RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2024, **14**, 23574

Organo NHC catalyzed aqueous synthesis of 4β-isoxazole-podophyllotoxins: *in vitro* anticancer, caspase activation, tubulin polymerization inhibition and molecular docking studies†

Rajkumar Nagavath,[a] Murali Krishna Thupurani,[b] Vinitha Badithapuram,[a] Ravinder Manchal,[a] Chandra Sekhar Vasam*[c] and Narasimha Swamy Thirukovela *[a]

Received 12th June 2024
Accepted 8th July 2024

DOI: 10.1039/d4ra04297b

rsc.li/rsc-advances

We present, for the first time, the organo-*N*-heterocyclic carbene (NHC) catalyzed 1,3-dipolar cycloaddition of 4β-*O*-propargyl podophyllotoxin (**1**) with *in situ* aromatic nitrile oxides to afford regioselective 4β-isoxazolepodophyllotoxin hybrids (**6a–n**) in benign aqueous-organic media. Preliminary anticancer activity results showed that compound **6e** displayed superior activity against MCF-7, HeLa and MIA PaCa2 human cell lines compared with podophyllotoxin. Compounds **6j** and **6n** showed greater activity against the MCF-7 cell line than the positive control. Caspase activation studies revealed that compound **6e** at 20 μg ml^{-1} concentration had greater caspase 3/7 activation in MCF-7 and MIAPaCa2 cells than podophyllotoxin. Furthermore, *in vitro* tubulin polymerization inhibition studies revealed that compound **6e** showed comparable activity with podophyllotoxin. Finally, *in silico* molecular docking studies of compounds **6e**, **6j**, **6n** and podophyllotoxin on α,β-tubulin (pdb id 1SA0) revealed that compound **6n** showed excellent binding energies and inhibition constants compared with podophyllotoxin.

Introduction

Nature has consistently offered us a wide variety of bioactive products to treat serious diseases such as cancer, immune system diseases, neurological conditions, and infections.[1] Among these products is podophyllotoxin (**1**), one of the most prevalent naturally occurring cyclolignans isolated from *Podophyllum peltatum* L. and *Podophyllum hexandrum*,[2] which has been broadly used in clinical studies on diverse malignancies. The mechanisms of action of semi-synthetic derivatives etoposide (**2**) and teniposide (**3**) are significantly different from those of the parent podophyllotoxin.[3,4] These two semi-synthetic derivatives inhibit DNA topoisomerase II, whereas the parent podophyllotoxin inhibits the assembly in the microtubulin.[5,6] Even yet, there have been reports on etoposide's toxicity and limitations, including its moderate efficacy, low solubility in water, potential for drug resistance, metabolic inactivation, and other adverse consequences.[7,8]

These findings have prompted numerous investigations into the structural modification of etoposide, which includes etopophos (**4**), that addresses the bioavailability aspect. The substitution at the 4β-position resulting in strong inhibition of topoisomerase II was the most significant change. After a study[9,10] on the substitution of heterocycles for etoposide's C-4 sugar unit, MacDonald and colleagues[11] produced a composite pharmacophore model that identified the C-4 molecular area of podophyllotoxin (**1**) as a potentially variable position for further research. Bulky substituents at C-4 may be advantageous for DNA topo-II inhibition, as further evidenced by comparative molecular field analysis (CoMFA) models revealed by Lee and the group.[11,12] These hypotheses align with the outstanding activity profiles of GL-331 (**5**), TOP-53 (**6**), and NK 611 (**7**).[13] Interestingly, drug-resistance profiles of GL-331 and TOP-53 differed significantly from that of the parent compound **1**, and both compounds demonstrated increased DNA topo-II inhibition and antitumor potential. The activity profiles of these classes of compounds suggest that substitution at position C-4 plays a crucial role and rational modifications at the C-4 position are feasible.[14]

One of the main challenges in medicinal chemistry is the development of anticancer agents, as evidenced by severe side effects of current chemotherapeutics, which include non-specific targeting, low solubility and incapability to enter tumour cells.[15] These factors suggest the need for ongoing efforts to develop desired anti-cancer drug-like candidates with minimal side

[a]*Department of Chemistry, Chaitanya (Deemed to be University), Himayathnagar (V), Moinabad (M), Ranga Reddy (D), Hyderabad, India. E-mail: thirukovelann@chaitanya.edu.in*

[b]*Department of Biotechnology, Chaitanya (Deemed to be University), Himayathnagar (V), Moinabad (M), Ranga Reddy (D), Hyderabad, India*

[c]*Department of Pharmaceutical Chemistry, Telangana University, Nizamabad, TS, India. E-mail: csvasam@telanganauniversity.ac.in*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4ra04297b

effects.[16,17] As a result, current research is concentrated on the development of novel, safer therapeutic agents that are crucial for clinical use.[18,19] Remarkably, because of their numerous biological uses, *N*-heterocycles with an oxygen atom are regarded as an important class of compounds in medicinal chemistry.[20] In view of this, the selection of an isoxazole ring in the design and synthesis of biologically active compounds is considered to be a better choice, since in isoxazole both O and N atoms are present in adjacent positions and have low bond dissociation energy.[21] Also, isoxazole consists weak basic character and due to a weak N–O bond, this ring breaks simply by photolysis and thermolysis. Predominantly, deprotonation of isoxazole leads to ring-opening and additional substitutions would lead to well therapeutic activity.[22] Many research groups have been working on the development of new isoxazole-based anticancer active compounds (Fig. 1).[23]

