Chemistry and Biology of Fascaplysin, a Potent Marine-Derived CDK-4 Inhibitor

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Abstract: Marine natural products offer an abundant source of pharmacologically active agents with great diversity and complexity, and the potential to produce valuable therapeutic entities. Indole alkaloids is one of the important class of marine-derived secondary metabolites, with wide occurrence amongst variety of marine sources such as sponges, tunicates, algae, worms and microorganisms and have been extensively studied for their biological activities. Among this chemical family, a sponge-derived bis-indole alkaloid fascaplysin (**1**) exhibited broad range of bioactivities including antibacterial, antifungal, antiviral, anti-HIV-1-RTase, p56 tyrosine kinase inhibition, antimalarial, anti-angiogenic, antiproliferative activity against numerous cancer cell lines, specific inhibition of cyclin-dependent kinase-4 (IC_{50} 350 nM) and action as a DNA intercalator. In the present review, the chemical diversity of natural as well as synthetic analogues of fascaplysin has been reviewed with a detailed account on synthetic reports and pharmacological studies. Our analysis of the structure-activity relationships of this family of compounds highlights the existence of various potential leads for the development of novel anticancer agents.

Keywords: Fascaplysin, anticancer, cyclin-dependent kinase, CDK-4, marine natural products, anti-angiogenic.

1. INTRODUCTION

Oceans are unique resources that provide a diverse array of natural products, primarily from invertebrates such as sponges, tunicates, bryozoans, and molluscs, and from marine bacteria and cyanobacteria [1]. These marine sources produce an unprecedented molecular diversity by the incorporation of elements like bromine, chlorine or iodine that are not readily available in metabolites of terrestrial species. Marine organisms produce structurally distinct secondary metabolites, possibly due to factors unique to marine environments such as high salinity and pressure and constant temperature. A variety of marine sources including sponges, tunicates, red alga, acorn worms, and symbiotic bacteria have been shown to generate indole alkaloids, which represent the largest number and most complicated of the marine alkaloids ($1/4^{th}$ of total alkaloids). Due to structural complexity of marine metabolites, their structure elucidation, chemical modification, stereochemistry, synthesis, and pharmacology have received a great deal of interdisciplinary attention from areas of research other than chemistry and include pharmacology, physiology, and medicine [2].

Hundreds of these bis-indole molecules have been isolated from natural sources, and many of these molecules have potent medicinal properties. Biosynthetically bis-indoles have been produced from the two molecules of the amino acid L-tryptophan [3]. Fascaplysin (**1**), a bis-indole alkaloid isolated from a marine sponge *Fascaplysinopsis* Bergquist sp. showed selective inhibition of cyclin-dependent kinase-4 [4, 5] and also blocked the growth of cancer cells [4, 6-9]. Few reviews have been published on fascaplysin or bis-indole alkaloids. Mahale and Chaudhuri (2007) [10] in their book discussed about developments of fascaplysin-based CDK inhibitors as anticancer agents. He *et al.* (2008) [11] published a short review on synthetic aspects of fascaplysin comprising 18 references and was written in a Chinese language. Gul and Hamann (2005) [2] reviewed indole alkaloids derived from marine sources possessing different therapeutic activities. Several natural as well as synthetic analogs of fascaplysins are known in the literature and several reports exist on their pharmacological studies; however no any comprehensive review is available on the topic. Thus, in this paper, we have reviewed the natural product chemistry, synthetic chemistry and pharmacology of fascaplysin (**1**) and its natural and synthetic analogs. The review has been divided into five sections: Fascaplysin and its natural analogs; structural basis for CDK-4 selectivity of fascaplysin; total syntheses of fascaplysins; fascaplysin related other marine natural products; and fascaplysin-inspired synthetic analogs as CDK-4 inhibitors.

2. FASCAPLYSIN (1) AND ITS NATURAL ANALOGS

Fascaplysin (**1**) was first isolated in 1988 by Roll *et al* [12] from marine sponge *Fascaplysinopsis* Bergquist sp. collected in the South Pacific near the Fiji island as unusual antimicrobial pigment. It showed inhibition of the growth of several microbes, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Saccharomyces cerevisiae*. It showed suppression in the proliferation of mouse leukemia cells L-1210 with ED_{50} = 0.2 μM/mL [12] and also exhibited selectivity in murine tumor cytotoxicity assay [13]. Fascaplysin exhibited anti-proliferation effect towards human cervical cancer HeLa cells through induction of apoptosis *via* extrinsic death pathway and mitochondrial

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pathway, but not arresting cell cycle progression at G1 phase [14].

Angiogenesis is a complex process that is mediated by the endothelial cells that line blood vessels, and regulated by a number of stimulators such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and endostatin [15]. Due to extensive chemical and biological diversity of the marine environment, it is proved to a prolific resource for discovery of novel angiogenesis inhibitors. Amongst secreted growth factors, vascular endothelial growth factor (VEGF) is mainly responsible for formation of new capillaries, thus therapeutic intervention of VEGF has proved beneficial in suppression of tumor growth. Fascaplysin (**1**) exhibited anti-angiogenesis activity *via* VEGF blockage and through direct effects of cell cycle arrest and apoptosis on human umbilical vein endothelial cells (HUVEC) [16, 17]. No antitumor effect was observed *in vivo* with doses of 5-20 mg/kg in a model of Ehrlich ascitic carcinoma in mice due to observed suppressive action on immunocompetent cells [18]. Recently Yan *et al* (2011) showed that fascaplysin (**1**) inhibits the growth of S180 cell implanted tumor possibly via apoptosis, anti-angiogenesis, or cell cycle arrest mechanism [19].

Fascaplysin showed promising specific CDK-4 inhibitory activity with IC_{50} of 0.35 μM and it also blocked the growth of cancer cells at the G0/G1 phase of the cell cycle [4-8]. However, poor activity was observed against other CDKs with IC_{50} of > 100 μM for CDK1/cyclin B, IC_{50} >50 μM for CDK-2/cyclin A and CDK-2/cyclin E, IC_{50} 20 μM for CDK-5/p35 [4]. Fascaplysin showed ten-fold more specificity towards CDK4/D1 than CDK6/D1. Interestingly, CDK4/D2 and CDK4/D3 were not inhibited at >100 μM concentrations while CDK6/D2 was inhibited with an IC_{50} of 35 μM. This may reflect the way different cyclins bind to their CDK partners and in doing so subtly alter the three dimensional geometry of the active site. Fascaplysin have not shown inhibition of other prototype tyrosine kinases (IC_{50} for IGF-1R and v-abl is >10 μM) [4]. Fasacaplysin also showed DNA intercalating activity [20]. The binding mode and affinity constants of fascaplysin were found to be comparable to those of other typical DNA intercalators. Computational studies shown that the predicted binding mode of fascaplysin (**1**) in the ATP binding site of a CDK4 homology model is a double hydrogen bonding to Val 96 [21, 22].

Jimenez *et al.* (1991) [23] isolated fascaplysin pigment, with the counterion dehydroluffariellolide diacid monoanion (Fig. **1**), from the Fijian sponge *F. reticulata,* along with other novel β-carbolines homofascaplysin A (**5**) (accompanied by counterion), homofascaplysin B (**9**), homofascaplysin C (**13**), and secofascaplysin A [23]. Fascaplysin analogs have been classified into three types based on their structural features *viz.* type Ia, Ib and Ic as depicted in Fig. (**1**). Type Ia consists of fascaplysin (**1**) and its bromo substituted analogs, 3-bromofascaplysin (**2**), 10-bromofascaplysin **(3)** and 3,10-dibromofascaplysin (**4**). Type Ib analogs includes alcohol analogs of fascaplysin *viz*. homofascaplysin A (**5**), homofascaplysate A (**6**), 3-bromohomofascaplysin A (**7**) and a recently isolated aromatic substituted analog thorectandramine (**8**). Dicarbonyl ester or formyl substituted analogs are part of type Ic compounds. These include homofascaplysin B (**9**), homofascaplysin B-1 (**10**), 3-bromohomofascaplysin B (**11**), 3-bromohomofascaplysin B-1 (**12**), homofascaplysin C (**13**) and 3-bromohomofascaplysin C (**14**).

