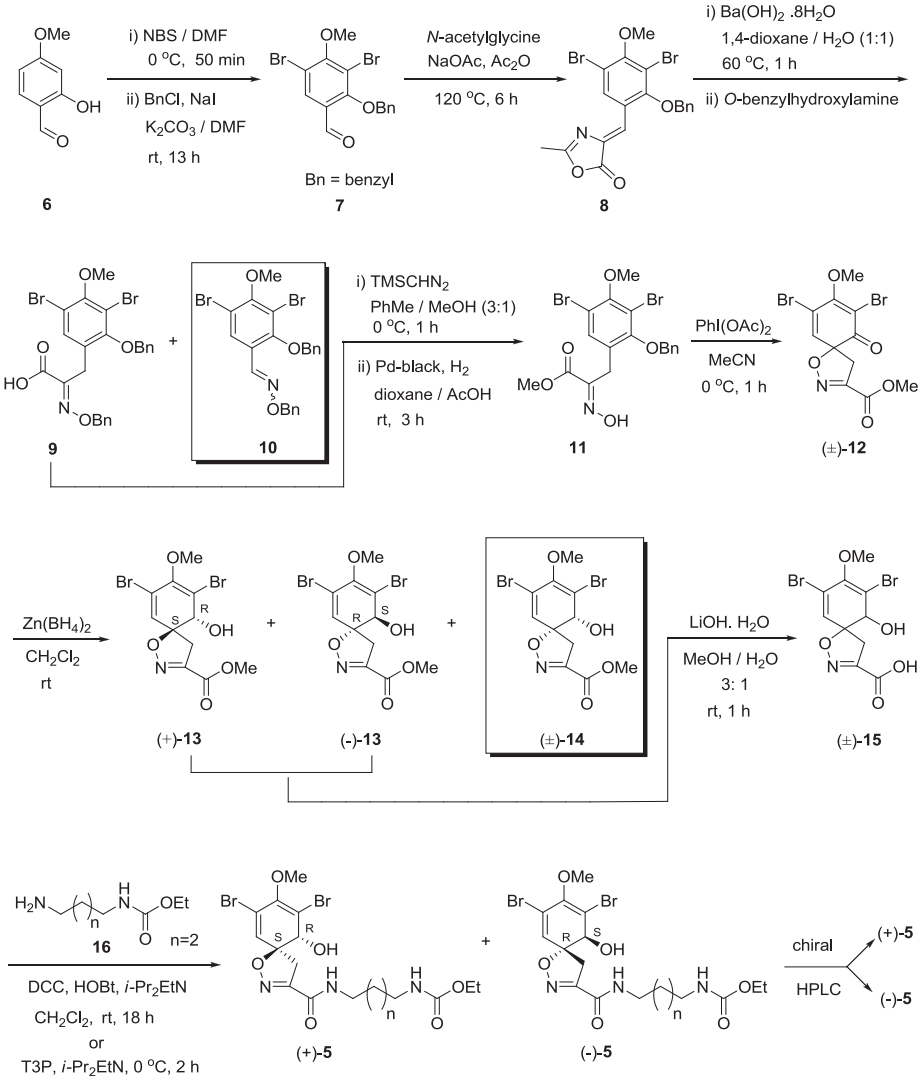


Fig. 5. Structure of (+)-trans-trans aerothionin.

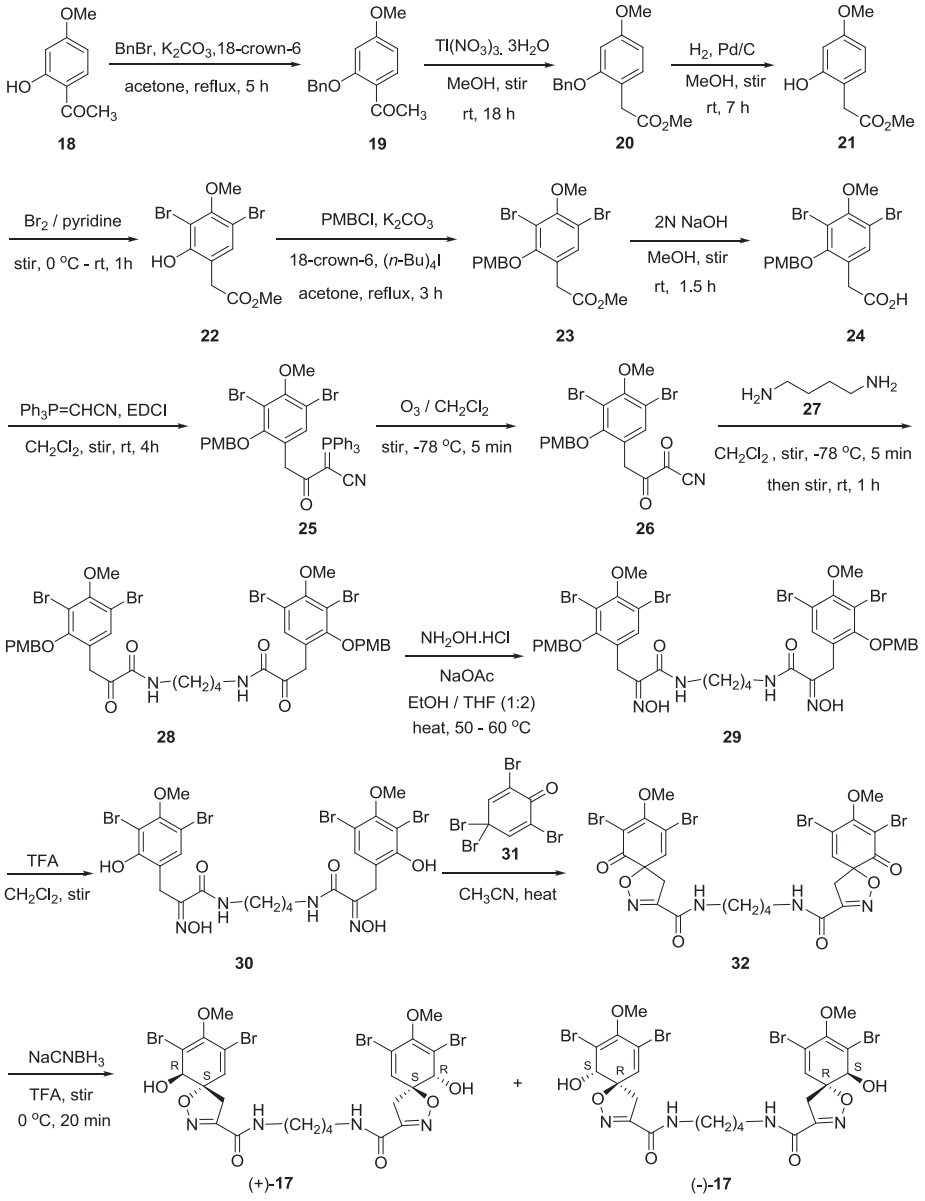
dichloromethane/methanol extract of marine Crinoid *Himerometra magnipinna* [33].

It was found that (+)-trans-trans aerothionin **17** (trans-vicinal relationship between hydroxyl group and oxygen atom in the spiroisoxazoline unit) having S-configuration at both spirocentres exhibited moderate cytotoxicity with EC_{50} value 42 μM against the benchmark HeLa cell line [29]. However, cytotoxic effect of (−)-trans-trans aerothionin **17**, a non natural isoxazoline derivative bearing R-configuration at both spirocentres is still not clear.

Wasserman et al. reported the synthesis of (±)-trans-trans aerothionins **17** using the cyano ylide coupling methodology (Scheme 2) [34]. In this approach, protection of phenolic hydroxyl was achieved by treating 2-hydroxy-4-methoxyacetophenone **18** with a mixture of benzyl bromide (BnBr), 18-crown-6 and potassium carbonate using acetone as a solvent to obtain benzyl ether **19** (97% yield). The rearrangement of **19** in the presence of thallium nitrate trihydrate afforded methyl[2-(benzyloxy)-4-methoxyphenyl]acetate **20** in 76% yield which underwent deprotection on treatment with Pd/C catalyst under hydrogen atmosphere to furnish methyl-(2-hydroxy-4-methoxyphenyl)acetate **21**. Dibromo derivative **22** was formed in 90% yield by the treatment of compound **21** with bromine using pyridine. This on further reaction with *p*-methoxybenzyl chloride (PMBCl) in the presence of