Synthesis of isoxazole derivatives is extensively carried out through[24] cyclomerization, cycloaddition, condensation and functionalization *etc.* In particular, 1,3-dipolar cycloaddition of nitrile oxides with alkynes has good synthetic value, since it produces biologically useful isoxazoles.[22,23] Thus, Cu(I) and Ru(II) catalyzed 1,3-dipolar cycloaddition of nitrile oxides with terminal alkynes to access regioselective 3,5-di- and 3,4-disubstituted isoxazoles correspondingly have been well-developed.[24,25] However, considering the probable disproportionation in metal catalysis and advantages observed with organo-catalysis in homogeneous catalysis,[26] in 2011, our group developed an organo-*N*-heterocycliccarbenes (NHCs) catalyzed 1,3-dipolar cycloaddition of nitrile oxides with alkynes to obtain regioselective 3,5-di- and 3,4,5-trisubstituted isoxazoles in DCM solvent,[27] since, NHCs are distinct Lewis base (nucleophilic) organocatalysts, they have both σ-basicity and π-acidity properties.[28] Starting from initial studies on thiamine-derived NHCs in benzoin,[28a] and Stetter reactions,[28b] the mechanistic variety of NHCs contingent on their properties has led to the progress of numerous extraordinary C–C and C–X (X = heteroatom) bond formations. Certainly, the isolation of the first stable NHC by Arduengo in 1991 (ref. 29) from imidazolium salts revealed their tunable steric and electronic properties by changing *N*-substituents on the imidazole ring. In addition, the imidazolium salts, precursors to NHCs, remain stable and easy to handle.

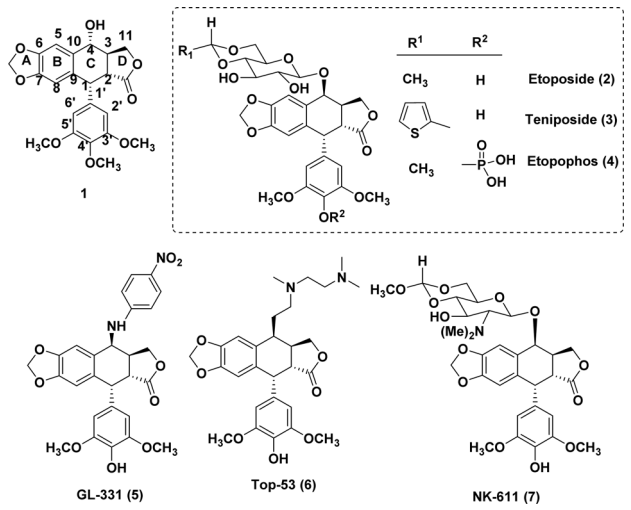


Fig. 1 Structures of a few anticancer-active podophyllotoxin derivatives.

Conversely, organocatalytic reactions have appeared as a substitute synthetic strategy that may eventually lead to large-scale pharmaceutical synthesis applications.[30] The development of organocatalytic reactions in aqueous media is especially promising because, generally speaking, organocatalysts are stable in the presence of aqueous media. The use of water as the solvent in *N*-heterocyclic carbene (NHC)-catalyzed polarity inversion reactions is restricted when compared to enamine catalysis.[28c,31] In 2004, Bode revealed the use of aq. organic (THF–H_2O, 10 : 1) in organo-NHC-catalyzed addition of enals and aldehydes.[28p,32] The similar group then used a stoichiometric amount of water as a reagent or co-solvent in the organo-NHC catalyzed reaction of formylcyclopropanes and α-chloroaldehyde bisulfite salts.[33] In 2010, Rovis and group developed an organo-NHC catalyzed reaction of α-chloro aldehydes in toluene–water solvent media comprising around 1.0 equivalent of water.[34]

In another study, Hoveyda and group developed an enantioselective addition between dimethylphenylsilyls and to α,β-unsaturated carbonyls in the aqueous medium using organo-NHC-catalysis and results displayed that water is comfortable to organo-NHC catalysis.[35] Certainly, traces of water are usually presumed to exist even when anhydrous organic solvents are utilized in organo-NHC-catalyzed reactions. Also, results of the experimental and theoretical studies of Amyes, Diver, Gudat, and Nyulaszi groups show that NHCs are reasonably stable in aqueous environment.[36]

Considering that thiamine, an NHC precursor, is indispensable for several biological processes that arise mainly in an aqueous atmosphere.[37] In 2013, Y. R. Chi and co-workers first time utilized catalytic efficacy of organo-NHC to promote the reaction of enals with enones in pure water.[38] Following nature's lead, the use of aqueous solvent media would be an appropriate choice in NHC-catalyzed reactions.

Based on all the above findings and in our ongoing effort to develop aqueous organic synthesis[39a–d] and anticancer agents,[39e–k] we report an organo-NHC catalyzed 1,3-dipolar cycloaddition between *in situ* nitrile oxides and 4β-*O*-propargyl podophyllotoxin to construct new C4-modified isoxazole linked podophyllotoxins in aq. MecCN media. As well, we also investigated the *in vitro* anticancer activity, caspases activation and *in vitro* tubulin polymerization of newly synthesized isoxazole-linked podophyllotoxins. Finally, molecular docking studies were carried out for the most potent compounds found in *in vitro* activity studies. To the best of our knowledge, this is the first report concerning the organo-NHC catalyzed 1,3-dipolar alkyne-nitrile oxide cycloaddition in aqueous organic media to obtain new biologically active isoxazole compounds.

Results and discussion

At first, the synthesis of the key starting materials of the current work such as 4β-*O*-propargyl podophyllotoxin (**1**)[39f,40] and

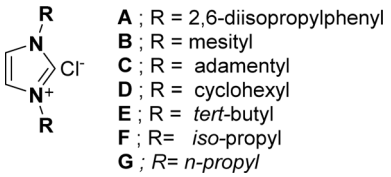


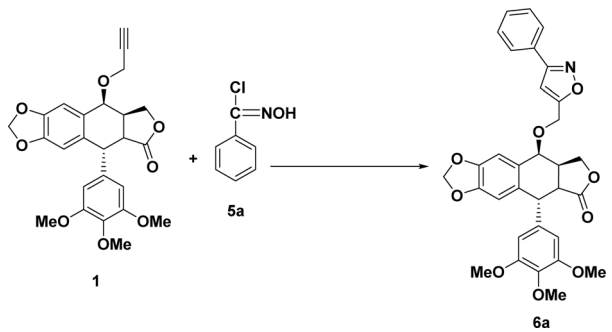
Fig. 2 Structures of NHC pre-catalysts used in the present study.

chlorooximes (hydroximoyl chlorides) (**6a–n**)[41] was achieved according to literature procedures.