Segraves *et al* [6] isolated fascaplysin (**1**) along with several of its natural analogs **2-6, 9-12** from sponge collections (*Fascaplysinopsis reticulata*) and two tunicate collections (*Didemnum* sp.). Cytotoxicities of compounds **1-3** were assessed against murine and human tumor cell lines using the disk diffusion soft agar assay. Fascaplysin (**1**), the most potent compound tested, exhibited murine tumor

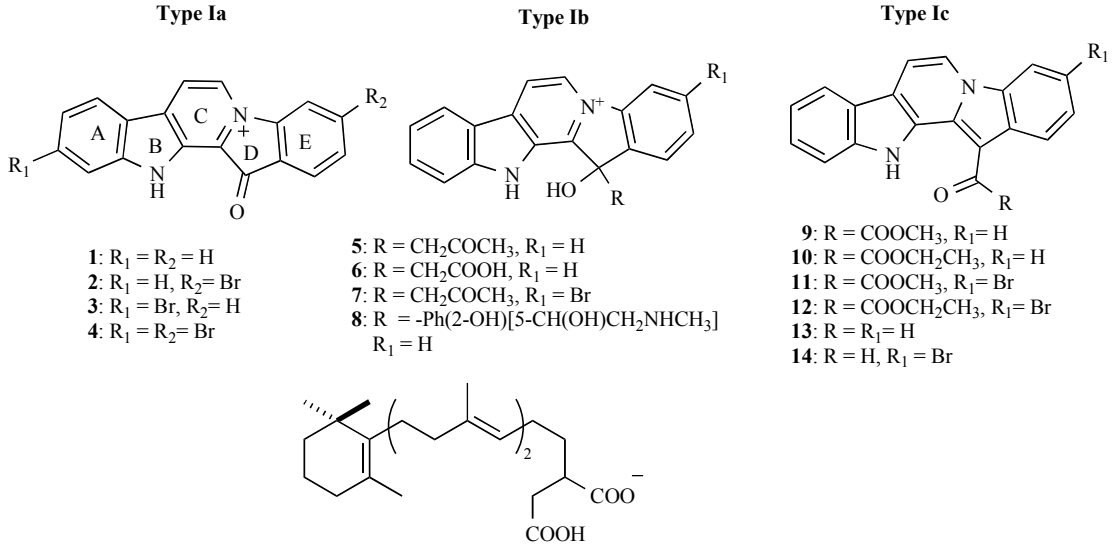


Fig. (1). Chemical structures of fascaplysin (**1**), its natural analogs **2-14** and dehydroluffarielloide diacid monoanion.

selectivity. However, 10-bromofascaplysin (**3**) showed the most interesting activity and had potency similar to that of **1** but with human solid tumor selectivity, which **1** lacks. Fascaplysin (**1**) and 3-bromofascaplysin (**2**) were also screened against 60 NCI cell lines. The overall level of activity reveals that **1** was active, but the presence of the sesterterpene anion causes activity in some cell lines where **1** is inactive. Interestingly, the presence of bromine in **2** produces an overall increase in activity as compared to **1** but also causes inactivity in cell lines that are sensitive to **1**. Fascaplysin showed promising anticancer activity against colon cancer cell line HCT-116 and melanoma cancer cell lines MALME-3M and M-14 with IC_{50} values of 600, 360 and 920 nM respectively; however IC_{50} values of 1-4 μM were observed against other cell lines tested *viz*. COLO-205 (colon), HCC-2998 (colon), SF-295 (CNS), U251 (CNS), OVCAR-3 (ovarian), RXF-393 (renal), SK-MEL-5 (melanoma). No activity was observed against breast cancer cell lines HS-578T and BT-549 [6].

Recently Lu *et al* [24] reported isolation of 3-bromohomofascaplysin A (**7**), a fascaplysin analogue from a Fijian ascidian *Didemnum sp*. Homofascaplysin A (**5**) isolated from the sponge *Hyrtios erecta* and from *Fascaplysinopsis reticulata* showed inhibition of p56lck tyrosine kinase [25, 26]. A hexacyclic quaternary β-carbolinium derivative, thorectandramine (**8**), isolated by antiproliferative activity guided fractionation of the organic extract of the sponge *Thorectandra sp*. showed cytotoxicity in MCF-7, OVCAR-3 and A549 cell lines with EC_{50} 27.0–55.0 μg/mL [27]. Bromo derivatives of fascaplysin, 3- and 10-bromofascaplysins (**2-3**) were effective at sub-micromolar concentrations as anticancer agents, which exerted their action, at least in part, through the induction of caspase-8, -9, -3-dependent apoptosis [28]. Chemical structures and biological source for natural fascaplysins are mentioned in Fig. (**1**) and Table **1** respectively.

Studies on the reactivity of fascaplysin (**1**) to various nucleophilic and electrophilic reactions has also been reported [29, 30]. Reversible deprotonation of fascaplysin was achieved with non-nucleophilic bases. Under basic aqueous conditions, opening of ring D of fascaplysin occurred, yielding zwitter-ionic reticulatine, whereas, in a methoxide containing methanol solution, an unexpected addition of three molecules of methanol to the pyridinium ring produced an isomer mixture of a trimethoxy-substituted compound. Transformation of the keto group of fascaplysin to the oxime took place in the presence of pyridine as base. Grignard and alkyllithium reagents added as expected to the keto group of fascaplysin, providing tertiary alcohols. Electrophilic aromatic substitutions, such as halogenation and sulfonation, occur primarily para to the indole N-atom at C (9) of the unique 12H-pyrido[1,2-a:3,4-b']-diindole ring system of fascaplysin.

3. STRUCTURAL BASIS FOR CDK-4 SELECTIVITY OF FASCAPLYSIN (1)

Docking studies of fascaplysin (**1**) in the ATP binding pocket of CDK2 and CDK4 identified the structural basis for CDK4 selectivity. The structural basis for selectivity of **1** for CDK-4 was not immediately apparent from examination of its docked poses in CDK2 and CDK4. Fascaplysin (**1**), which contains a quaternary nitrogen at the intersection of two of its aromatic rings, binds in a similar conformation in both CDK2 and CDK4 with the lactam NH and carbonyl

Table 1. List of Fascaplysin and its Natural Analogs with their Biological Source

Entry	Name	Source	Ref.
1	Fascaplysin	*Fascaplysinopsis reticulata, Hyrtios erecta, Thorectandra* sp., *Didemnum* sp.	[5, 6, 12, 25, 26, 60-62]
2	3-Bromofascaplysin	*Didemnum* sp.	[6]
3	10-Bromofascaplysin	*Fascaplysinopsis reticulata*	[6]
4	3,10-Dibromofascaplysin	*Fascaplysinopsis reticulata*	[6]
5	Homofascaplysin A	*Fascaplysinopsis reticulata, Hyrtios erecta*	[25, 26]
6	Homofascaplysate A	*Fascaplysinopsis reticulata, Didemnum* sp.	[6]
7	3-Bromohomofascaplysin A	*Didemnum sp.*	[24]
8	Thorectandramine	*Thorectandra* sp.	[27]
9	Homofascaplysin B	*Fascaplysinopsis reticulata*	[25]
10	Homofascaplysin B-1	*Didemnum* sp.	[6]
11	3-Bromo-homofascaplysin B	*Didemnum* sp.	[6]
12	3-Bromo-homofascaplysin B-1	*Didemnum* sp.	[6]
13	Homofascaplysin C	*Fascaplysinopsis reticulata*	[6, 25]
14	3-Bromohomofascaplysin C	*Didemnum* sp.	[6]

groups forming a donor-acceptor hydrogen bond pair to the hinge region (L83CDK2 [V96CDK4]) as shown in Fig. (**2**). Such an interaction involving the backbone connecting the N- and C-terminal kinase domain lobes is observed with all CDK2 inhibitors and many other kinase ATP antagonists. Relative to the CDK4 binding mode, the CDK2-docked structure projects slightly further into the base of the ATP cleft and hence makes more complementary van der Waals contacts with V18CDK2, F80CDK2, and L134CDK2, which is reflected in the more favorable van der Waals interaction energy. The contribution of the Coulombic term to the enthalpy of binding, on the other hand, was strikingly different between CDK2 and CDK4 (+28 versus –60 kcal/mol, respectively) and resulted in a much more favorable energy for binding to CDK4 (–86 kcal/mol more favorable). The difference in non-bonded energies correlates well with the observed potency differences (fascaplysin is at least 100-fold more active against CDK4) and examination of the catalytic sites of both enzymes explains the structural basis for the variation. In CDK4, an acidic residue (E144) replaces Q131CDK2, whereas a neutral residue (T102CDK4) is substituted for the positively charged K89CDK2. These differences lead to a two-unit increase in the formal charge of the ATP binding pocket of CDK2 relative to CDK4 and explain the more favorable electrostatic energy with the later kinase. The quaternary nitrogen of fascaplysin (**1**) is in close proximity to E144CDK4, resulting in the increased contribution to the Coulombic term. Additionally, the proximity of the ligand quaternary N to K89CDK2 leads to less favorable binding to CDK2. Interestingly, the steric constraint of the larger K89CDK2 side chain (salt bridged to D86CDK2) results in contacts with the lipophilic rings of fascaplysin (**1**) and also in better complementarity with the lipophilic part of the cleft relative to CDK4 (as shown by the more favorable van der Waals interaction energy); however, this in turn results in weaker H bonding with the CDK2 hinge region (as shown by Ludi H bonding scores of 62 for CDK2 versus 78 for CDK4). The combination of less-optimal H bonds and unfavorable electrostatic interactions thus offsets the increase in van der Waals energy and results in the two orders of magnitude decrease in potency of fascaplysin against CDK2 compared to CDK4 [22].