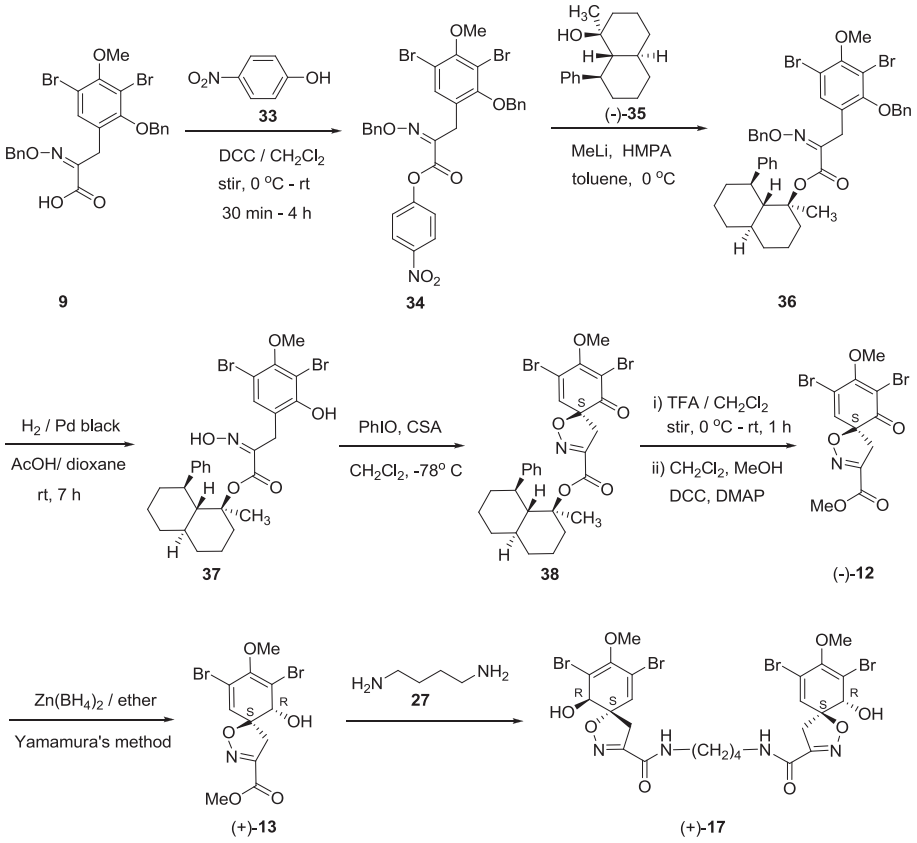
Scheme 1. First total synthesis of (+)-subereamolline A **5**.



Scheme 2. Synthesis of (±)-trans-trans aerothionins **17**.

potassium carbonate, 18-crown-6 and tetra butyl ammonium iodide using acetone as a solvent gave methyl-[3,5-dibromo-2-(*p*-methoxybenzyloxy)-4-methoxyphenyl]acetate **23**. Hydrolysis of ester **23** to obtain **24** was readily achieved by treatment with sodium hydroxide in methanol. The coupling of compound **24** with (triphenylphosphoranylidene)acetonitrile using 1-ethyl-3-(3-dimethyl-aminopropyl) carbodimide (EDCI) afforded ylide **25** which was further transformed to diketonitrile **26** by reacting it with ozone in dichloromethane at a very low temperature. *Bis*-α-ketoamide **28** obtained by treating **26** with 1,4-diaminobutane **27** was further converted into *bis*-*E*-oxime **29** in 95% yield using hydroxylamine hydrochloride in the presence of sodium acetate. Deprotection of **29** with trifluoroacetic acid yielded substrate **30** which underwent intramolecular ring closure to form *bis*-spiro isoxazolidine **32** by the reaction of **30** with 2,4,4,6-tetrabromo-2,5-cyclohexadienone **31** in acetonitrile. The reduction of cyclohexadienone ketonic centres of **32** underwent in a stereospecific manner in order to generate both the hydroxyl groups particularly in trans relationship with respect to the corresponding spiroisoxazoline rings to form racemic mixture of (±)-trans-trans aerothionins **17** in 25% yield was achieved by using sodium cyanoborohydride in trifluoroacetic acid (Scheme 2).

Asymmetric synthesis of (+)-aerothionin **17** via the synthesis of enantiomerically enriched cyclohexadienone spiroisoxazoline (−)-**12** has been reported by Murakata et al. (Scheme 3) [35]. In this method, 2-[(benzyloxy)imino]-3-[2-(benzyloxy)-3,5-dibromo-4-methoxyphenyl]propanoic acid **9** was treated with *p*-nitrophenol **33** in the presence of *N,N'*-Dicyclohexylcarbodiimide (DCC) using a mixture of dichloromethane/methanol as solvent to produce *p*-nitrophenyl ester **34** in 91% yield. The transesterification of **34** with lithiated (−)-**35** as a chiral auxiliary gave 1-methyl-1-decalyl ester **36** in 85% yield which underwent hydrogenolysis in the presence of palladium catalyst using a mixture of acetic acid-dioxane (1:1) solvent system to produce *O*-phenolic oxime ester **37** in 91% yield. The compound **37** so obtained was further reacted with iodosylbenzene (PhIO) in the presence of camphor-sulfonic acid (CSA)



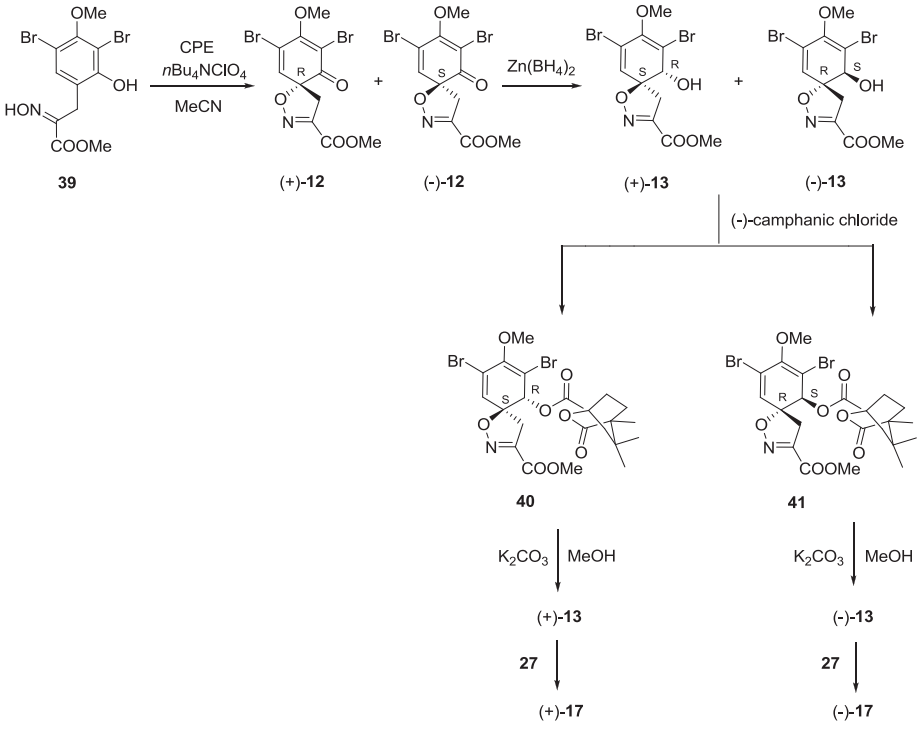
Scheme 3. Asymmetric synthesis of (+)-trans-trans aerothionin **17**.

using dichloromethane to obtain spiroisoxazoline **38** in 83% yield. Chiral auxiliary was easily removed by treating **38** with trifluoroacetic acid at room temperature for 1 h followed by the formation of methyl ester (−)-**12** in 71% yield with 74% enantiomeric excess by the use of combination of *N,N′*-Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in the mixture of methanol and dichloromethane. The reduction of (−)-**12** with $Zn(BH_4)_2$ in ether furnished cyclohexadienylisoxazoline (+)-**13**, which on amidation with butanediamine **27** afforded (+)-**17** with *S*-configuration at both the spirocentres [36].

Ogamino et al. reported a highly efficient synthesis of optically pure (+)-**17** and (−)-**17** from the racemic spiroisoxazoline derivative (±)-**13** [37]. In this approach, synthesis of compound (±)-**13** was efficiently achieved by electrochemical oxidation of **39** using constant potential electrolysis (CPE) to produce (±)-**12** followed by its reduction with $Zn(BH_4)_2$ [38,39]. The treatment of (±)-**13** with (−)-camphanic chloride resulted in the formation of camphanic acid esters **40** and **41** as two diasteromers in 46% and 47% yield, respectively which were easily separated by silica gel chromatography. The solvolysis of **40** and **41** using potassium carbonate as a base furnished optically active spiroisoxazoline (+)-**13** and (−)-**13**, respectively. Finally, condensation of (+)-**13** and (−)-**13** with 1,4-diaminobutane produced optically pure (+)-**17** and (−)-**17** in 28% and 33% yield, respectively (Scheme 4).

Another approach for the synthesis of (±)-trans-trans aerothionins **17** via phenolic oxidation of methyl pyruvate **39** with thallium (III) trifluoroacetate as a key step has been reported by Yamamura et al. [40]. Alkaline hydrolysis of azalactone **8** with potassium hydroxide using a mixture of dioxane-water followed by the oximation with hydroxylamine hydrochloride and benzylation in the presence of potassium carbonate in *N,N*-dimethylformamide (DMF) afforded tribenzyl derivative **42** in 31% yield. Transesterification of **42** in methanol containing potassium carbonate base furnished the corresponding methyl ester which further on hydrogenolysis in the presence of catalytic palladium-carbon (10%) using a mixture of dioxane-acetic acid gave the desired dihydroxy derivative **39** in moderate yield. The compound so obtained was further oxidized by thallium (III) trifluoroacetate in trifluoroacetic acid (TFA) to afford compounds **43**, (±)-**12** and **44** in 21%, 27% and 3% yield, respectively. The reduction of racemate (±)-**12** by excess of $Zn(BH_4)_2$ in a mixture of chloroform and ether yielded a mixture of (±)-**13** trans–trans and (±)-**14** cis–cis products in ratio (1.3:1) with 70% yield. Finally, amidation of (±)-**13** with 1,4-butanediamine **27** furnished (±)-trans-trans aerothionins **17** in 18% yield (Scheme 5).