Later, we concentrated on the development of the optimization reaction conditions from 1,3-dipolar cycloaddition of terminal alkyne **1** with *N*-hydroxybenzimidoyl chloride (**6a**) as a model reaction using K_2CO_3 and diverse 5 mol% NHC pre-catalysts (**A–G**) at RT in (7 : 3) H_2O/MeCN solvent media.

The results revealed that the NHC pre-catalysts (imidazolium salts) (**A–G**) (Fig. 2) used in this reaction gave desired cycloaddition product (5*R*,8*aR*,9*S*)-9-((3-phenylisoxazol-5-yl)methoxy)-5-(3,4,5-trimethoxyphenyl)-5,8,8*a*,9-tetrahydrofuro[3′,4′:6,7] naphtha [2,3-*d*][1,3]dioxol-6(5*aH*)-one (**6a**) in 22–76% yields

Table 1 Optimization of reaction conditions[a]



Entry	Catalyst precursor (*n* mmol)	Base	Solvent	Time (h)	Yield[b] (%)
1	**A** (5)	K_2CO_3	H_2O/MeCN	2	71
2	**B** (5)	K_2CO_3	H_2O/MeCN	2	76
3	**C** (5)	K_2CO_3	H_2O/MeCN	3	46
4	**D** (5)	K_2CO_3	H_2O/MeCN	3	40
5	**E** (5)	K_2CO_3	H_2O/MeCN	5	31
6	**F** (5)	K_2CO_3	H_2O/MeCN	5	22
7	**G** (5)	K_2CO_3	H_2O/MeCN	5	27
8	**B** (3)	K_2CO_3	H_2O/MeCN	5	35
9	**B** (7)	K_2CO_3	H_2O/MeCN	2	76
10	**B** (5)	$KHCO_3$	H_2O/MeCN	3	38
11	**B** (5)	DIEA	H_2O/MeCN	2	56
12	**B** (5)	Et_3N	H_2O/MeCN	3	52
13	**B** (5)	DBU	H_2O/MeCN	2	68
14	**B** (5)	K_2CO_3	H_2O/DCM	6	Trace
15	**B** (5)	K_2CO_3	H_2O/toluene	6	Trace
16	**B** (5)	K_2CO_3	H_2O/MeOH	2	62
17	**B** (5)	K_2CO_3	H_2O/EtOH	2	65
18	**B** (5)	K_2CO_3	H_2O	3	31

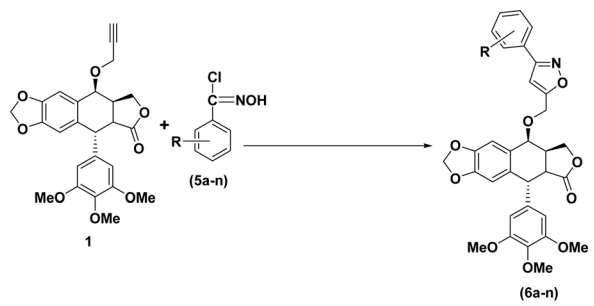
[a] Reaction conditions: (i) intermediate **1** (1 mmol), NHC pre-catalysts **A–G** (0.05 mmol), base (0.15 mmol), (7 : 3) aqueous/organic solvent media RT and 20 min. (ii) **5a** (1 mmol), base (1.5 mmol) and remaining time according to Table 1. [b] Isolated yields after column chromatography.

(Table 1, entries 1–7). Out of all, NHC pre-catalyst **B** gave the best yield (76%) of **6a** (Table 1, entry 2). The molecular structure of NHC pre-catalysts had a robust effect on the catalytic efficacy. The NHC pre-catalysts comprising bulky aromatic groups provided good yields of **6a** (Table 1, entries 1–2), while, NHC pre-catalysts containing aliphatic groups gave low to moderate yields of **6a** (Table 1, entries 3–7). Moreover, lowering the NHC pre-catalyst **B** concentration from 5 mol% led to a lower yield of **6a** (Table 1, entry 8), whereas, increasing NHC pre-catalyst **B** loading from 5 mol% product did not show any effect on the yield of the product **6a** (Table 1, entry 9). Using the optimal NHC pre-catalyst **B** (Table 1, entry 2), the effect of the bases on product **6a** yield was also examined. The results revealed that $KHCO_3$, DIEA and Et_3N provided low yields (38–52%) of **6a** (Table 1, entries 10–12), while, DBU gave a moderate (68%) yield of **6a** (Table 1, entry 13). Interestingly, K_2CO_3 was found to be suitable, as it provided a good yield (76%) of **6a** (Table 1, entry 2).

Finally, we investigated the effect of the solvent system on the yields of **6a**, under optimized reaction conditions. The trace amount of **6a** was isolated using bi-phasic systems such as aq. DCM (Table 1, entry 14) and aq. toluene (Table 1, entry 15). The yield of **6a** was slightly good in the case of aq. MeOH (Table 1, entry 16) and aq. EtOH (Table 1, entry 17). However, the use of pure water gave a low yield (31%) of the product **6a** (Table 1, entry 18).

With the above optimal conditions (5 mol% NHC pre-catalyst **B**, K_2CO_3 and 7 : 3 water/MeCN) in our hand, the current approach was extended to a variety of aromatic aldehydes. In general, aromatic aldehydes containing electron-withdrawing groups provided better yields of the corresponding isoxazoles (Scheme 1, entries **6f–n**) than the remaining aldehydes (Scheme 1, entries, **6a–g**).