4. TOTAL SYNTHESES OF FASCAPLYSINS

Fascaplysin (**1**) and its natural analogs were synthesized by several researchers and more than ten syntheses have been reported so far. Amongst 14 fascaplysins, the total synthesis of only 6 of them *viz*. **1-4**, **9** and **13** have been reported. Gribble's group (1990, 1992)[31, 32] synthesized fascaplysin (**1**) for the first time in seven steps starting from indole (**15**) in overall yield of 55%, the key step being a trifluoroacetic acid ring closure - Pd/C dehydrogenation step (Fig. **3**). The pivotal intermediate diindole **18** was synthesized from indole (**15**) in 3 steps. Indole (**15**) and oxalyl chloride smoothly react to form 3-indolylglyoxylyl chloride (**16**), which on treatment with the sodium salt of indole gave keto amide **17** in 86% yield. Reduction of the carbonyl groups of **17** was problematic due to the well-known propensity of N-acylindoles to suffer reductive c1eavage. Treatment of **17** with $LiAlH_4$ resulted in cleavage

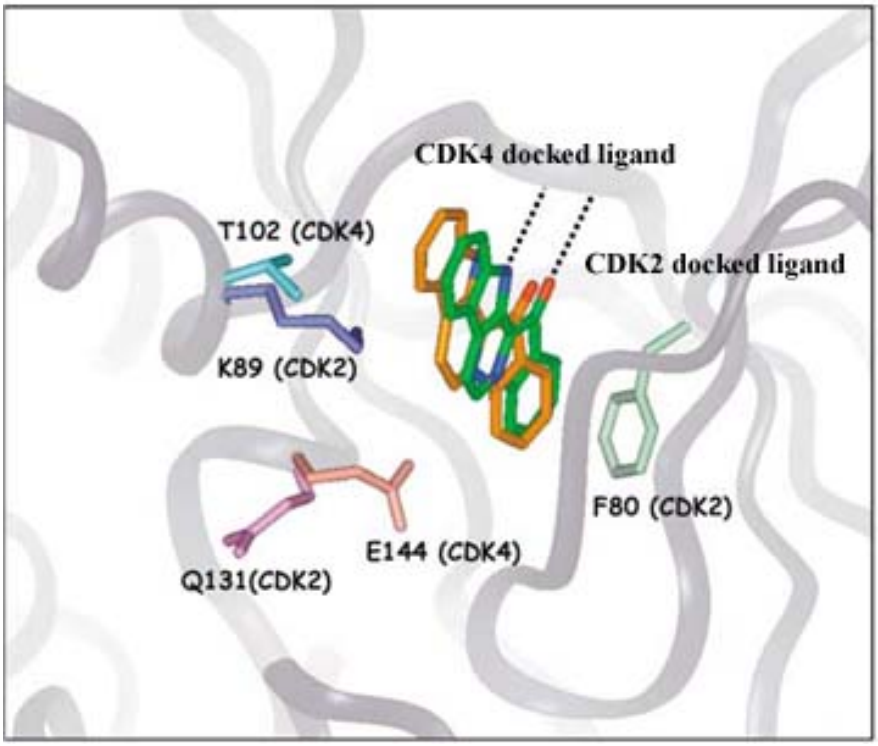


Fig. (2). Superposition of the models of fascaplysin (**1**) within the ATP binding sites of CDK2 and CDK4. The CDK2 docked ligand is towards left side whereas the CDK4 ligand is towards right. Only the backbone ribbon of CDK4 is shown along with the major residues differing in the two proteins. F80CDK2 and the hinge region H bonds are shown as reference points. The position of E144CDK4 is induced by electrostatic interaction with the ligand [22].

of the amide bond to give **15** and tryptophol. However, this cleavage reaction could be thwarted by the use of sodium (mono)trifluoroacetoxyborohydride, which afforded **18** in 60% yield (47% overall yield from indole **15**). Alternative route for synthesis of diindole **18** is also reported. Compound **18** on treatment with TFA at room temperature resulted in smooth cyclization to a 10: 1 mixture of **22** and **23** in high yield. Treatment of the **22-23** crude mixture with 10% Pd/C in refluxing 2-ethoxyethyl ether (180-190 °C) gave the fully aromatic pentacycle **24** in 93% yield from **18**. Treatment of **24** with m-chloroperbemic acid, magnesium monoperoxy-phthalate, H_2O_2, or, preferably, peracetic acid in methanol, followed by treatment with aqueous conc. HC1, afforded fascaplysin (**1**) as a red powder in 85% yield. Treatment of **24** with the Vilsmeier reagent gave homofascaplysin C (**13**) in 88% yield. Treatment of **24** with oxalyl chloride followed by methanol gave homofascaplysin B (**9**) in essentially quantitative yield from **24**.

Rocca *et al*. (1993) [33] synthesized fascaplysin (**1**) starting from phenyl boronic acid **25** and iodopyridine **26**. Palladium catalyzed cross-coupling reaction between boronic acid **25** and iodopyridine **26** using Suzuki's procedure gave the biaryl **27**. Regioselective metalation of **27** with n-butyllithium in THF at low temperature and reaction of the resulting lithio derivative with 2-fluorobenzaldehyde afforded the corresponding trisubstituted pyridine **28** in 95% yield. Oxidation of **28** by MnO_2 in refluxing toluene led to the carbonyl derivative **29** in very high yield. The one-pot double cyclization of **29** to fascaplysin **(1)** was best achieved by treatment with pyridinium chloride at 170 °C followed by a basic workup (Fig. **4**).

Molino *et al*. (1994) [34] synthesized fascaplysin (**1**) starting from N-protected 3-formyl indole **30** as depicted in Fig. (**5**). Conversion of the N-methoxymethyl-3-formyl-indole (**30**) into the key intermediate iminophosphorane **31** was performed by sequential treatment with ethyl azidoacetate and triphenylphosphine. Further an aza-Wittig/electrocyclic ring-closure of β-(3-indolyl)vinyl iminophosphorane **31** gave l-substituted β-carboline **32**. This further on ester hydrolysis and nitro group reduction led to

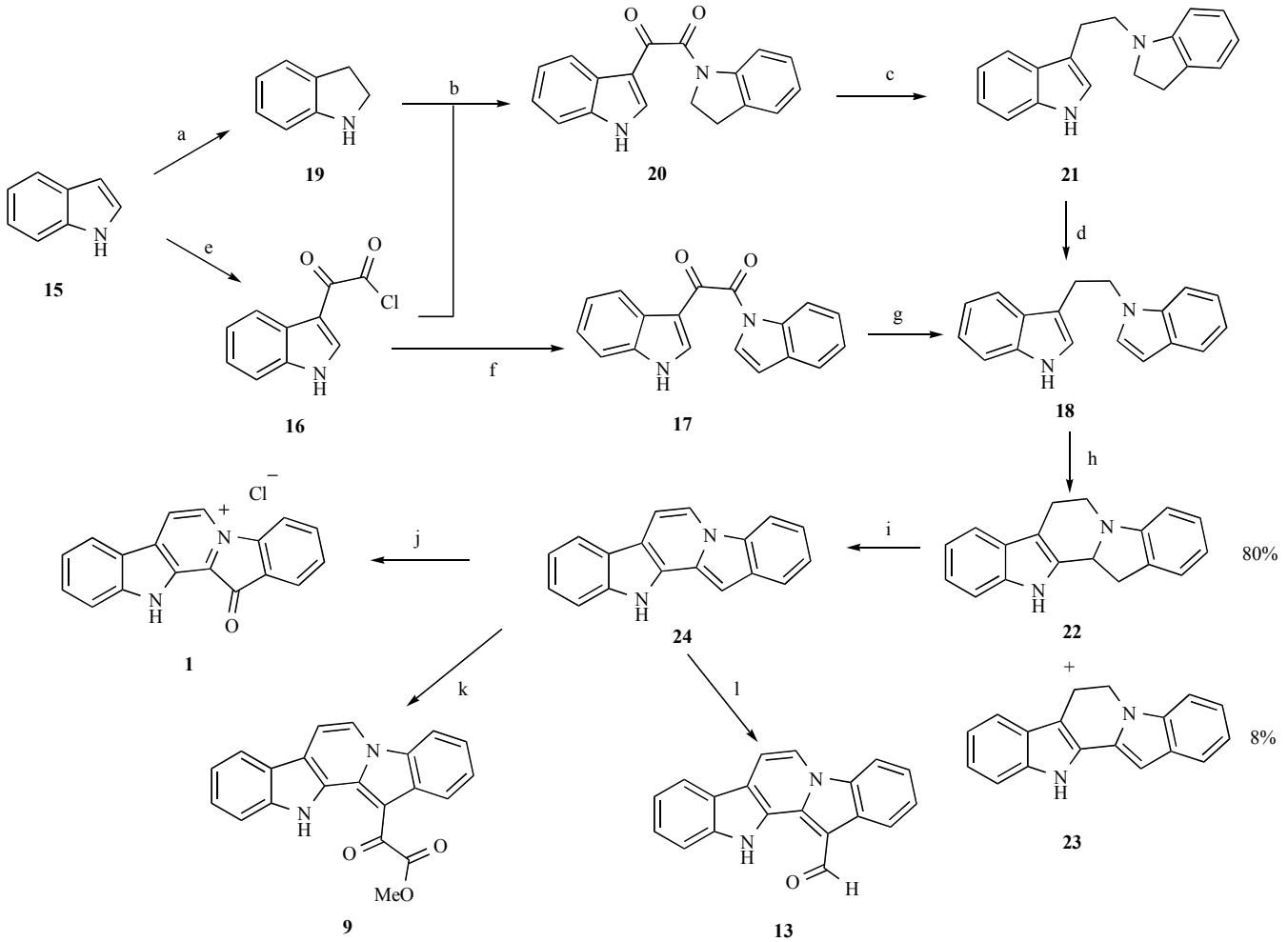


Fig. (3). Gribble's (1990, 1992) synthesis of fascaplysin (**1**), homofascaplysins B (**9**) and C (**13**) [31, 32]. Reagents and conditions: (a). $NaBH_3CN$, HOAc, 15 °C, 94%; (b). K_2CO_3, THF, rt, 2 h, 93%; (c). AlH_3, THF, rt, 75 min, 97%; (d). MnO_2, $CHCl_3$, Heat, 4 h, 99%; (e). $COCl_2$, Et_2O, 5 °C, 92%; (f). Indole, NaH, THF, DMF, rt, 90 min, 86%; (g). $NaBH_3OCOCF_3$, THF, Heat, 20 h, 60%; (h). TFA, rt, 30 min, 80%; (i). Pd/C, $(EtOCH_2CH_2)_2O$, Heat 6 h,93%; (j). CH_3CO_3H, THF, 0 °C, HCl, EtOH, 85%; (k). $(COCl)_2$, MeOH, 99%; (l). $POCl_3$, DMF, 88%.