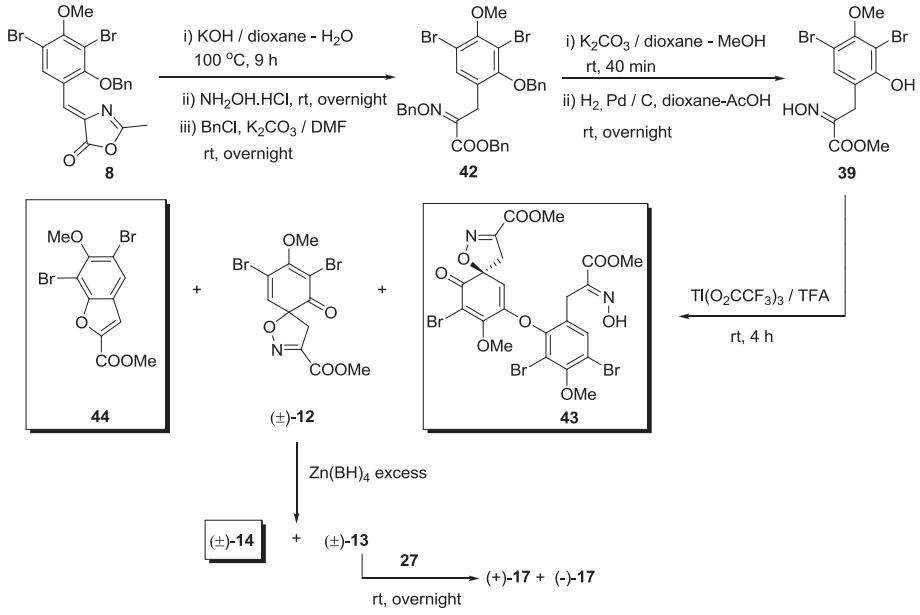
An efficient method for the synthesis of (±)-trans-trans aerothionins **17** by adopting a new route for oxidative spirocyclization of phenolic oxime ester has been developed by Boehlow et al. [41]. The bromination of 2-hydroxy-4-methoxybenzaldehyde **6** with NBS in DMF resulted in the formation of dibromoaldehyde **45** in 97% yield. The compound **45** so obtained was further treated with methyl chloromethyl ether (MOMCl) in the presence of diisopropylethylamine using tetrahydrofuran (THF) to afford the protected aldehyde **46** in 99% yield which on further reaction with 2-(*tert*-butyldimethylsilyloxy)-2-(dimethylphosphono)acetate using lithiumdiisopropylamide (LDA) and THF yielded silyl enol ether **47** in 92% yield. Deprotection of **47** by $Et_3N.HF$ in methanol followed by immediate addition of hydroxylamine hydrochloride furnished oxime **48** in 90% yield. The treatment of **48** with catalytic amount of *p*-toluenesulfonic acid (TsOH) in methanol resulted in removal of MOM group to produce phenolic oxime **39** in 100% yield. The oxidative cyclization of oxime ether **39** using polymer-supported



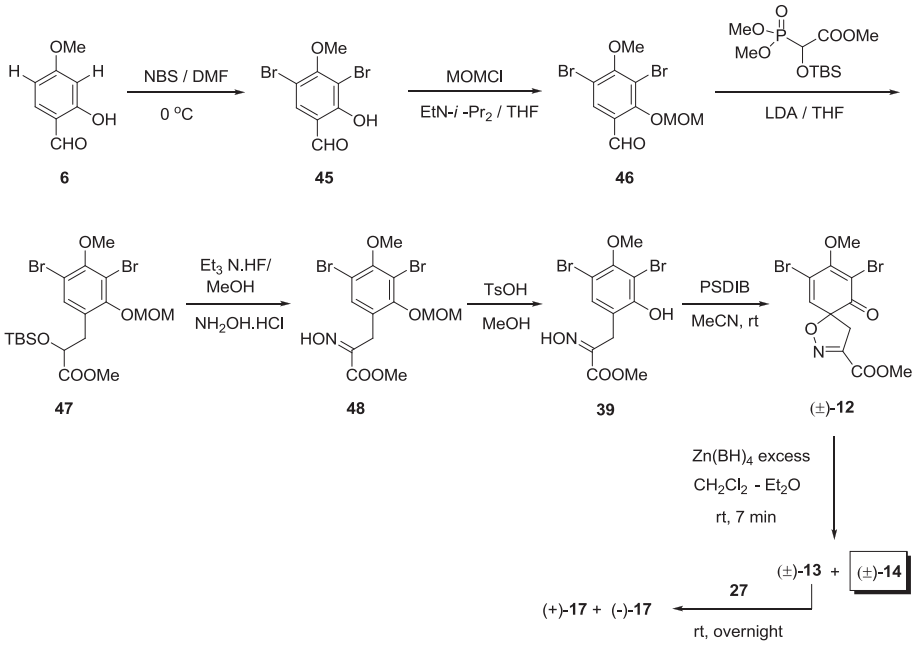
Scheme 4. A highly efficient synthetic approach to optically pure (+)-**17** and (−)-**17**.

(diacetoxyiodo)benzene (PSDIB) reagent in acetonitrile afforded spiroisoxazoline (±)-**12** with high yield and purity. Spiroisoxazoline (±)-**12** so obtained was easily transformed into (±)-**17** by the same procedure as adopted by Yamamura et al. [40] (Scheme 6).

Harburn et al. have developed a novel synthetic approach to (±)-**17** through acylation of amine with coumarin as a key step [42]. In this method, dibromosalicylaldehyde **45** was treated with *N*-acetylglycine in the presence of sodium acetate using acetic anhydride to afford acetamido coumarin **49** in 53–90% yield. Hydrolysis of **49** with an ethanolic solution of sulfuric acid gave enol **50** in 90% yield which on reaction with 8.5 equivalent of hydroxylamine hydrochloride in 80% ethanol under reflux produced oximino coumarin **51** in 69% yield. The compound **51** was reacted with 1,4-butanediamine **27** using triethylamine as a base in methanol at 60 °C under reflux to afford amide **52** derivative in 68% yield. The treatment of **52** with PSDIB using acetonitrile resulted in the oxidative spirocyclization which led to the formation of aerothionin precursor **32** which might be further converted into (±)-**17** by the same procedure as adopted by Wasserman et al. [34] (Scheme 7).



Scheme 5. Synthesis of (±)-trans-trans aerothionins **17** via phenolic oxidation as a key step.

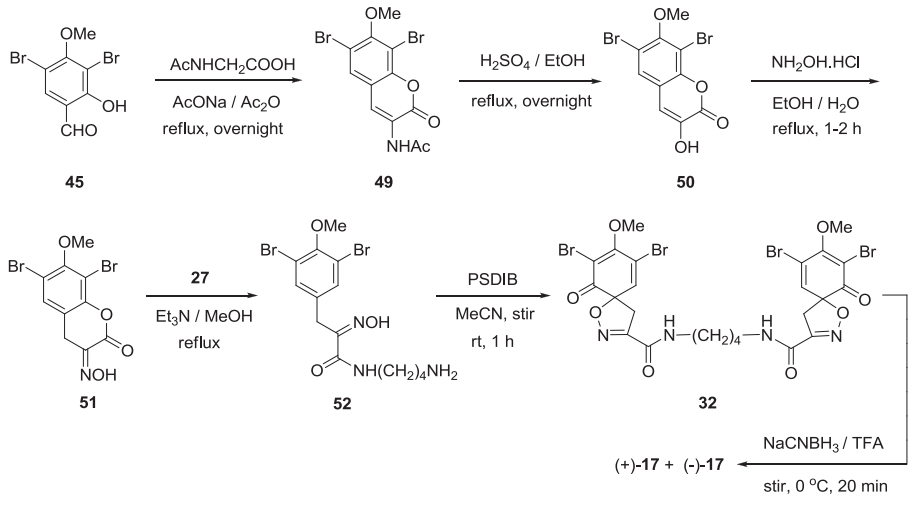


Scheme 6. A new synthetic route to (±)-trans-trans aerothionins **17** using oxidative spirocyclization.