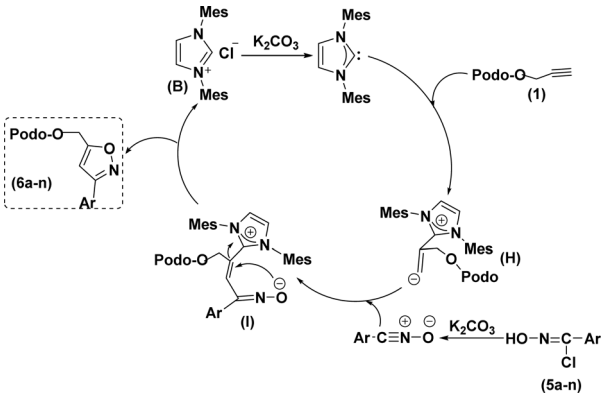
Based on the literature survey,[27,42] we propose a plausible mechanism as shown in Scheme 2. At first, an *in situ* organo-NHC catalyst (obtained from pre-catalyst **B** and K_2CO_3) would react with alkyne (**1**) to form the zwitterion intermediate (**H**). Later this reactive intermediate interacts with *in situ* nitrile oxides (obtained from the reaction between chlorooximes



Reaction conditions: (i) Intermediate **1** (1.0 mmol), pre-catalyst B (0.05 mmol) and K_2CO_3 (0.15 mmol), (7:3) H_2O/MeCN, RT and 20 min.
(ii) Chlorooximes (**5a-n**) (1 mmol) and K_2CO_3 (1.5 mmol) and 100 min.

**R = H; 6a; 76%. R = 4-Me; 6b; 76%. R = 3-Me; 6c; 77%.
R = 3,5-diMe; 6d; 73%. R = 4-OMe; 6e; 70%. .
R = 4-Cl; 6f; 82%. R = 4-Br; 6g; 78%. R = 4-F; 6h; 83%.
R = 4-CN; 6i; 83%. R = 4-NO_2; 6j; 85%. R = 3-Cl; 6k; 79%.
R = 3-CN; 6l; 81%.R = 3,5-diCl; 6m; 77%. R = 3,5-diCN; 6n; 77%.**

Scheme 1 Synthesis of 4β-isoxazolepodophyllotoxin hybrids (**6a–n**).



Scheme 2 A possible mechanism.

(**5a–n**) and K_2CO_3) through a nucleophilic attack to give additional zwitterion intermediate (**I**), which, finally undergo C–O hetero-cyclization to give regioselective 3,5-di-substituted 4β-isoxazolepodophyllotoxin hybrids (**6a–n**).

In vitro anticancer activity

All the above 4β-isoxazolepodophyllotoxin hybrids (**6a–n**) were examined for their *in vitro* anticancer activity against three human cancer cell lines (MCF-7, HeLa and MIAPACA) using podophyllotoxin as a positive control. The results (Table 2) revealed that compound **6e** (GI_{50} = 0.18–0.32 μM) showed greater activity than the podophyllotoxin (GI_{50} = 0.31–0.51 μM) against three cell lines. As well, compounds **6j** and **6n** exhibited greater activity (GI_{50} values 0.23 and 0.12 μM, respectively) than the positive control (GI_{50} = 0.31 μM) against MCF-7, while, the same compounds (GI_{50} = 1.03–1.54 μM) had promising activity in comparison to podophyllotoxin (GI_{50} = 0.51–0.74 μM) against HeLa and MIAPACA. Furthermore, compounds **6i** (GI_{50} = 0.42 μM) and **6m** (GI_{50} = 0.36 μM) had almost similar activity with the podophyllotoxin (GI_{50} = 0.31 μM) against MCF-7.

Table 2 *In vitro* anticancer activity of newly developed podophyllotoxin compounds (**6a–n**) with GI_{50} in μM[a]

Compound	R	MCF-7[b]	HeLa[c]	MIAPACA[d]
6a	H	2.19 ± 0.33	4.45 ± 1.17	3.98 ± 1.26
6b	4-CH_3	3.08 ± 0.54	3.92 ± 1.18	3.22 ± 1.02
6c	3-CH_3	3.92 ± 0.82	3.83 ± 1.54	4.67 ± 1.93
6d	3,5-diCH_3	1.34 ± 0.23	2.74 ± 0.52	2.28 ± 0.29
6e	**4-OCH_3**	**0.18 ± 0.07**	**0.32 ± 0.11**	**0.17 ± 0.05**
6f	4-Cl	2.13 ± 0.37	3.05 ± 1.02	3.14 ± 0.32
6g	4-Br	4.32 ± 0.83	7.48 ± 0.69	6.84 ± 2.01
6h	4-F	4.87 ± 0.79	6.12 ± 1.08	13.13 ± 3.27
6i	4-CN	**0.42 ± 0.15**	3.64 ± 0.93	4.02 ± 0.79
6j	4-NO_2	**0.23 ± 0.12**	1.11 ± 0.29	1.54 ± 0.18
6k	3-Cl	3.15 ± 0.43	9.68 ± 3.12	18.32 ± 2.87
6l	3-CN	2.52 ± 0.91	12.25 ± 2.24	10.22 ± 3.06
6m	3,5-diCl	**0.36 ± 0.17**	2.19 ± 0.84	2.33 ± 0.18
6n	3,5-diCN	**0.12 ± 0.08**	1.28 ± 0.25	1.03 ± 0.19
Podophyllotoxin		0.31 ± 0.19	0.74 ± 0.23	0.51 ± 0.12

[a] 50% growth inhibition and values are the mean of three independent experiments. [b] Breast cancer. [c] Cervical cancer. [d] Pancreatic cancer.

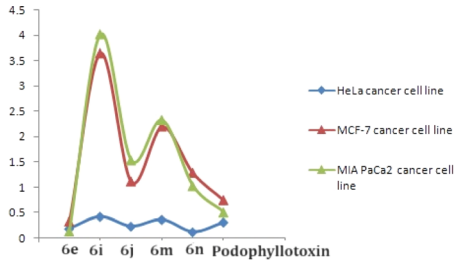


Fig. 3 GI_{50} curves of compounds **6e**, **6i**, **6j**, **6m** and **6n** and podophyllotoxin.

The nature of the phenyl ring that is attached to isoxazole moiety, affecting *in vitro* anticancer activities was revealed using structure–activity relationship (SAR) studies. Introducing a strong electron-releasing group (OCH_3) at the 4th position resulted in compound **6e** displayed enhanced activity. The compound **6d** containing a weak electron-donating group at 3rd and 5th positions (3,5-diCH_3) was ranked second in this category. However, simple phenyl ring compound **6a** or compounds **6b** and **6c** bearing mono-CH_3 groups irrespective of their positions had reduced potency as compared to compounds **6d** and **6e** (Fig. 3).