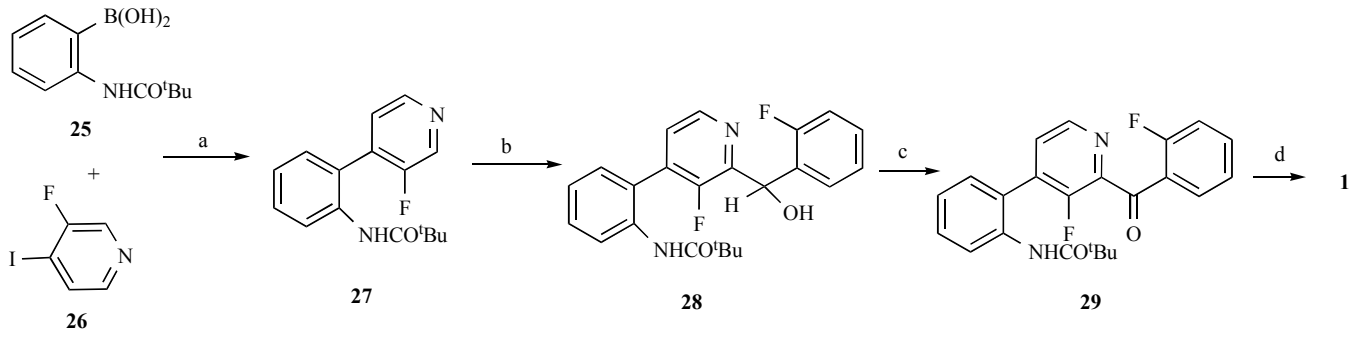


Fig. (4). Total synthesis of fascaplysin (**1**) by Rocca *et al* (1993) [33]. Reagents and conditions: (a). $Pd(PPh_3)_4$/ 2M K_2CO_3, toluene, reflux, 48 h, 98%; (b). BuLi, THF, -75 °C, 1 h, 2-F-PhCHO, 1 h, -75 °C, 95%; (c). MnO_2, toluene, reflux, 2 h, 99%; (d). Pyridine, HCl, 170 °C, 10 min, NH_4OH, ice.

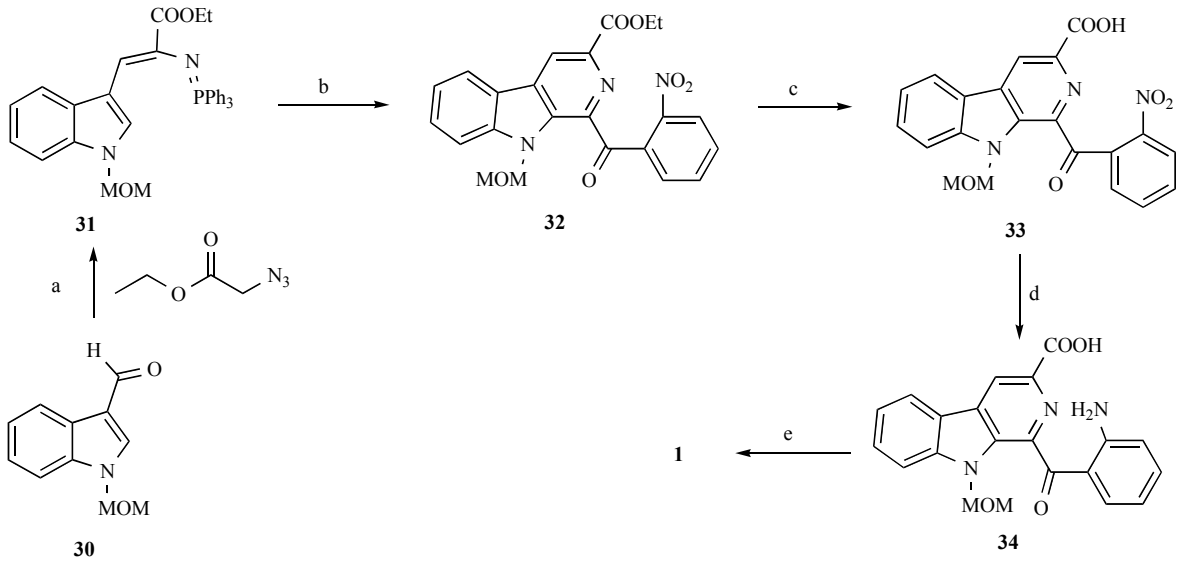


Fig. (5). Total synthesis of fascaplysin (**1**) by Molina *et al* (1994) [34]. Reagents and conditions: (a). PPh_3; (b). $C_6H_4(o\text{-}NO_2)COCHO$, toluene,160 °C, 60-65%; (c). LiOH, THF/H_2O, rt, quantitative yield; (d). H_2, PtO_2, MeOH, 80%; (e). $NaNO_2$, $MeOH\text{-}H_2O$, HCl, 0 °C to reflux, 60%.

formation of compound **34**. Direct conversion of **34** into fascaplysin (**1**), which involves formation of the cationic 5-membered ring. Deprotection and decarboxylation, was performed in 60% yield by diazotization and further heating of the resulting diazonium salt.

Radchenko *et al*. (1997) [35] synthesized fascaplysin (**1**) in five-steps starting from tryptamine (**35**) in 44% overall yield (Fig. **6**). Acylation of tryptamine (**35**) with ortho-bromo phenylacetic acid (**37**) gave the corresponding amide **39** which was converted into dihydro-carboline **43** by treatment with $POCl_3$ (Bischler–Napieralski cyclization). Treatment of **43** with usual black MnO_2 in $CHC1_3$ at room temperature led to formation of **47**. Reaction of **43** (or **47**) with MnO_2 in $CHCl_3$ under reflux produced acyl substituted β-carboline **51** with high yield. Short-run heating of **51** yielded the pyridodiindole quaternary salt which was converted into fascaplysin (**1**) by treatment with dry HCI in MeOH. Garcia *et al* (2006) [36] and Zhidkov *et al* (2007) [37] synthesized bromofascaplysins **2-4** using same synthetic route as depicted in Fig. (**6**) starting from 6-bromotryptamine (**36**).

Recently Zhidkov *et al* (2010) [38] reported new route for total synthesis of fascaplysin *via* a simple and practical approach to pyrido[1,2-a:3,4-b']diindole ring system **58-59** formation starting from indoloketones **55-56** as depicted in Fig. (**7**). The five ring membered key intermediate **59** was synthesized from indoloketone **56** starting material. Dehydrogenation of compound **59** was performed over Pd/C in preparative yield. Compound **60** on treatment with perbenzoic acid led to formation of fascaplysin (**1**). The key intermediate **58** on treatment with DDQ led to formation of homofascaplysin C (**13**). Homofascaplysin (**13**) on treatment with meta-chloroperbenzoic acid also led to formation of **1**.