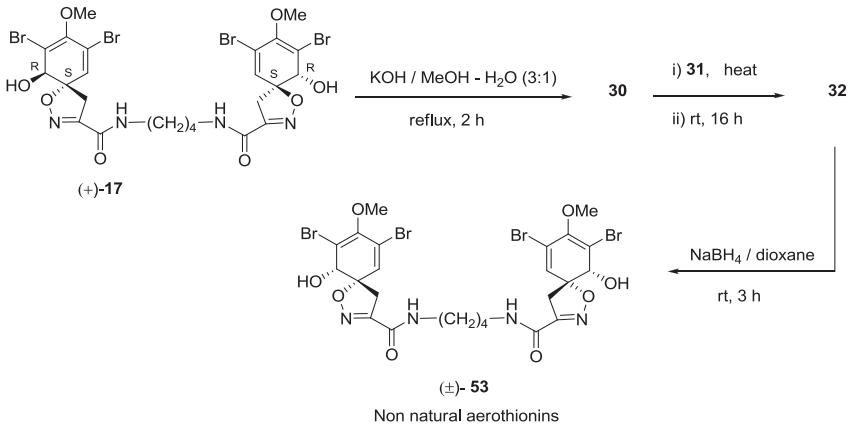
A successful conversion of natural (+)-trans-trans aerothionin **17** into (±)-cis,cis-aerothionins **53** (a non natural isoxazoline derivatives in which cis vicinal relationship between a hydroxyl group and an oxygen atom in the spiroisoxazoline unit is present) has efficiently been achieved by Thomson et al. [43]. Alkaline hydrolysis of (+)-**17** in the presence of potassium hydroxide using a mixture of methanol and water (3:1) as a solvent resulted in the formation of *bis*-oxime *bis*-phenol **30** in quantitative yield. Transformation of **30** into *bis*-spiro isoxazolidine **32** in 64% yield was easily carried out by heating **30** with 2,4,4,6-tetrabromo-2,5-cyclohexadienone **31**. The compound **32** was then reduced by sodium borohydride in dioxane to afford a mixture of non natural aerothionins (±)-**53** in 43% yield. However, the cytotoxic effect of racemic isoxazoline derivative (±) **53** is still unexplored (Scheme 8).

Synthesis of a racemic mixture of (+)-calafianin **54** (natural product) and (−)-calafianin **54** which displayed significant antimicrobial activity was readily achieved using (±)-trans-trans aerothionins **17** as a starting material [44,45]. In this strategy, (±)-**17** was treated with methanesulfonic acid (MsOH) at 0 °C followed by the treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford (±)-**54** with 11% yield (Scheme 9).

On the other hand, a bromotyrosine derived secondary metabolite, 11-oxoaerothionin **55** was isolated from the methanol/chloroform extract of Caribbean sea sponge *Aplysina lacunosa* collected from the west coast of Puerto Rico Fig. 6 [31]. It has also been isolated from the methanol/dichloromethane extract of the Carribbean sponge *Aplysina fistularis insularis* collected from the central coast of Venezuela [28] and methanol/chloroform extract of the sponge *Aplysina cauliformis* collected from Puerto Rico [46]. The compound **55** was screened for its selective anticancer activity against the panel of four human tumor cell lines including three solid tumors i.e. breast (MCF-7), melanoma (SK5-MEL) and colon (HCT 116), and one leukemic line (T cell leukemia) [31]. For this study, five different concentrations of drug (0.01–100 μg/ml) were



Scheme 7. Synthesis of (±)-**17** via acylation of amine with coumarin.



Scheme 8. Conversion of natural (+)-trans-trans aerothionin **17** into (±)-cis-cis aerothionins **53**.

used and found that at a very low concentration range (0.01–0.1 μg/ml), **55** displayed selective cytotoxicity against only one human colon (HCT 116) cell line. However, at concentration equal to or greater than 20 μg/ml, it displayed cytotoxic activity against all the tested human tumor cell lines. Due to its selective cytotoxic effect, compound **55** was chosen as a reliable candidate for further testing as a potential antineoplastic agent. The IC_{50} value of compound **55** against MCF-7, SK5-MEL, HCT 116 and T cell leukemia cell line were found to be 20, 10, 10, and 3.5 μg/ml, respectively and the assays were based on the potentiality of living tumor cells to reduce tetrazolium dye (XTT) to a soluble purple formazan metabolite [31]. The compound **55** differs from (+)-**17** in configuration at one spirocentre and one chiral centre to which hydroxyl group was attached (Fig. 6). It has one carbonyl group flanked between two amide groups in place of methylene group as in (+)-**17**. These structural differences might be responsible for strong cytotoxic effect of **55** in comparison to (+)-**17**, which were found against four cancer cell lines with more selectivity towards HCT cell line.

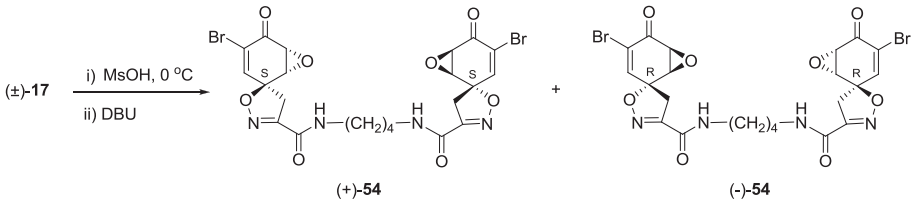
2.3. Aplysinones A–D

Four dibromotyrosine-derived metabolites, aplysinones A–D **56**–**59** have been isolated from the acetone/methanol extract of sponge *Aplysina gerardogreeni* collected at the Gulf of California Fig. 7 [47]. Cytotoxicity of these compounds were tested against MDA-MB-231 (breast adenocarcinoma), A-549 (lung carcinoma) and HT-29 (colon adenocarcinoma) human cell lines using Pharma Mar, a colorimetric type assay using sulforhodamine B reaction for the quantitative measurement of cell growth and viability [47].

From this investigation, it has been found that aplysinones A **56**, B **57** and D **59** displayed significant growth inhibitory activity with most of the GI_{50} (concentration that causes 50% growth inhibition) values lower than 5 μM against all the three tested lines. Moreover, compounds **56** and **57** resulted in total growth inhibition of the three tested lines with TGI_{50} (concentration that causes total growth inhibition) values lower than 5 μM and compound **59** was found to be active as total growth inhibitors against MDA-MB-231 and HT-29 cell lines. The main growth inhibitory effect of compound **58** was found in MDA-MB-231 cells. Among these compounds, **57** was observed to be a highly potent growth inhibitor and also exhibited a significant cell killing activity with LC_{50} (concentration that causes 50% cell killing) value ranging from 3.0 to 4.1 μM against all the three cell lines. The reported cytotoxic assay of aplysinones **A-D** against three tested cell lines with reference to Doxorubicin is summarized in Fig. 8. Aplysinones **56**–**59** possess S-configuration at the isoxazoline linked spirocentre and R-configuration at the other chiral centre. Among all, **57** displayed the strong cytotoxic potential which might be due to the presence of carbonyl group at position-3 instead of methoxy group as in all other derivatives.

2.4. Aplysinamisines (I–III)

On the other hand, from methanol/chloroform extract of sponge *Aplysina cauliformis*, three bromotyrosine-derived alkaloids aplysinamisines (I–III) **60**–**62** have been isolated Fig. 9 [46]. The compound **61** was also isolated from the dichloromethane/methanol extract of Australian sponge *Suberea clavata* [48]. All the isolated products have been tested against three human-tumor cell lines and it was found that compound **62** showed cytotoxicity against all the three cell lines i.e. human breast (MCF-7), T cell leukemia (CCRF-CEM) and human colon (HCT 116) with IC_{50} value 30, 6, and 10 μg/ml, respectively [46]. While aplysinamisines (II) **61** displayed selective cytotoxicity against HCT 116 cell line with $IC_{50} = 10$ μg/ml [46]. However, **60** was found to be inactive against all tested cell lines [46]. All aplysinamisines **60**–**62** bear S-configuration at the isoxazoline linked spirocentre and R-configuration at the other chiral centre. Therefore, the difference in their cytotoxicity may be attributed to the presence of differently functionalized side chains linked to nitrogen atom of the amide group. It has been found that



Scheme 9. Synthesis of (±)-calafianin using (±)-trans-trans aerothionins **17**.

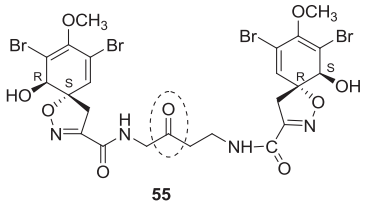


Fig. 6. Structure of 11-oxoaerothionin.

compound **60** containing (2-amino-1*H*-imidazol-4-yl)allyl substituent would be completely devoid of cytotoxicity while 5-guanidinopentyl substituent in **61** resulted in selective cytotoxicity against a cancer cell line [46]. However, **62** having phenoxypropyl substituent attached to amide nitrogen displayed strong cytotoxic effect against the three cancer cell lines.

2.5. Psammaplysin A–C and E

Three dibromotyrosine-derived metabolites psammaplysin A–C **63**–**65** belongs to Druinella family were isolated from methanol/chloroform extract of the sponge *Psammaplysilla Purpurea* [49]. Compounds **63**–**65** were found to exhibit *in vitro* cytotoxicity towards the human colon tumor HCT 116 cell line with IC_{50} values, 6, 3 and 3 μg/ml, respectively [49]. The compounds **63** and **64** have also been isolated from the methanol/ethylacetate extract of Guam sponge *Suberea* sp. [50], and 95% ethanol extract of Palau sponge *Psammaplysilla Purpurea* [51]. Badr et al. isolated compound **63** from the methanol extract of sponge *Pseudoceratina arabica* collected from EI-Sheikh at the Egyptian Red Sea coast [52]. Another metabolite, Psammaplysin E **66** was also isolated with **63** from the methanol extract of sponge *Pseudoceratina Purpurea* collected from the Hachijo Jima Island, 300 km south of Tokyo [53]. Compounds **63**–**66** have also been isolated from the combined methanol and methanol/dichloromethane extract of *Aplysinella* sp. of sponge (order Verongida, family Aplysinellidae) collected from the Chuuk, Federated States of Micronesia Fig. 10 [54]. Moreover, compounds **63**, **64** and **66** have also been isolated from the dichloromethane/methanol extract of Balinese marine sponge *Aplysinella strongylata* (order Verongida, family Aplysinellidae) collected at Tulamben, Bali, Indonesia [55].