With respect to the electron-withdrawing group series, compounds **6j** and **6n** bearing 4-NO_2 and 3,5-diCN groups, respectively, displayed improved activity. The next better activity was shown by compound **6m** having the 3,5-diCl group. Compounds **6f** and **6i** with 4-Cl and 4-CN groups, respectively, had slightly weaker activity than the compound **6m**. However, compounds **6g**, **6h** and **6k** containing mono-halogen groups such as 4-Br, 4-F and 3-Cl, respectively, or compound **6l** with 3-CN group displayed poorer activity than the remaining compounds in this series (Fig. 4).

Caspases activation study

The caspase protease family of enzymes is essential for both the start and completion of apoptosis. When they become active, they cleave many regulatory and structural proteins, which causes the cell to break down internally.[43] The traditional indicators of apoptosis, including nuclear condensation, DNA

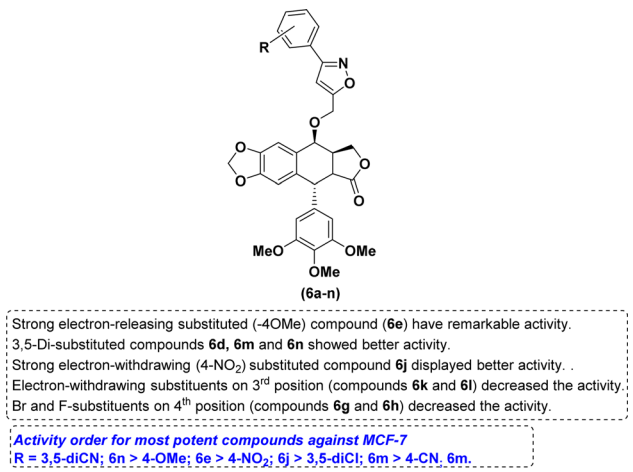


Fig. 4 SAR summary.

fragmentation, and plasma membrane blebbing, are caused by these proteolytic processes.[44] The elimination of damaged cells and preservation of cellular homeostasis are two processes in which apoptosis is essential.[45] The terms "intrinsic pathway" and "extrinsic pathway" refer to the two primary caspase activation mechanisms that lead to apoptosis; they are so named because pressures from the outside or inside of the cell, respectively, often activate them. Cellular stressors such as end plasmicreticulum stress, metabolic stress, and DNA damage are the main causes of the intrinsic pathway's activation. This route is activated by several chemotherapy medications that are used to treat different types of cancer. When these stimuli come together, the mitochondria experience outer membrane permeabilization (MOMP), which releases cytochrome c from the intermembrane space of the mitochondria into the cytosol.

Hence the podophyllotoxin and most potent compounds **6e**, **6i**, **6j**, **6m** and **6n** observed in the above *in vitro* anticancer activities were further tested for their caspase activation and results are shown in Table S1† (Fig. 5–9). Out of all, compound **6e** showed paramount caspase activation. In accordance with the results, we noted that the activation of caspases by the compounds was found to be concentration-dependent. Among all, compound **6e** highly activated caspase 3/7 in MCF-7 cells with 94.5%, which is even higher than the percentage of the podophyllotoxin 91.9% recorded at 20 μg ml^{-1}. Subsequently, the 3/7 caspase activation by **6e** was also found significant in MIA PaCa2 cells with 92.3%, which is higher than the percentage value of podophyllotoxin 88.6% recorded at 20 μg ml^{-1}. On the other hand, the caspase 3/7 activation by **6e** in HeLa cells was found slightly lower at 81.3% compared to podophyllotoxin at 86.9% recorded at 20 μg ml^{-1}. These results suggest that compound **6e** is a good selection for the activation of 3/7 caspase. Next to **6e**, compounds **6j** and **6i** also highly induced caspase 3/7 activation in MCF-7 cells with 90.1% and 89.3%, respectively, recorded at 20 μg ml^{-1}. The results are compared with 91.9% recorded with podophyllotoxin at 20 μg ml^{-1}. While the different caspase action by the remaining compounds was found average or least. Compound **6m** exhibited the least caspase 3/7, 8, and 9 activations with 44.8%, 53.4, 65.0%, 63.0%, 41.9% and 51.7%, respectively, in HeLa, MCF-7 and MIA PaCa2 cancer cells.

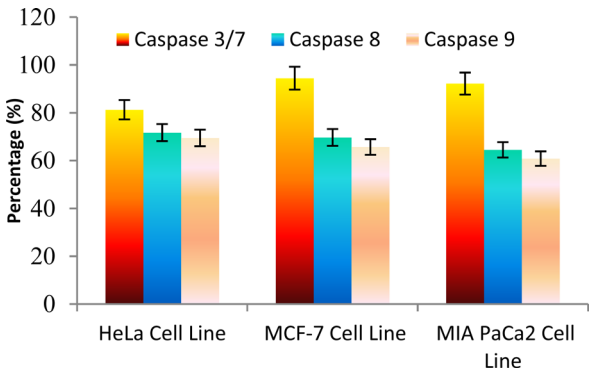


Fig. 5 Effect of compound **6e** against the activation of different caspases in selected cancer cell lines.

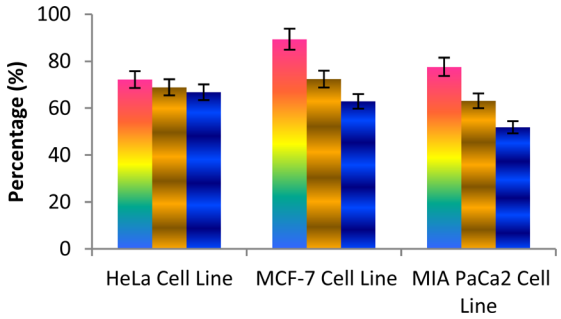


Fig. 6 Effect of compound **6i** against the activation of different caspases in the selected cancer cell lines.