Recently Waldman (2010) [39] reported total synthesis of fascaplysin (**1**) and homofascaplysin C (**13**) from boc-protected 3-ethynyl-indole-2-carbaldehyde (**61**) as a common precursor (Fig. **8**). The microwave assisted silver catalyzed cascade cyclization of **61** with aniline **64** yielded the pentacyclic core **65** in high yield after acidic work-up. Partial reduction of the tert-butyl ester by means of *in-situ* generated lithium diisobutylpiperidinohydroaluminate provided the natural product homofascaplysin C (**13**) in 48% overall yield

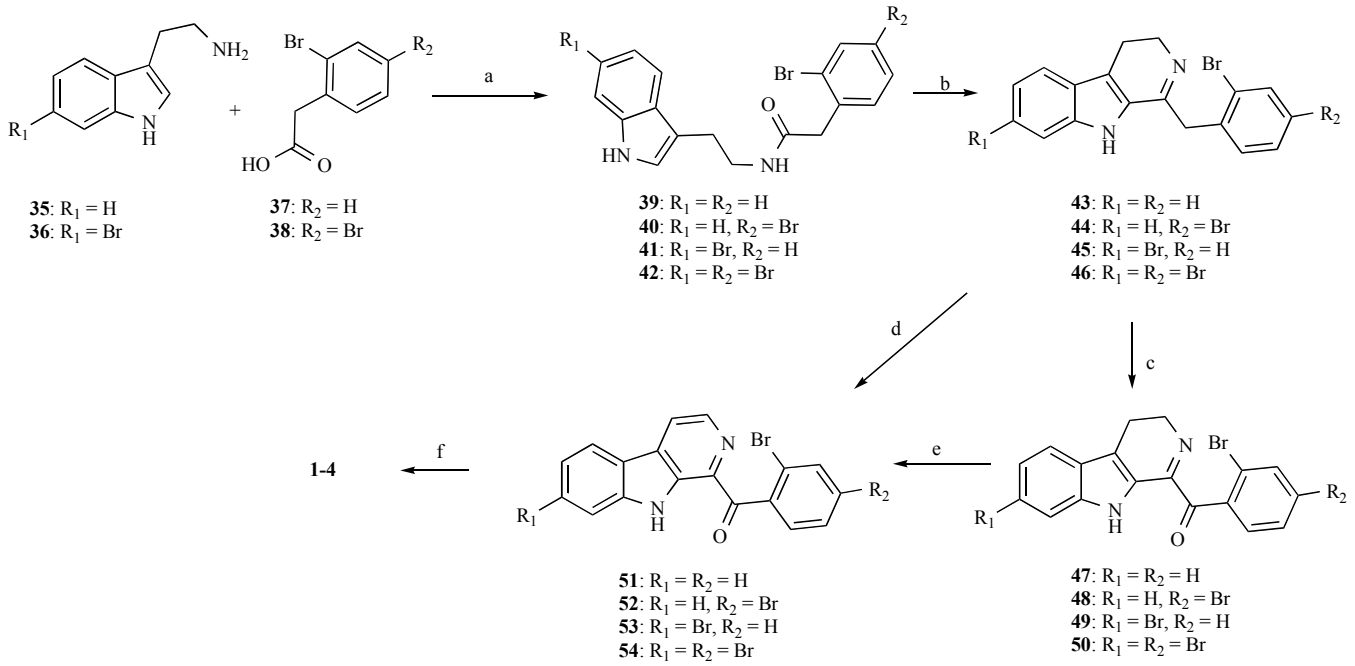


Fig. (6). Total synthesis of fascaplysin (**1**) by Radchenko *et al* (1997) [35] and bromofascaplysins **2-4** by Garcia *et al* (2006) [36] and Zhidkov *et al* (2007) [37]. Reagents and conditions: (a). Tetralin, azeotropic distilation, 40 min, 83% for **39**; DCC, CH_3CN, reflux, 30 min, 88-89% for **40-42**; (b). $POCl_3$, PhH (or ACN), reflux, 30 min, 68%; (c). MnO_2, $CHCl_3$, rt, 3 h, 94%; (d). MnO_2, $CHCl_3$, reflux, 3 h, 86%; (e). MnO_2, $CHCl_3$ (or PhH), reflux, 3 h, 91%; (f). 220 °C, 20 min, HCl/MeOH, 90%.

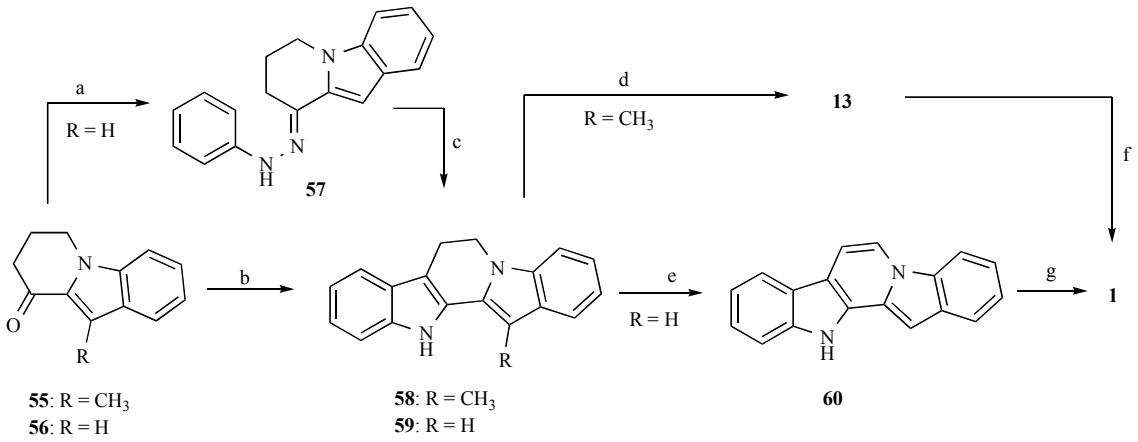


Fig. (7). Total synthesis of fascaplysin (**1**) and homofascaplysin C (**13**) by Zhidkov *et al* (2010) [38]. Reagents and conditions: (a). Ph-NH-NH_2, HCl , EtOH, rt, 2 h, 78%; (b). Ph-NH-NH_2, HCl, AcOH, Heat 4 h, 91%; (c). TsOH, PhH, Heat, 20 min, 60%; (d). DDQ, 1,4-dioxane, heat, 2 h, 50%; (e). Pd/C, $(EtOCH_2CH_2)_2$, heat, 6 h, 75%; (f). m-CPBA, EtOAc, rt, 24 h, 67%; (g). CH_3CO_3H, MeOH, 0 °C, 45 min, 85%

over four steps from **61**. Pentacyclic core **63** was synthesized from boc-protected 3-ethynyl-indole-2-carbaldehyde **(61)** by treatment with aniline **62** in 61% yield. Formylation of **63** with $POCl_3$ cleanly provided homofascaplysin C (**13**) with an overall yield of 53%. The pentacyclic core **63** was efficiently transformed to the natural product fascaplysin (**1**) by oxidation with peracetic acid, followed by salt formation in 52% overall yield.

5. FASCAPLYSIN RELATED OTHER MARINE NATURAL PRODUCTS

Large number of bis-indole marine natural products have been reported so far. Since our focus in the present review is fascaplysin (**1**), only natural products which bear five-membered ring scaffold of fascaplysin are discussed herein. Other bis-indoles in which two indoles are linked *via* different linkers and do not possess a fused ring skeleton are not a part of this review. Staurosporine (**66**), an indolocarbazole alkaloid isolated from a marine ascidian *Eudistoma toealensis* inhibited the cellular proliferation of twelve human leukaemia cell lines, the cell lines differed in their sensitivity towards the individual staurosporine derivatives. Staurosporine showed potent inhibition of PKC. It is not selective PKC inhibitor as it also inhibited cAMP-dependent protein kinases, cGMP-dependent protein kinases (PKG) and tyrosine protein kinases at similar concentrations

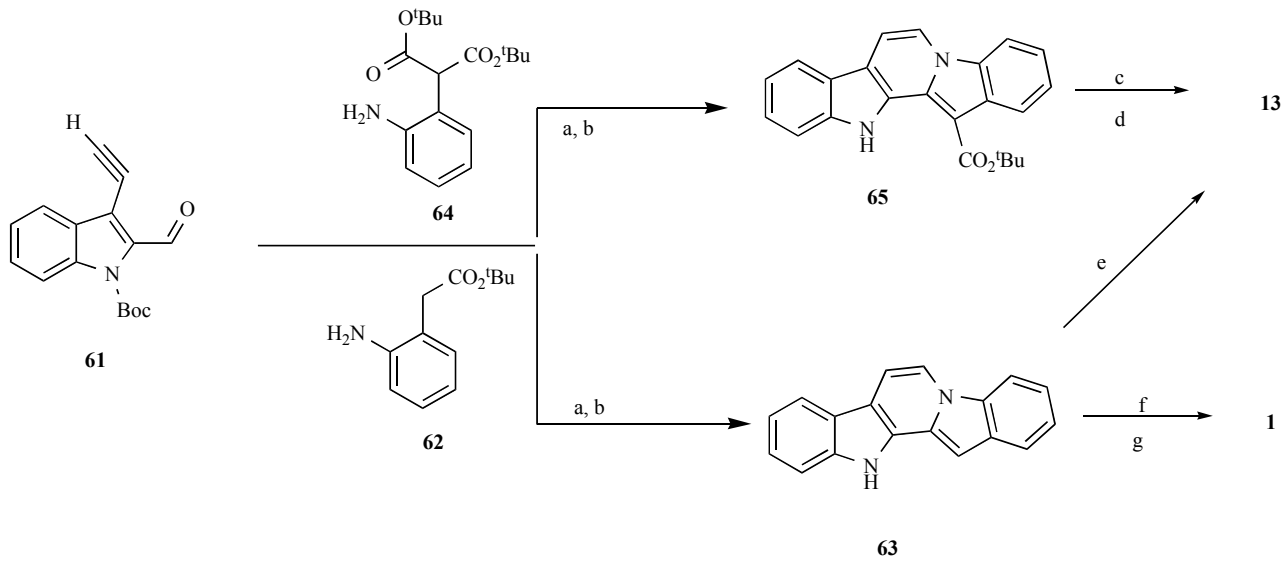


Fig. (8). Total synthesis of fascaplysin (**1**) and homofascaplysin C (**13**) by Waldman *et al* (2010) [39]. Reagents and conditions: (a). AgOTf (2.5 mol%), 2,6 -lutidine (10 mol%), MW (150 Watts), EtOH, 150 °C, 45 min; (b). HCl, 24 h, 91% for compound **65** and 61% for compound **63** from step a and b; (c). piperidine, n-BuLi, DIBAH, THF, 0 °C; (d). 1N HCl, 53% from step c and d; (e). $POCl_3$, DMF, 85%; (f). CH_3CO_3H, MeOH, HOAc, 0 °C; (g). Conc. HCl, 85% from step f and g.