It has been observed that compound **66** was found to display significant cytotoxic activity against KB (human oral, epidermoid carcinoma) and LoVo (human colon, adrenocarcinoma) cells at 5 μg/ml [56]. Moreover, **66** also exhibited potent cytotoxicity

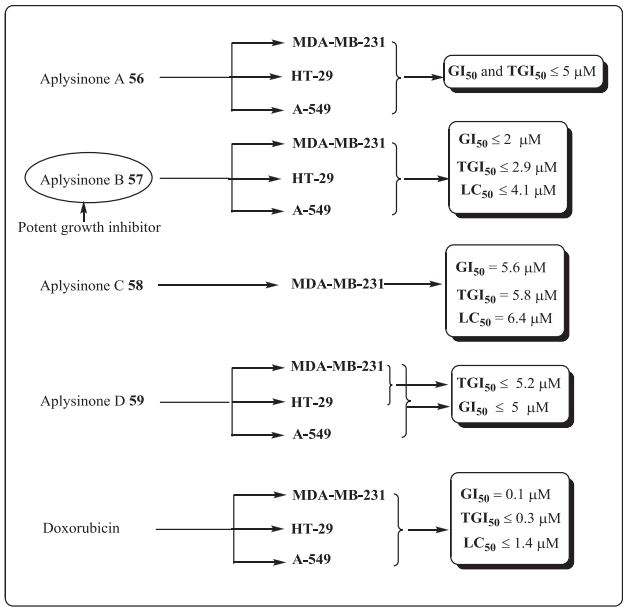


Fig. 8. Cytotoxic potential results of aplysinones A–D.

against P-388 murine leukemia cells with IC_{50} value 2.1 μg/ml [53]. The psammaplysins **63**–**66** possess S-configuration at the isoxazoline linked spirocente as well as at other chiral centre. Among all, the strong cytotoxic effect of **66** might be due to the attachment of 2,5-dioxocyclopent-3-en-1-methylidene moiety to the amide nitrogen of the amide chain.

2.6. Purealidin P, Purealidin Q and Purealidin S

From the ethyl acetate soluble part of the methanolic extract of Okinawan marine sponge *Psammaplysilla purea*, two cytotoxic isomeric bromotyrosine alkaloids purealidin P **67** [57] and purealidin Q **68** were isolated Fig. 11 [57,58]. However, **67** has also been isolated from the dichloromethane/methanol extract of sponge *Psammaplysilla purpurea* Carter (Aplysinellidae) collected from the Mandapam coast of the Gulf of Manner in South India [59]. Both compounds exhibited significant *in vitro* cytotoxicity against murine lymphoma L1210 cells with IC_{50} values of 2.8 and 0.95 μg/ml, respectively [57]. Moreover, these alkaloids **67** and **68** also displayed significant cytotoxicity against human epidermoid carcinoma KB cells with IC_{50} values, 7.6 and 1.2 μg/ml, respectively [57].

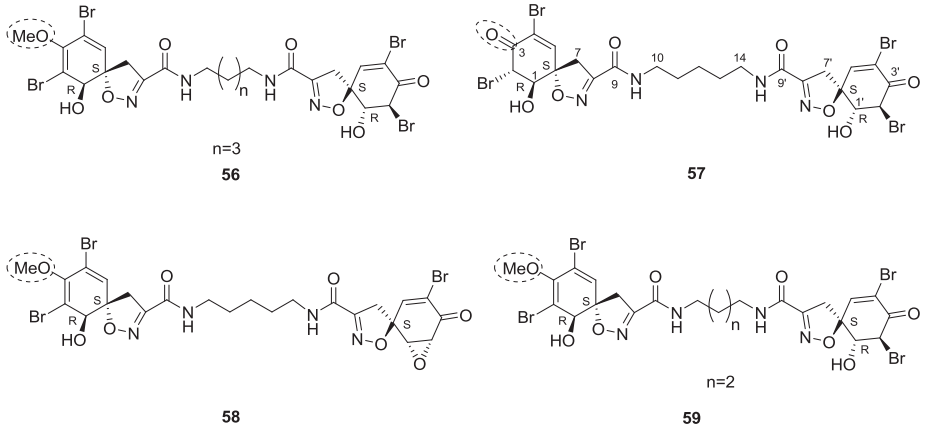


Fig. 7. Structures of aplysinones A–D.

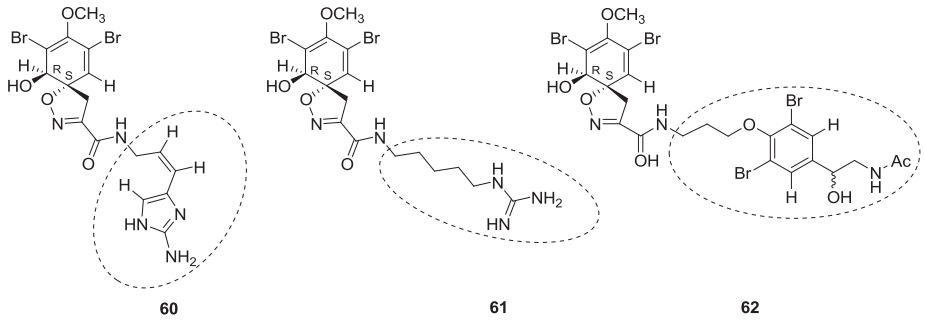


Fig. 9. Structures of aplysinamisines I–III.

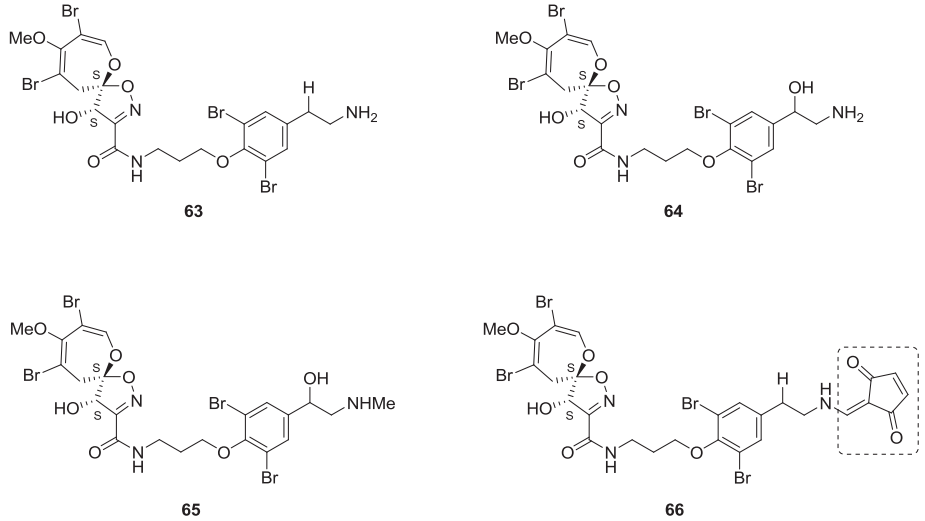


Fig. 10. Structures of psammaplysin A–C and E.

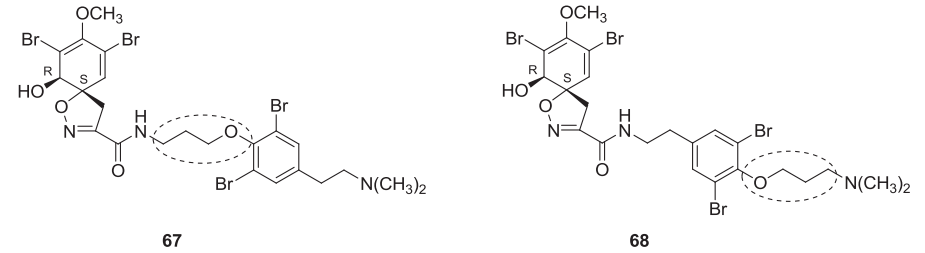


Fig. 11. Structures of purealidin P and purealidin Q.

Tabudravu et al. had isolated two bromotyrosine alkaloids purealidin Q **68** and purealidin S **69** from the methanol/dichloromethane extract of Fizian marine sponge *Druinella* sp. Fig. 12 [60]. The same authors also reported the cytotoxic effects of **69** against the ovarian tumor (A2780) and leukemia (K562) cell lines [60].

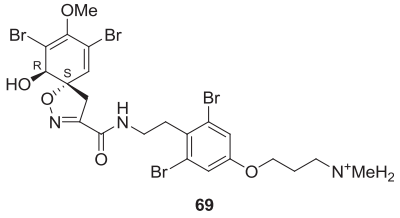


Fig. 12. Structure of purealidin S.

From the bioassay data, it was concluded that compound **69** showed moderate cytotoxicity with ID_{50} values of 7.44 and 6.02 μg/ml against cell lines, A2780 and K562, respectively [60]. Purealidins **67**–**69** have S-configuration at the spirocentre and R-configuration at the other chiral centre. Therefore, high cytotoxic potential of **68** was perhaps due to attachment of oxygen atom to the dimethyl amino group containing side chain instead of attachment of oxygen atom to side chain bearing amide functionality as in compound **67**.