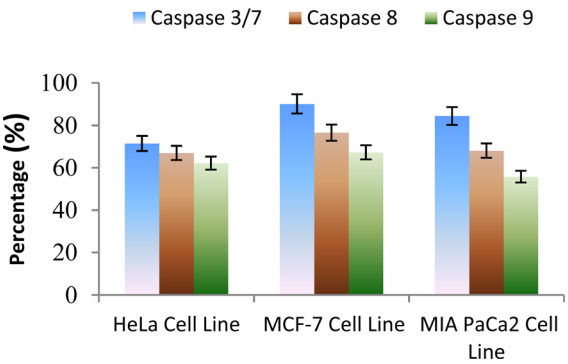


Fig. 7 Effect of compound **6j** against the activation of different caspases in selected cancer cell lines.

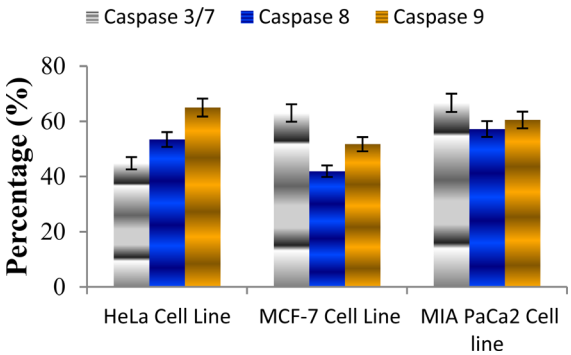


Fig. 8 Effect of compound **6m** against the activation of different caspases in selected cancer cell lines.

Tubulin polymerization inhibition

Targeting the microtubules in the development of anticancer drugs was considered to be an important goal in current medicinal chemistry,[46] as they are involved in a few cellular functions, such as cell division, organelle transport, motility and keeping signal transduction.[47] A few antimitotic agents such as Vinblastine, Colchicine, and Vincristine inhibit tubulin polymerization *via* their binding to the colchicine or vinca-binding sites of tubulins.[48] Curiously, for the past few years, few anti-mitotic agents such as taxanes and vinca alkaloids were used for clinical trials of varied cancer patients.[46,49] However,

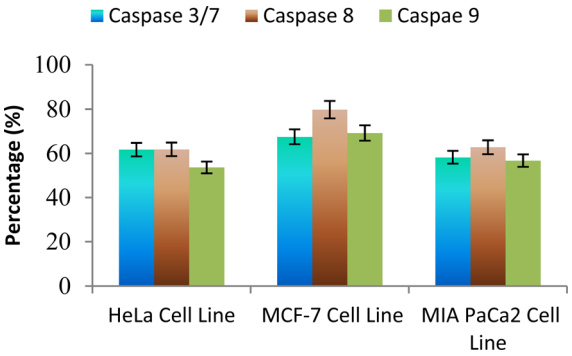


Fig. 9 Effect of compound **6n** against the activation of different caspases in selected cancer cell lines.

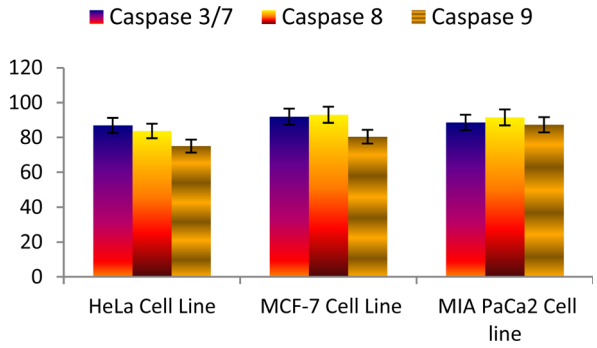


Fig. 10 Effect of podophyllotoxin against the activation of different caspases in the selected cancer cell lines.

Table 3 Tubulin polymerization inhibition studies of compounds **6e**, **6i**, **6j**, **6m** and **6n**

Compound	IC_{50} (μM)
6e	0.88
6i	4.85
6j	1.32
6m	3.79
6n	1.43
Podophyllotoxin	0.84

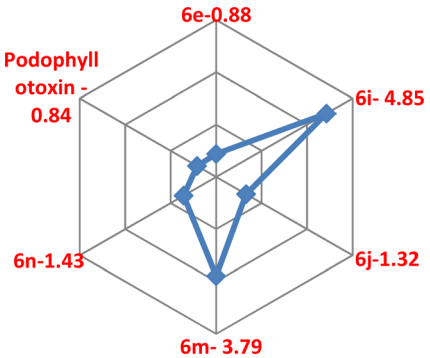


Fig. 11 Tubulin polymerization inhibition studies.

little solubility and oral bioavailability and slightly high toxicity make these anti-mitotic agents less in clinical trials of cancer,[46–50] which were made a crucial need to develop novel anti-mitotic agents. Interestingly, a few reports of C4-ring-modified podophyllotoxin[51] and a few isoxazole[52] derivatives have been recognised to inhibit tubulin polymerization.

Hence, we studied the *in vitro* tubulin polymerization inhibition studies of compounds **6e**, **6i**, **6j**, **6m** and **6n** using a tubulin assembly assay. The results revealed that compound **6e** (IC_{50} = 0.88 μM) showed comparable activity with the podophyllotoxin (IC_{50} = 0.84 μM). As well, compounds **6j** (IC_{50} = 1.32 μM) and **6n** (IC_{50} = 1.43 μM) have shown promising potency in comparison to positive control. However, compounds **6i** (IC_{50} = 4.85 μM) and **6m** (IC_{50} = 3.79 μM) had moderate activity as compared to the positive control (Table 3).

Molecular docking studies

The molecular docking studies of most potent compounds **6e**, **6j** and **6n** found in the above *in vitro* studies and positive control podophyllotoxin were carried out by taking α,β-tubulin (pdb id 1SA0) as target[53] and results are shown in Table 4. It was found that compound **6n** displayed greater binding energy (−9.39 kcal mol^{-1}) and showed 130.79 nM inhibition constant. Compound **6e** was ranked second in this study, with its binding energy as −7.99 kcal mol^{-1} and 1.38 μM as the inhibition constant. Compound **6j** displayed binding energy (−7.84 kcal mol^{-1}) and showed a 1.84 μM as the inhibition constant. On the other aspect, positive control podophyllotoxin showed binding energy = −9.22 kcal mol^{-1} and 173.47 nM as the inhibition constant.