[40]. Differences in efficacy of the staurosporine derivatives in modulating growth may result from differences in their ability to inhibit certain kinases involved in cell growth and tumour promotion [41]. De-*O*-methylstaurosporine (**67**) [42], 11-hydroxystaurosporine (**68**) [43] isolated from ascidian *Eudistoma sp*. are also inhibitors of protein kinase C [42, 43]. Structurally similar indolocarbazole alkaloids antibiotic K252b (**69**) and its methyl ester (K252a, **70**) isolated from from the cultures of *Nocardiopsis sp*. and *Actinomadura sp.* are very potent inhibitors of protein kinase C in cell-free systems and the inhibition is observed at low nanomolar concentrations [44, 45]. K-252a (**70**) inhibits cyclic nucleotide-dependent kinases with similar potency, whereas K-252b (**69**) showed somewhat weaker activity [46]. The inhibition of protein kinase C by K-252a and K-252b is reversible by higher concentrations of ATP, indicating competitive interaction with the ATP binding site of the enzyme [46, 47]. The aglycone of the alkaloid staurosporine, K-252c (**71**, also termed as staurosporinone) isolated from the culture broth of *Nocardiopsis sp. K-290* was found to inhibit PKC with an IC_{50} of 0.214 μM. K-252c (**69**) exhibited a more potent inhibitory activity (about 10-fold) against PKC than against PKA. In other investigations K-252c inhibited cell-adhesion of the EL-4-IL-2 cell line and expressed activity in the K562 bleb and neutrophil assays, in addition to showing micromolar and submicromolar inhibition of enzyme activity against seven PKC isoenzymes [48, 49]. Antibiotic K-252d (**72**) which contain a rhamnopyranosyl moiety was isolated from the *Nocardiopsis sp.* K290e also showed PKC inhibition [48]. Tjipanazole G1 (**73**), a minor alkaloid isolated from blue-green alga *Tolypothrix tjipanasensi* showed activation of protein kinase C [50]. Another bis-indole alkaloid rebeccamycin (**74**) isolated from *Nocardia aerocoligenes* showed antitumor activity [51]. Fifteen N-glycosides of indolo[2,3-α]carbazoles tjipanazoles A1-I isolated from extract of the cyanophyte *Tolypothrix tjipanasensis* showed antifungal activities [50]. Structure of tjipanazole D (**75**) and other bis-indoles is shown in Fig. (**9**).

6. FASCAPLYSIN (1) INSPIRED SYNTHETIC ANALOGS AS CDK-4 INHIBITORS

Fascaplysin (**1**) itself cannot be used as an anticancer drug because of its high toxicity, thought to be due to the fact that its flat structure can act as a DNA intercalator [20]. Thus, extensive amount of synthetic studies have been reported on non-planar (non-toxic) analogs of fascaplysin for discovery of potent CDK-4 inhibitors. In the present section, most of the synthetic analogs are non-planar and differ by linkage pattern of two indole rings *viz.* 1,3-linked analogs; 1,1-linked analogs. Analogs bearing tryptamine and β-carboline skeletons are also reported. Examples of fascaplysin-inspired synthetic analogs with their CDK-4 and CDK-2 activity are listed in Table **2**.

Researchers from University of Leichester have synthesized series of fascaplysin-inspired compounds for discovery of non-toxic and highly selective CDK-4 inhibitors. A series of 1,3-linked bis-indoles represented by compounds **76** and **77** have been reported. The most potent CDK-4/cyclin D1 inhibitor in the series was **77** with IC_{50} of 38 μM (19-fold selectivity over CDK-2/cyclin A) [52, 53]. Series of 1,3-linked bis-indoles varying in substitution pattern on both the aromatic rings have been synthesized following the total synthesis route of fascaplysin reported by Gribble's group in 1990 (see synthesis of compound **18** from indole **15** in Fig. (**3**)) [52, 53]. Same group also synthesized series of 1,1-linked bis-indole class of fascaplysin-inspired compounds. A series represented by compounds **78-80** with CDK-4 inhibition up to IC_{50} of 37 μM are reported. Analogs containing ethyl linker are most potent amongst synthesized compounds in the series [53]. The synthetic scheme for preparation of 1,1-linked bis-indoles **78-80** is depicted in Fig. (**10**) [53].

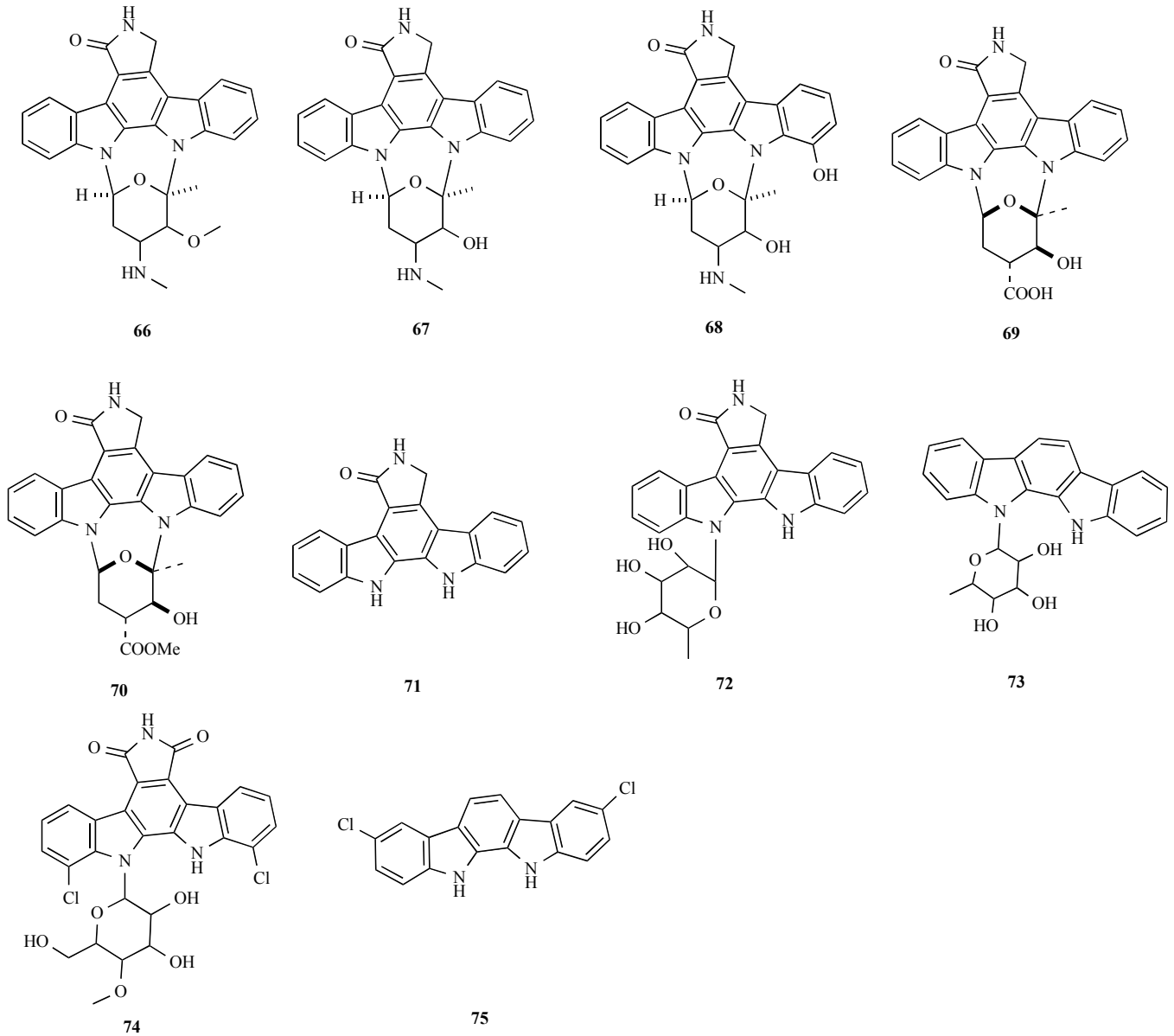


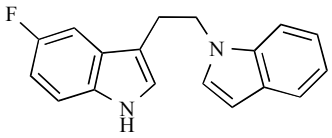
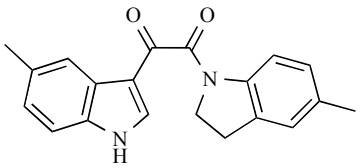
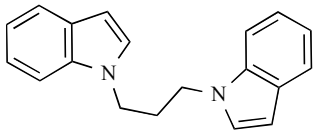
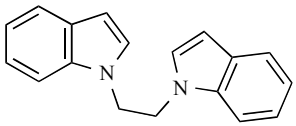
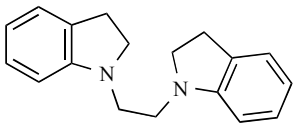
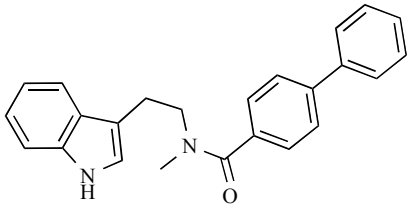
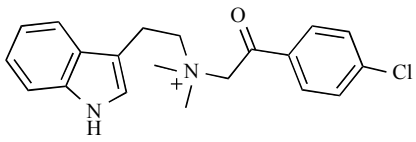
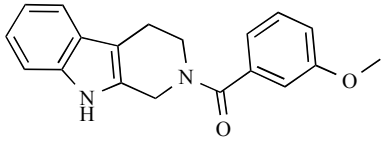
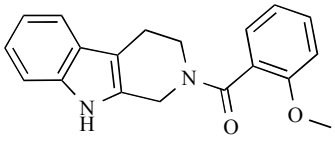
Fig. (9). Fascaplysin related marine natural products.