2.7. Fistularin-3,11-ketofistularin,11-deoxyfistularin-3 and 11,19-dideoxyfistularin-3

Two bromotyrosine metabolites, Fistularin-3 **70** and 11-ketofistularin **71** have been isolated from ethyl acetate soluble material of methanol/toluene extract of marine sponge, *Aplysina archeri* of the *Aplysinidae* family Fig. 13 [61]. Fistularin-3 **70** was also

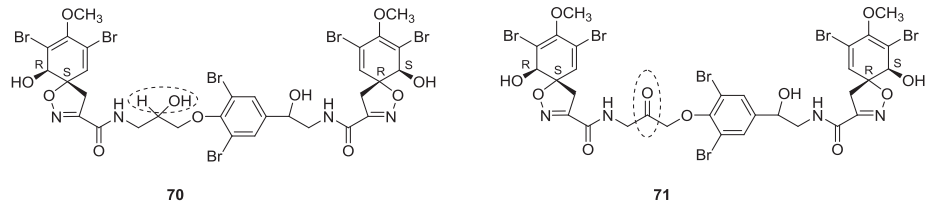
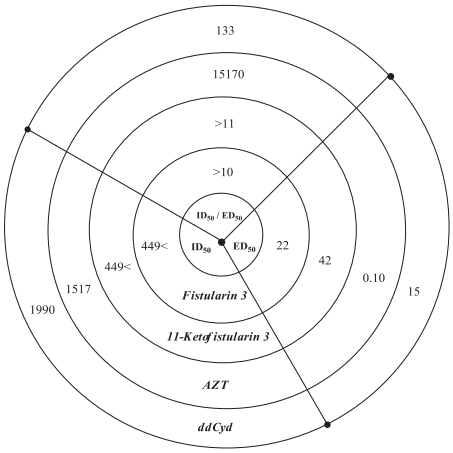


Fig. 13. Structures of fistularin 3 and 11-ketofistularin.

isolated from the methanol/chloroform extract of Caribbean Sea sponge *Aplysina lacunosa* collected off the west coast of Puerto Rico [31] and methanol/dichloromethane extract of Australian marine sponge *Pseudoceratina verrucosa* [62]. However, Gopichand et al. isolated **70** from the specimen *Aplysina fistularis* forma fulva collected from St. Thomas, Virgin Island [63]. Moreover, **70** was also isolated from acetonitrile extract of marine sponge *Aplysina cauliformis* collected from Brazil [64], dichloromethane/methanol extract of sponge *Aplysina fulva* collected from Key Largo Florida [64] and ethanol extract of sponge *Aiolochroia crassa* collected from the coast of South Water Key, Belize [65].

Feline leukemia virus (a gamma retrovirus that is a significant cause of neoplastic-related disorders affecting cats worldwide) activity of both compounds **70** and **71** were compared with two more efficient anti HIV agents, 3′-Azido-3′-deoxythymidine (AZT) and 2′,3′-Dideoxycytidine (ddCyd) and it was concluded that both compounds inhibited the growth of feline leukemia virus with ED_{50} values of 22 μM (4.8 μg/200 μl) and 42 μM (9.3 μg/200 μl), respectively [61]. Comparative results are summarized in Fig. 14.

Another bromotyrosine derivative, 11-deoxyfistularin-3 **72** was isolated along with **70** from the methanol/dichloromethane extract of Caribbean sponge *A. fistularis insularis* Fig. 15 [28]. Michael et al. have recently evaluated antiproliferative and pro-apoptotic effects of fistularin 3 **70** and 11-deoxyfistularin **72** on the two cell lines i.e. Jurkat E6.1 and U937 using the MTT method and annexin V/propidium iodide by flow cytometry [66]. From this analysis, it has been found that inhibition response was concentration and time dependent, and IC_{50} values for **70** and **72** against Jurkat and U937 cell lines were found to be 7.39 and 8.10 μM, respectively. Both compounds induced upto 35% annexin V increase in the U937 cell line after incubation for 24 h and 48 h and necrosis was not reported in any of the cases [66]. Compound **70** also induced a decrease in the number of cells in the S phase and increase in the G0/G1 phase in both the cell lines, while there was an increase in the number of cells in the G2/M phase in the Jurkat cell line [66]. Authors also reported that fistularin-3 **70** was found to be more active than 11-deoxyfistularin **72** in repressing the cell cycle and inducing apoptosis [66]. Moreover, both the compounds hold potential to be used in the development of new drugs to treat hematologic malignancies [66]. Cytotoxic effect of compound **72** was also tested against six cell lines i.e. X-17, HeLa, Hep-2, RD, Lovo and MCF-7 using MTT method [28]. Compound **72** was found to be highly cytotoxic against MCF-7 (human breast carcinoma) with LD_{50} value 17 μg/ml in comparison to rest of the cell lines in which LD_{50} value exceeded 50 μg/ml [28].



ED_{50} (μM) = Effective dose required to reduce viral proliferation by 50%.
ID_{50} (μM) = Inhibitory dose required to reduce cell viability by 50%
ID_{50} / ED_{50} = Therapeutic index

Fig. 14. Comparative feline leukemia virus activity study of fistularin-3 and 11-ketofistularin with AZT and ddCyd.

From the dichloromethane and methanol extract of verongid sponge *Pseudoceratina durissima* collected from the Great Barrier Reef, a novel bromotyrosine derived metabolite 11,19-dideoxyfistularin-3 **73** was isolated Fig. 15 [27,29]. This alkaloid was also isolated from the methanol/chloroform extract of sponge *Aplysina lacunosa* Larmarck (family Aplysinellidae) [31] and methanol extract of Red Sea sponge *Suberea mollis* [22]. The results of the cytotoxic assays disclosed that compound **73** displayed moderate cytotoxicity against the HeLa cell line with EC_{50} value of 2.6 μM [29]. Compounds **70**–**73** possess S-configuration at one spirocentre and R-configuration at other spirocentre but **70** was found to be more active than **71** against feline leukemia virus (even more effective than **72**) in inducing apoptosis and repressing cell cycle. The high potency of **70** might be due to the presence of hydroxy group in side chain linked to amide nitrogen in comparison to the presence of carbonyl and methylene group as in **71** and **72**, respectively.

2.8. Ianthesine E and other bromotyrosine alkaloids

Kalaitzis et al. had isolated ianthesine E **74** from the methanol extract of Great Barrier Reef marine sponge *Pseudoceratina* sp. Fig. 16 [29]. The natural product **74** bear S-configuration at spirocentre was found to display cytotoxicity against the HeLa cell line with EC_{50} value 60 μM. In addition, **74** was also tested for its inhibitory action to [^{3}H] DPCPX binding to adenosine A_1 receptors in a whole cell binding assay with inhibited 61% inhibition at 100 μM [29].

Other bromotyrosine derivative **75** was isolated from the methanol/toluene extract of Caribbean sponge *Aplysina cauliformis* Fig. 16 [67]. The cytotoxic effect of **75** was tested against HeLa cell lines which inhibited cell proliferation with IC_{50} value of 50 μg/ml [67]. The compound **75** having S-configuration at isoxazoline linked spirocentre displayed a low potential in comparison to (+)-**5** which might be due the presence of ethyl carbamate group in (+)-**5** which makes it more active even at nanomolar dose level than methyl carbamate group in **75**. Moreover, **75** also inhibited the mammalian protein synthesis in a cell free system [67].

3. Conclusion

The applications of isoxazoline containing natural products in the field of anticancer research have been greatly envisaged

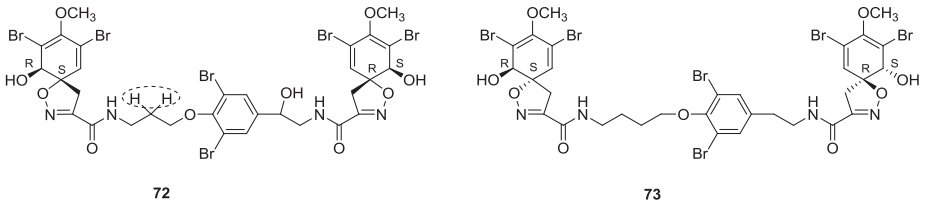


Fig. 15. Structures of 11-deoxyfistularin and 11,19-dideoxyfistularin-3.

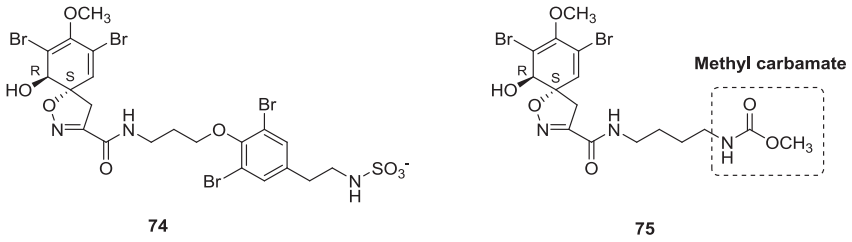


Fig. 16. Structures of ianthesine E and other bromotyrosine alkaloid.

through this review as it further elucidates the effect of structural features and absolute configurations essential for the anticancer potential of such compounds. Most of the isoxazoline containing natural products possessing S-configuration at isoxazoline linked spirocentre were found to be cytotoxic against different cancer cell lines particularly MCF-7 and HCT-116 cell lines, further strengthening the remarkable potential of these natural isoxazolines. On the basis of the existing synthetic approaches and anticancer properties of such heterocycles, this field of research is quite open to synthetic chemists and biologists to design some novel entities with improved performance through chemical transformations and development of new isoxazoline containing moieties having broad spectrum therapeutic implications.