With respect to binding interaction, podophyllotoxin showed π–π stacking with TYR224 residue and compound **6j** formed salt bridge with ARG2 residue.

Overall results revealed that the compound **6n** showed an encouraging binding energy and inhibition constant than the podophyllotoxin (Fig. 10 and 11).

Table 4 Molecular docking results of **6e**, **6j** and **6n** and podophyllotoxin

Entry	Binding energy (kcal mol^{-1})	No. of H-bonds	Inhibition constant
6e	−7.99	—	1.38 μM
6j	−7.84	—	1.80 μM
6n	−9.39	—	130.79 nM
Podophyllotoxin	−9.22	—	173.47 nM

Conclusions

Herein, we, first time developed an aqueous organo-NHC catalyzed 1,3-dipolar cycloaddition between 4β-*O*-propargyl podophyllotoxin (**1**) and *in situ* nitrile oxides to afford regioselective 3,5-di-substituted 4β-isoxazolepodophyllotoxin hybrids (**6a–n**). Compound **6e** was the most potent analogue showing greater anti-cancer activity against MCF-7, HeLa and MIAPACA cell lines with GI_{50} values of 0.18, 0.32 and 0.17 μM, respectively,

compared to the standard drug podophyllotoxin (GI_{50}; 0.31, 0.74 and 0.51 μM, respectively). Compounds **6j** and **6n** showed greater activity (GI_{50} = 0.23 and 0.12 μM, respectively) than the positive control (GI_{50} = 0.31 μM) against the MCF-7 cell line. Furthermore, compounds **6i** (GI_{50} = 0.42 μM) and **6m** (GI_{50} = 0.36 μM) had closer activity to the podophyllotoxin (GI_{50} = 0.31 μM) against the MCF-7 cell line. The results of *in vitro* tubulin polymerization inhibition revealed that compound **6e** (IC_{50} = 0.88 μM) displayed almost similar activity with podophyllotoxin (IC_{50} = 0.84 μM). The molecular docking studies of potent compounds **6e**, **6j**, **6n** and podophyllotoxin as tubulin polymerization inhibitors were found to be supportive of the corresponding *in vitro* activity data.

Data availability

Data will be made available on the reader's request.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors are thankful to Chaitanya (Deemed to be University), Himayathnagar (V), Moinabad (M), Ranga Reddy (D), Hyderabad, India, for providing research facilities.

Notes and references

1 L. M. L. Slevin, *Cancer*, 1991, **67**, 319–329.
2 K. R. Hande, *Eur. J. Cancer*, 1998, **34**, 1514–1521.
3 B. A. Caner and D. L. Longo, *Cancer therapy and biotherapy*, Lippincott-Raven Publishers, New York, 1996.
4 L. Huang, R. L. Wu and A. M. Xu, *Am. J. Transl. Res.*, 2015, **7**, 2141–2158.
5 S. Desben and S. G. Renault, *Curr. Med. Chem.*, 2002, **2**, 71–90.
6 D. Leroy, A. V. Kajava, C. Frei and S. M. Gasser, *Biochemistry*, 2001, **40**, 1624–1634.
7 K. Kobayashi and M. J. Ratain, *Cancer Chemother. Pharmacol.*, 1994, **34**, S64–S68.
8 J. Y. Chang, F. S. Han, S. Y. Liu, Z. Q. Wang, K. H. Lee and Y. C. Cheng, *Cancer Res.*, 1991, **51**, 195–198.
9 R. M. Moraes, F. E. Dayan and C. Canel, *Stud. Nat. Prod. Chem.*, 2002, **26**, 149–182.
10 T. L. MacDonald, E. K. Lehnert, J. T. Loper, K. C. Chow and W. E. Ross, Oxford University Press, New York, 1991, p. 119.
11 S. J. Cho, A. Tropsha, M. Suffness, Y. C. Cheng and K. H. J. Lee, *Med. Chem.*, 1996, **39**, 1383–1395.
12 Z. Xiao, Y. D. Xiao, J. Feng, A. Golbraikh, A. Tropsha and K. H. J. Lee, *Med. Chem.*, 2002, **45**, 2294–2309.
13 T. Terada, K. Fujimoto, M. Nomura, J. Yamashita, K. Wierzba, R. Yamazaki, J. Shibata, Y. Sugimoto, Y. Yamada, T. Kobunai, S. Takeda, Y. Minami, K. Yoshida and H. J. Yamaguchi, *Med. Chem.*, 1993, **36**, 1689–1699.
14 (*a*) A. Kamal, B. A. Kumar, M. Arifuddin and S. G. Dastidar, *Bioorg. Med. Chem.*, 2003, **11**, 5135–5142; (*b*) A. Kamal, N. L. Gayatri, D. R. Reddy, P. S. M. M. Reddy, M. Arifuddin, S. G. Dastidar, A. K. Kondapi and M. Rajkumar, *Bioorg. Med. Chem.*, 2005, **13**, 6218–6225; (*c*) A. Kamal, E. Laxman, G. B. R. Khanna, P. S. M. M. Reddy, T. Rehana, M. Arifuddin, K. Neelima, A. K. Kondapi and S. G. Dastidar, *Bioorg. Med. Chem.*, 2004, **12**, 4197–4201; (*d*) A. Kamal, B. A. Kumar, M. Arifuddin and S. G. Dastidar, *Lett. Drug Des. Discovery*, 2006, **3**, 205–209; (*e*) A. Kamal and B. A. Kumar, WO136018, 2008; (*f*) A. Kamal, B. A. Kumar and M. Arifuddin, WO07339, 2004.
15 P. Dong, K. Rakesh, H. Manukumar, Y. H. E. Mohammed, C. Karthik, S. Sumathi, P. Mallu and H. L. Qin, *Bioorg. Chem.*, 2019, **85**, 325–336.
16 X. Zhang, K. Rakesh, C. Shantharam, H. Manukumar, A. Asiri, H. Marwani and H. L. Qin, *Bioorg. Med. Chem.*, 2018, **26**, 340–355.
17 W. Y. Fang, L. Ravindar, K. Rakesh, H. Manukumar, C. Shantharam, N. S. Alharbi and H. L. Qin, *Eur. J. Med. Chem.*, 2019, **173**, 117–153.
18 H. Liu, S. Long, K. Rakesh and G. F. Zha, *Eur. J. Med. Chem.*, 2020, **185**, 111804.
19 M. Xu, Y. Peng, L. Zhu, S. Wang, J. Ji and K. P. Rakesh, *Eur. J. Med. Chem.*, 2019, **180**, 656–672.
20 (*a*) M. R. Sivala, V. Chintha, K. M. Potla, S. Chinnam and N. R. Chamarthi, *Signal Transduction Res.*, 2020, **40**, 486–492; (*b*) Md. Elagawany, L. Maram and B. Elgendy, *J. Org. Chem.*, 2023, **88**, 17062–17068.
21 F. Hu and M. Szostak, *Adv. Synth. Catal.*, 2015, **357**, 2583–2614.
22 G. Boyd, E. Fangheanel, D. Gudat, D. Kikelj and C. T. Pedersen, Hetarenes and Related Ring Systems, in *Science of Synthesis: Houben-Weyl Methods of Molecular Transformations*, vol. 11, ed. E. Schaumann, Thieme Chemistry, 2014.
23 (*a*) J. Zhua, J. Moa, H. Z. Lina, Y. Chenb and H. Suna, *Bioorg. Med. Chem.*, 2018, **26**, 3065–3075; (*b*) G. Ch. Arya, K. Kaur and V. Jaitak, *Eur. J. Med. Chem.*, 2021, **221**, 113511; (*c*) A. Sysak and B. O. Mrukowicz, *Eur. J. Med. Chem.*, 2017, **137**, 292–309.
24 F. Hua and M. Szostak, *Adv. Synth. Catal.*, 2015, **357**, 2583–2614.
25 (*a*) G. Scott and V. V. Fokin, *Angew. Chem.*, 2008, **120**, 8409–8411; (*b*) F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless and V. V. Fokin, *J. Am. Chem. Soc.*, 2005, **127**, 210–216; (*c*) T. V. Hansen, P. Wu and V. V. Fokin, *J. Org. Chem.*, 2005, **70**, 7761–7764.
26 (*a*) B. List, *Chem. Rev.*, 2007, **107**, 5413–5415; (*b*) S. Bertelsen and K. A. Jorgensen, *Chem. Soc. Rev.*, 2009, **38**, 2178–2189; (*c*) C. Grondal, M. Jeanty and D. Enders, *Nat. Chem.*, 2010, **2**, 167–178; (*d*) W. Liu, H. Cao, H. Zhang, H. Zhang, K. H. Chung, C. He, H. Wang, F. Y. Kwong and A. Lei, *J. Am. Chem. Soc.*, 2010, **132**, 16737–16740; (*e*) L. S. Hegedus, *J. Am. Chem. Soc.*, 2009, **131**, 17995–17997.
27 S. Kankala, R. Vadde and C. S. Vasam, *Org. Biomol. Chem.*, 2011, **9**, 7869–7876.