Further these researchers discovered non-planar non-toxic fascaplysin-inspired tryptamine class of synthetic analogs (e.g. **84** and **85**) and identified the most potent analog named CA224 (**84**) with CDK4/D1 IC_{50} of 6.2 μM and 84-fold selectivity over CDK-2/cyclin A [21, 54, 55]. Molecule **84** was predicted to be located in the ATP binding site of the CDK4 homology model in a similar fashion to fascaplysin (**1**) but with the double hydrogen-bonding interaction being with the backbone of His 95/Val 96 and an extra stabilization arising from a p–p stacking interaction between the biphenyl moiety of the ligand with the side chains of Phe 93 and Phe 159 [21]. Synthetic scheme for tryptamine analogs **84** [56] and **85** [55] is depicted below in Fig. (**11**).

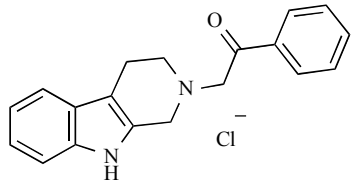
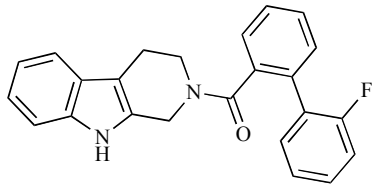
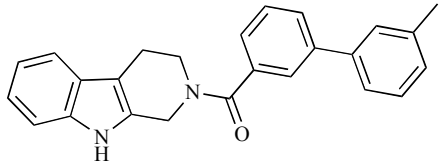
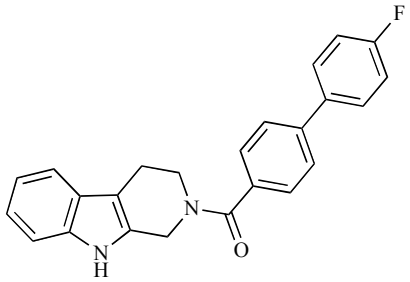
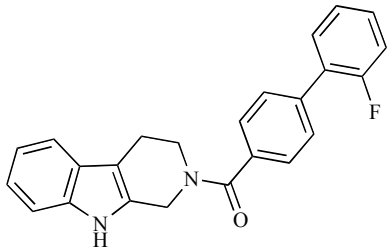
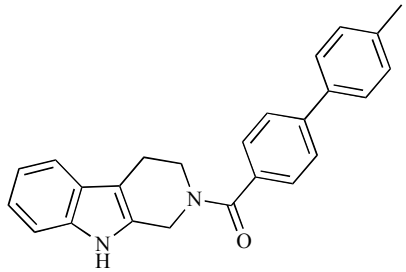
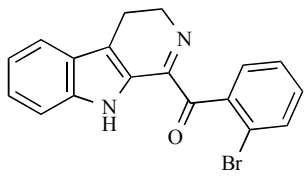
Several N-substituted β-carboline class of fascaplysin-inspired analogs (e.g. **92-99**) have also been reported. A β-carboline analog CA199 (**92**) displayed 32-fold specificity for Cdk4-cyclin D1 (IC_{50} 24 μM) compared with CDK2 (IC_{50} 766 μM) [21, 57]. CA199 (**92**) showed inhibition of the growth of different cancer cell lines at concentrations ranging from 10–40 μM. It also blocked growth of asynchronous cells at G0/G1 in a retinoblastoma protein (pRb) dependent manner. Moreover, CA199 blocked growth only at early G1 in synchronised cells released from a mimosine-induced G1/S block [57]. Biphenyl analogs **95-99** showed potent and highly selective CDK-4 inhibition. Compound **99** possessed most potent activity against CDK-4 with IC_{50} values of 9 μM and 82-fold selectivity over CDK-2. Synthesis of compounds **92-99** is depicted in Fig. (**12**).

A series of C-linked β-carbolines (e.g. **47** and **51**) exhibiting promising CDK-4 inhibitory activity (analog **47**, IC_{50} 11 μM) have been reported. These analogs also showed greater selectivity towards CDK-4. Compound **47** was 74-fold selective for CDK-4 [58]. These type of compounds,

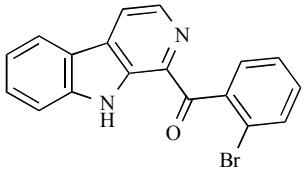
Table 2. Fascaplysin Inspired Synthetic Analogs and their CDK-4 and CDK-2 Activity

Sub-class	Entry	Structure	IC_{50} (μM)		Selectivity*	Ref
			CDK-4/D1	CDK-2/A		
1,3-Linked bis-indoles	**76**		50	200	4	[52, 53]
	77		38	710	19	[53]
1,1-Linked bis-indoles	**78**		38	1070	28	[53]
	79		37	940	25	[53]
	80		66	1230	19	[53]
Tryptamines	**84**	 CA224	6.2	521	84	[21, 54]
	85		50	#	#	[55]
β-Carbolines	**92**	 CA199	24	766	32	[21, 57]
	93		26	861	33	[21]

(Table 2). Contd…..

Sub-class	Entry	Structure	IC_{50} (μM)		Selectivity*	Ref
			CDK-4/D1	CDK-2/A		
	94		34	1467	43	[21]
	95		19	434	23	[56]
	96		11	465	42	[56]
	97		12	438	37	[56]
	98		17	310	18	[56]
	99		9	736	82	[56]
	47		11	818	74	[58]

(Table 2). Contd…..

Sub-class	Entry	Structure	IC_{50} (μM)		Selectivity*	Ref
			CDK-4/D1	CDK-2/A		
	51		14	940	67	[58]

*Selectivity for CDK-4 versus CDK-2; # CDK-2 activity not provided in original paper.

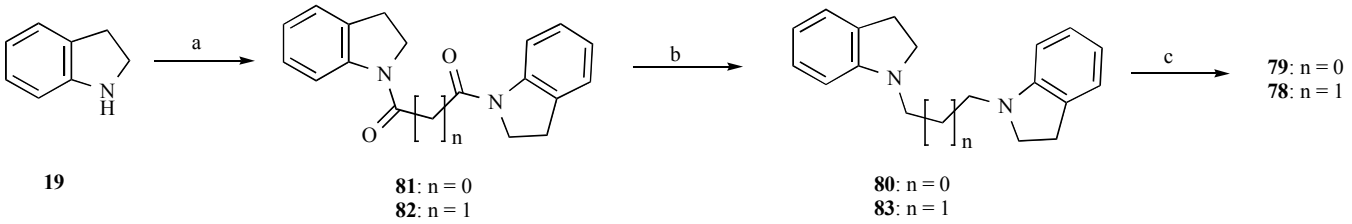


Fig. (10). Synthesis of 1,1-linked fascaplysin analogs **78-80** [53]. Reagents and conditions: (a). $ClCO(CH_2)_nCOCl$, K_2CO_3, anhydrous THF, N_2, 0 °C, 1.5 h, 69–87%; (b). $LiAlH_4$, anhyd.THF, anhyd. ether, N_2, reflux, over night, 36–76%; (c). activated MnO_2, $CHCl_3$, reflux, 60 h, 30–75%.