Conflictions

None.

Acknowledgments

We thank the Department of Science and Technology (DST), New Delhi, for awarding INSPIRE-DST fellowship to Ms. Kamalneet Kaur.

References

[1] L. Shi, R. Hu, Y. Wei, Y. Liang, Z. Yang, S. Ke, Anthranilic acid-based diamides derivatives incorporating aryl-isoxazoline pharmacophore as potential anticancer agents: design, synthesis and biological evaluation, European Journal of Medicinal Chemistry 54 (2012) 549–556.
[2] P. Govind, Malnutrition leading to cancer by some environmental hazards, IJRAP 1 (2010) 287–291.
[3] J. Kim, E.J. Park, Cytotoxic anticancer candidates from natural resources, Current Medicinal Chemistry – Anti-Cancer Agents 2 (2002) 485–537.
[4] S.H. Md, S. Fareed, S. Ansari, M.S. Khan, Marine natural products: a lead for anticancer, Indian Journal of Geomarine Sciences 41 (2012) 27–39.
[5] V.J. Ram, S. Kumari, Natural products of plant origin as anticancer agents, Drug News and Perspectives 14 (2001) 465–482.
[6] K. Petit, J.-F. Biard, Marine natural products and related compounds as anticancer agents: an overview of their clinical status, Anti-Cancer Agents in Medicinal Chemistry 13 (2013) 603–631.
[7] C. Martin-Cordero, A.J. Leon-Gonzalez, J.M. Calderon-Montano, E. Burgos-Moron, M. Lopez-Lazaro, Pro-oxidant natural products as anticancer agents, Current Drug Targets 13 (2012) 1006–1028.
[8] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs over the last 25 years, Journal of Natural Products 70 (2007) 461–477.
[9] K.-H. Altmann, J. Gertsch, Anticancer drugs from nature-natural products as a unique source of new microtubule-stabilizing agents, Natural Product Reports 24 (2007) 327–357.
[10] A.D. Kinghorn, E.J.C. de Blanco, H.-B. Chai, J. Orjala, N.R. Farnsworth, D.D. Soejarto, N.H. Oberlies, M.C. Wani, D.J. Kroll, C.J. Pearce, S.M. Swanson, R.A. Kramer, W.C. Rose, C.R. Fairchild, G.D. Vite, S. Emanuel, D. Jarjoura, F.O. Cope, Discovery of anticancer agents of diverse natural origin, Pure and Applied Chemistry 81 (2009) 1051–1063.
[11] A. Bhanot, R. Sharma, M.N. Noolvi, Natural sources as potential anti-cancer agents: a review, International Journal of Phytomedicine 3 (2011) 9–26.
[12] A.M. Vijesh, A.M. Isloor, P. Shetty, S. Sundershan, H.K. Fun, New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents, European Journal of Medicinal Chemistry 62 (2013) 410–415.
[13] M.I.L. Soares, A.F. Brito, M. Laranjo, J.A. Paixao, M.A. Botelho, T.M.V.D.P. e Melo, Chiral 6,7-*bis*(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles with anti-breast cancer properties, European Journal of Medicinal Chemistry 60 (2013) 254–262.
[14] X.-Q. Wang, L.-X. Liu, Y. Li, C.-J. Sun, W. Chen, L. Li, H.-B. Zhang, X.-D. Yang, Design, synthesis and biological evaluation of novel hybrid compounds of imidazole scaffold-bases 2-benzylbenzofuran as potent anticancer agents, European Journal of Medicinal Chemistry 62 (2013) 111–121.
[15] V. Zaharia, A. Ignat, N. Palibroda, B. Ngameni, V. Kuete, C.N. Fokunang, M.L. Moungang, B.T. Ngadjui, Synthesis of some *p*-toluenesulfonyl-hydrazinothiazoles and hydrazino-*bis*-thiazoles and their anticancer activity, European Journal of Medicinal Chemistry 45 (2010) 5080–5085.
[16] R. Ramachandran, M. Rani, S. Senthan, Y.T. Jeong, S. Kabilan, Synthesis, spectral, crystal structure and in vitro antimicrobial evaluation of imidazole/benzotriazole substituted piperdin-4-one derivatives, European Journal of Medicinal Chemistry 46 (2011) 1926–1934.
[17] Y.-C. Duan, Y.-C. Ma, E. Zhang, X.-J. Shi, M.-M. Wang, X.-W. Ye, H.-M. Liu, Design and synthesis of novel 1,2,3-triazole-dithiocarbamate hybrids as potential anticancer agents, European Journal of Medicinal Chemistry 62 (2013) 11–19.
[18] V. Kumar, K. Kaur, G.K. Gupta, A.K. Sharma, Pyrazole containing natural products: synthetic preview and biological significance, European Journal of Medicinal Chemistry 69 (2013) 735–753.
[19] A. Kamal, E.V. Bharathi, J.S. Reddy, M.J. Ramaiah, D. Dastagiri, M.K. Reddy, A. Viswanath, T.L. Reddy, T.B. Shaik, S.N.C.V.L. Pushpavalli, M.P. Bhadra, Synthesis and biological evaluation of 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids as anticancer agents, European Journal of Medicinal Chemistry 46 (2011) 691–703.
[20] A. Kamal, J.S. Reddy, M.J. Ramaiah, D. Dastagiri, E.V. Bharathi, M.A. Azhar, F. Sultana, S.N.C.V.L. Pushpavalli, M. Pal-Bhadra, A. Juvekar, S. Sen, S. Zingde, Design, synthesis and biological evaluation of 3,5-diaryl-isoxazoline/isoxazole-pyrrolobenzodiazepine conjugates as potential anticancer agents, European Journal of Medicinal Chemistry 45 (2010) 3924–3937.
[21] M.P. Sadashiva, Basappa, S.N. Swamy, F. Li, K.A. Manu, M. Sengottuvelan, D.S. Prasanna, N.C.A. Kumar, G. Sethi, K. Sugahara, K.S. Rangappa, Anti-cancer activity of novel dibenzo[*b*,*f*]azepine tethered isoxazoline derivatives, BMC Chemical Biology 12 (2012) 1–11.
[22] M.I. Abou-Shoer, L.A. Shalla, D.T.A. Youssef, J.M. Badr, A.-A.M. Habib, Bioactive brominated metabolites from the red sea sponge *Suberea mollis*, Journal of Natural Products 71 (2008) 1464–1467.
[23] L.A. Shaala, D.T.A. Youssef, M. Sulaiman, F.A. Behery, A.I. Foudah, K.A. EI Sayed, Subereamolline A as a potent breast cancer migration, invasion and