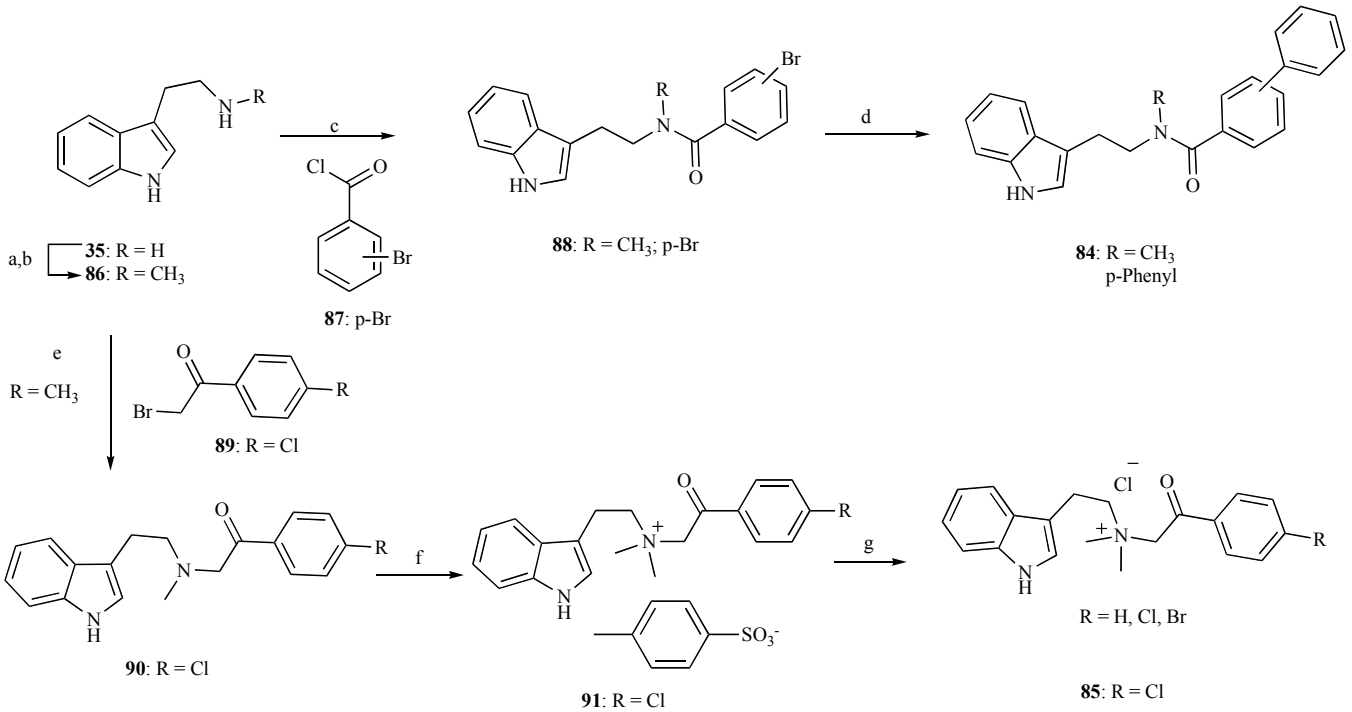


Fig. (11). Synthesis of tryptamine analogs **84-85** [21, 55, 56]. Reagents and conditions: (a). Ethyl chloroformate, 4 M NaOH, $CHCl_3$, 3 h, 95%; (b). $LiAlH_4$, THF, reflux, N_2, 1 h, 89%; (c) CH_2Cl_2, NaOH (4 M, aqueous, 1 equiv), 0 °C, 15 min then rt, 3 h, 48-86%; (d) $Pd(PPh_3)_4$, toluene, EtOH, Ar-$B(OH)_2$, K_2CO_3 (2 M, aq), 90 °C, 24 h, 16-65%; (e). toluene, N_2, $NaHCO_3$, Na_2SO_4, H_2O,4 h, 49-61%; (f). Methyl-p-toluene sulfonate, ACN, reflux, 4 h; (g). Dowex Cl, 1x 8-400 ion exchange resin, over night stirring and column, 46-53%.

reticulatines have also been reported from marine sponge *Fascaplysnopsis reticulate* [59]. Another structurally similar naturally occurring β-carboline alkaloid 1-deoxysecofascaplysin A isolated from marine sponge *Thorectandra sp*. showed potent anticancer activity in cell based assays [60]. Compounds **47** and **51** are synthetic intermediates in the total synthesis route for fascaplysin (**1**) as depicted in Radchenko *et al*. synthesis (Fig. **6**) [35, 58].

CONCLUSION

MNPs represent an interesting source of novel leads with potent chemotherapeutic or chemo-preventive activities.

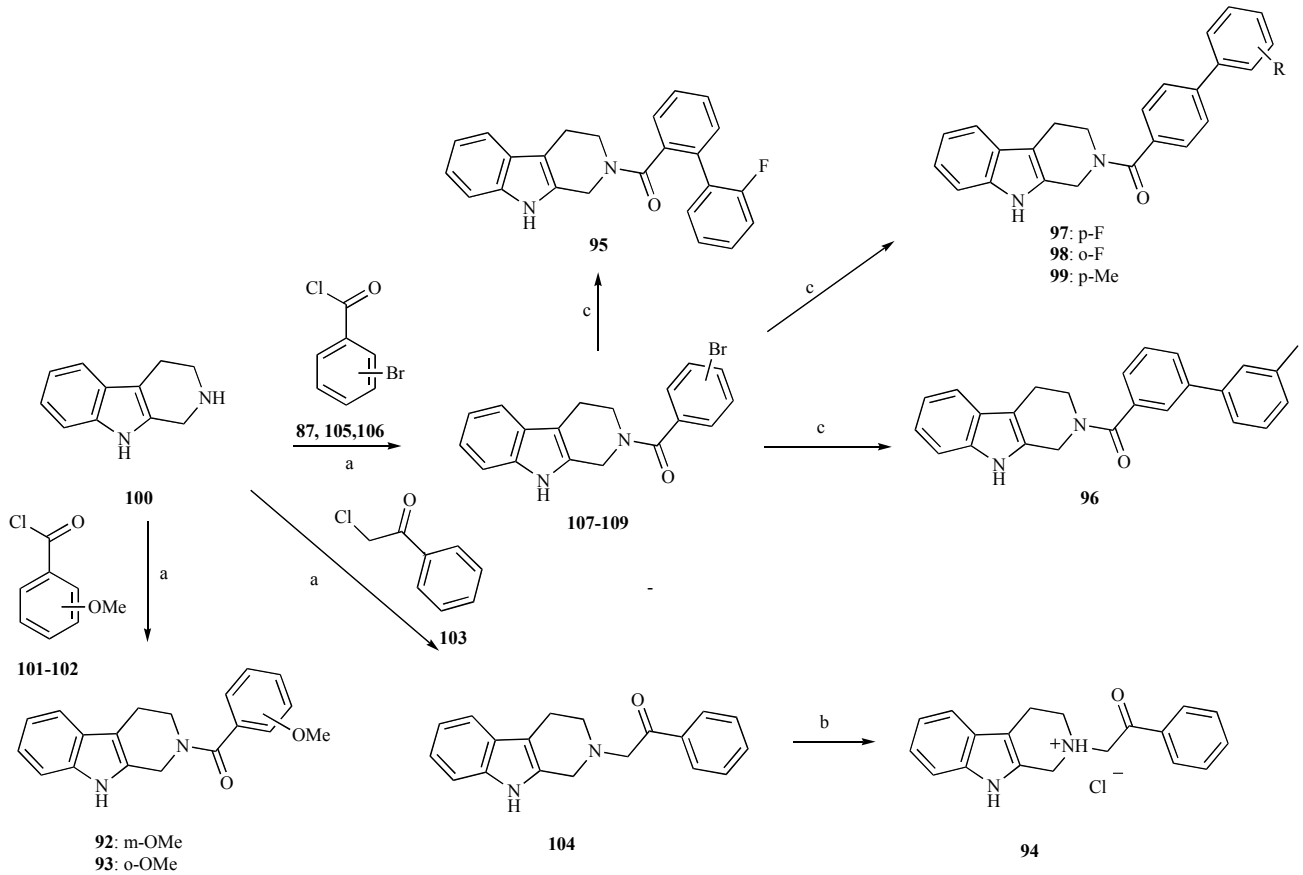


Fig. (12). Synthesis of β-carboline derivatives **92-99** [21, 56]. Reagents and conditions: (a) CH_2Cl_2, NaOH (4 M, aq, 1 equiv), 0 °C 15 min then rt, 3 h, 62-91%; (b). HCl gas, CH_2Cl_2, 3 min, 95%; (c). (c) $Pd(PPh_3)_4$, toluene, EtOH, Ar-$B(OH)_2$, K_2CO_3 (2 M, aq), 90 °C, 24 h, 56-97%.

Fascaplysin (**1**) is very promising marine-derived natural product lead compound with several biological activities and a promising CDK-4 inhibitory activity. Several natural analogs of fascaplysin have been reported but none of them have been evaluated for their CDK-4 activity. Thus there is a need to evaluate these natural compounds **2-14** for their CDK-4 inhibitory potential. Synthetic efforts on fascaplysin-inspired non-planar synthetic analogs led to discovery of potent and highly selective CDK-4 inhibitors **84** and **99** and are excellent synthetic leads for anticancer drug discovery. They have shown promising CDK-4 activity ($IC_{50} < 10$ μM) with >80 fold selectivity compared with CDK-2. Further, fascaplysin showed inhibition of VEGF, producing anti-angiogenesis activity. These promising biological activities and a selective inhibition of CDK-4 associated with fascaplysin has already led to discovery of few synthetic lead molecules and still there is much unexplored medicinal chemistry which may further lead to discovery of novel anticancer lead compounds.

CONFLICT OF INTEREST

Authors declare no any conflict of interest in connection with the present article.

ABBREVIATIONS

CDK = cyclin dependent kinase

VEGF = vascular endothelial growth factor

IGF-1R = insulin growth factor-1 receptor

NCI = national cancer institute

TFA = trifluoroacetic acid

MNPs = marine natural products.

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