proliferation inhibitor and bioactive dibrominated alkaloids from the red sea sponge *Pseudoceratina Arabica*, Marine Drugs 10 (2012) 2492–2508.
[24] J.W. Shearman, R.M. Myers, J.D. Brenton, S.V. Ley, Total syntheses of sub-ereamollines A and B, Organic and Biomolecular Chemistry 9 (2011) 62.
[25] E. Fattorusso, L. Minale, G. Sodano, Aerothionin, a tetrabromo-compound from *Aplysina aerophoba* and *Verongia thiona*, Journal of the Chemical Society D (1970) 752–753.
[26] K. Moody, R.H. Thomson, E. Fattorusso, L. Minale, G. Sadano, Aerothionin and homoaerothionin: tetrabromo spirohexadienylisoxazoles from *Verongia sponges*, Journal of the Chemical Society, Perkin Transactions 1 (1972) 18–24.
[27] M.R. Kernan, R.C. Cambie, Chemistry of sponges, VII. 11,19-dideoxyfistularin 3 and 11-hydroxyaerothionin, bromotyrosine derivatives from *Pseudoceratina durissima*, Journal of Natural Products 53 (1990) 615–622.
[28] R.S. Compagnone, R. Avila, A.I. Suarez, O.V. Abrams, H.R. Rangel, F. Arvelo, I.C. Pina, E. Merentes, 11-Deoxyfistularin-3, a new cytotoxic metabolite from the Caribbean sponge *Aplysina fistularis insularis*, Journal of Natural Products 62 (1999) 1443–1444.
[29] J.A. Kalaitzis, P.D.A. Leone, J.N.A. Hooper, R.J. Quinn, E. Ianthesine, A new bromotyrosine-derived metabolite from the Great Barrier Reef sponge *Pseudoceratina sp*, Natural Product Research 22 (2008) 1257–1263.
[30] Y. Venkateswarlu, M.R. Rao, U. Venkatesham, A new dibromotyrosine – derived metabolite from the sponge *Psammaplysilla purpurea*, Journal of Natural Products 61 (1998) 1388–1389.
[31] A.L. Acosta, A.D. Rodrigeuz, 11-oxoaerothionin: a cytotoxic antitumor bromotyrosine-derived alkaloid from the Caribbean marine sponge *Aplysina lacunosa*, Journal of Natural Products 55 (1992) 1007–1012.
[32] R.D. Encamacion, E. Sandoval, J. Malmstrom, C. Christophersen, Calafianin, a bromotyrosine derivative from the marine sponge *Aplysina gerardogreeni*, Journal of Natural Products 63 (2000) 874–875.
[33] N. Shao, G. Yao, L.C. Chang, Bioactive constituents from the marine crinoid *Himerometra magnipinna*, Journal of Natural Products 70 (2007) 869–871.
[34] H.H. Wasserman, J. Wang, Synthesis of the marine metabolite verongamine, hemibastadin-2 and aerothionin using the cyano ylide coupling methodology, Journal of Organic Chemistry 63 (1998) 5581–5586.
[35] M. Murakata, M. Tamura, O. Hoshino, Asymmetric oxidative cyclization of o-phenolic oxime esters: first synthesis of enantiomerically enriched spiroisoxazoline methyl esters, Journal of Organic Chemistry 62 (1997) 4428–4433.
[36] S. Nishiyama, S. Yamamura, Total syntheses of (±)-aerothionin and (±)-homoaerothionin, Tetrahedron Letters 24 (1983) 3351–3352.
[37] T. Ogamino, R. Obata, S. Nishiyama, Asymmetric synthesis of aerothionin, a marine dimeric spiroisoxazoline natural product, employing optically active spiroisoxazoline derivative, Tetrahedron Letters 47 (2006) 727–731.
[38] T. Ogamino, S. Nishiyama, A new ring opening access to aeroplysinin-1 a secondary metabolite of *Verongia aerophoba*, Tetrahedron 59 (2003) 9419–9423.
[39] T. Ogamino, Y. Ishikawa, S. Nishiyama, Electrochemical synthesis of spiroisoxazole derivatives and its application to natural products, Heterocycles 61 (2003) 73–78.
[40] S. Nishiyama, S. Yamamura, Total syntheses of (±)-aerothionin, (±)-homoaerothionin and (±)-aerophobin-1, Bulletin of the Chemical Society of Japan 58 (1985) 3453–3456.
[41] T.R. Boehlow, J. Harburn, C.D. Spilling, Approaches to the synthesis of some tyrosine-derived marine sponge metabolites: synthesis of verongamine and purealdin N, Journal of Organic Chemistry 66 (2001) 3111–3118.
[42] J.J. Harburn, N.P. Rath, C.D. Spilling, Efficient synthesis of tyrosine-derived marine sponge metabolites via acylation of amines with a coumarin, Journal of Organic Chemistry 70 (2005) 6398–6403.
[43] A.R. Forrester, R.H. Thomson, S.-O. Woo, Intramolecular cyclisation of phenolic oxime, III. A synthesis of *cis-cis* aerothionin, Justus Leibigs Annalen der Chemie (1978) 66–73.
[44] T. Ogamino, S. Nishiyama, Synthesis and structural revision of calafianin, a member of the spiroisoxazole family isolated from the marine sponge, *Aplysina gerardogreeni*, Tetrahedron Letters 46 (2005) 1083–1086.
[45] T. Ogamino, R. Obata, H. Tamoda, S. Nishiyama, Total synthesis, structural revision and biological evaluation of calafianin, a marine spiroisoxazoline from the sponge, *Aplysina gerardogreeni*, Bulletin of the Chemical Society of Japan 79 (2006) 134–139.
[46] A.D. Rodriguez, I.C. Pina, The structures of aplysinamisines I, II, III: new bromotyrosine-derived alkaloids from the Caribbean sponge *Aplysina cauliformis*, Journal of Natural Products 56 (1993) 907–914.
[47] C.J. Hernandez-Guerrero, E. Zubia, M.J. Ortega, J.L. Carballo, Cytotoxic dibromotyrosine-derived metabolites from the sponge *Aplysina gerardgreeni*, Bioorganic and Medicinal Chemistry 15 (2007) 5275–5282.
[48] M.S. Buchanan, A.R. Carroll, D. Wessling, M. Jobling, V.M. Avery, R.A. Davis, Y. Feng, J.N.A. Hooper, R.J. Quinn, Clavatadines C-E, guanidine alkaloids from the Australian Sponge *Suberea clavata*, Journal of Natural Products 72 (2009) 973–975.
[49] B.R. Copp, C.M. Ireland, Psammplysin C: a new cytotoxic dibromotyrosine-derived metabolite from the marine sponge Druinella (=*Psammaplysilla*) *Purpurea*, Journal of Natural Products 55 (1992) 822–823.
[50] A.D. Wright, P.J. Schupp, J.-P. Schror, A. Engemann, S. Rohde, D. Kelman, N. de Voogd, A. Carroll, C.A. Motti, Twilight zone sponges from guam yield theonellin isocyanate and psammaplysins I and J, Journal of Natural Products 75 (2012) 502–506.
[51] D.M. Roll, W.J. Chang, P.J. Scheuer, G.A. Gray, J.N. Shoolery, G.K. Matsumoto, G.D.V. Duyne, J. Clardy, Structures of the Psammaplysins, Journal of the American Chemical Society 107 (1985) 2916–2920.
[52] J.M. Badr, L.A. Shaala, M.I. Abou-Shoer, M.K. Tawfik, A.-A.M. Habib, Bioactive brominated metabolites from the red sea sponge *Pseudoceratina Arabica*, J. Nat. Prod 71 (2008) 1472–1474.
[53] S. Tsukamoto, H. Kato, H. Hirota, N. Fusetani, Ceratinamides A and B: new antifouling dibromotyrosine derivatives from the marine sponge *Pseudoceratina purpurea*, Tetrahedron 52 (1996) 8181–8186.
[54] S. Liu, X. Fu, F.J. Schmitz, M. Kelly-Borges, Psammaplysin F, a new bromotyrosine derivative from a sponge, *Aplysinella* sp. Journal of Natural Products 60 (1997) 614–615.
[55] I.W. Mudianta, T. Skinner-Adams, K.T. Andrews, R.A. Davis, T.A. Hadi, P.Y. Hayes, M.J. Garson, Psammaplysin derivatives from the balinese marine sponge *Aplysinella strongylata*, Journal of Natural Products 75 (2012) 2132–2143.
[56] T. Ichiba, P.J. Scheuer, Three bromotyrosine derivatives, one terminating in an unprecedented diketocyclopentenylidene enamine, Journal of Organic Chemistry 58 (1993) 4149–4150.
[57] J. Kobayashi, K. Honma, T. Sasaki, M. Tsuda, Purealidin J-R new bromotyrosine alkaloids from the okinawan marine Sponge *Psammaplysilla purea*, Chemical and Pharmaceutical Bulletin 43 (1995) 403–407.
[58] S. Tilvi, C. Rodrigues, C.G. Naik, P.S. Parameswaran, S. Wahidhulla, New bromotyrosine from the marine sponge *Psammaplysilla purpurea*, Tetrahedron 60 (2004) 10207–10215.
[59] Y. Venkateswarlu, U. Venkatesham, M.R. Rao, Novel bromine-containing constituents of the sponge *Psammaplysilla purpurea*, Journal of Natural Products 62 (1999) 893–894.
[60] J.N. Tabudravu, M. Jaspars, Purealidin S and purpuramine J bromotyrosine alkaloids from the fijian marine sponge *Druinella* sp. Journal of Natural Products 65 (2002) 1798–1801.
[61] S.P. Gunasekera, S.S. Cross, Fistularin 3 and 11-keto fistularin 3 feline leukemia virus active bromotyrosine metabolites from the marine sponge *Aplysina archeri*, Journal of Natural Products 55 (1992) 509–512.
[62] T.D. Tran, N.B. Pham, G. Fechner, J.N.A. Hooper, R.D. Quinn, Bromotyrosine alkaloids from the Australian marine sponge *Pseudoceratina verrucosa*, Journal of Natural Products 76 (2013) 516–523.
[63] Y. Gopichand, F.J. Schmitz, Marine natural products: fistularin-1, -2 and -3 from the sponge *Aplysina fistularis forma fulva*, Tetrahedron 20 (1979) 3921–3924.
[64] E.W. Rogers, M.F. de Oliveira, R.G.S. Berlinck, G.M. Konig, T.F. Molinski, Stereochemical heterogeneity in verongid sponge metabolites. Absolute stereochemistry of (+)-Fistularin-3 and (+)-11-epi Fistularin-3 by microscale LCMS-Marfey's analysis, Journal of Natural Products 68 (2005) 891–896.
[65] H. Gao, M. Kelly, M.T. Hamann, Bromotyrosine metabolites from the sponge *Aiolochroia crassa*, Tetrahedron 55 (1999) 9717–9726.
[66] M.R. Mizares, M. Ochoa, A. Barroeta, G.P. Martinez, A.I. Suarez, R.S. Compagnone, P. Chirinos, R. Avila, J.B. De Sanctis, Cytotoxic effects of fisturalin-3 and 11-deoxyfisturalin-3 on Jurkat and U 937 cell lines, Biomedical Papers of the Medical Faculty of the University Palacký, Olomouc, Czechoslovakia 156 (2012) 1–5.
[67] P. Ciminiello, C.D. Aversano, E. Fattorusso, S. Magno, M. Pansini, Chemistry of *Verongida sponges*. 9. Secondary metabolite composition of the Carribean sponge *Aplysina cauliformis*, Journal of Natural Products 62 (1999) 590–593.