28 (*a*) R. Breslow, *J. Am. Chem. Soc.*, 1958, **80**, 3719–3726; (*b*) H. Stetter, *Angew. Chem., Int. Ed. Engl.*, 1976, **15**, 639–647; (*c*) D. Enders, O. Niemeier and A. Henseler, *Chem. Rev.*, 2007, **107**, 5606–5655; (*d*) N. Marion, S. Diez-Gonzalez and S. P. Nolan, *Angew. Chem., Int. Ed.*, 2007, **46**, 2988–3000; (*e*) W. D. Jones, *J. Am. Chem. Soc.*, 2009, **131**, 15075–15077; (*f*) P. Chiang, M. Rommel and J. W. Bode, *J. Am. Chem. Soc.*, 2009, **131**, 8714–8718; (*g*) S. D. Sarkar and A. Studer, *Org. Lett.*, 2010, **12**, 1992–1995; (*h*) J. Pinaud, K. Vijayakrishna, D. Taton and Y. Gnanou, *Macromolecules*, 2009, **42**, 4932–4936; (*i*) N. E. Kamber, W. Jeong, S. Gonzalez, J. L. Hedrick and R. M. Waymouth, *Macromolecules*, 2009, **42**, 1634–1639; (*j*) E. M. Phillips, A. Chan and K. A. Scheid, *Aldrichim. Acta*, 2009, **42**, 55–66; (*k*) X. Bugaut, F. Liu and F. Glorius, *J. Am. Chem. Soc.*, 2011, **133**, 8130–8133; (*l*) A. T. Biju, N. E. Wurz and F. Glorius, *J. Am. Chem. Soc.*, 2010, **132**, 5970–5971; (*m*) J. Mahatthananchai, P. Zheng and J. W. Bode, *Angew. Chem., Int. Ed.*, 2011, **50**, 1673–1677; (*n*) V. Nair, S. Bindu and V. Sreekumar, *Angew. Chem., Int. Ed.*, 2004, **43**, 5130–5135; (*o*) A. B. Powell, Y. Suzuki, M. Ueda, C. W. Bielawski and A. H. Cowley, *J. Am. Chem. Soc.*, 2011, **133**, 5218–5220; (*p*) Y. Wang, Y. Xie, M. Y. Abraham, P. Wei, H. F. Schaefer III, P. v. R. Schleyer and G. H. Robinson, *J. Am. Chem. Soc.*, 2010, **132**, 14370–14372; (*q*) S. Kankala, R. Edulla, S. Modem, R. Vadde and C. S. Vasam, *Tetrahedron Lett.*, 2011, **52**, 3828–3831; (*r*) D. Du, L. Li and Z. Wang, *J. Org. Chem.*, 2009, **74**, 4379–4382.

29 (*a*) A. J. Arduengo, R. L. Harlow and M. Kline, *J. Am. Chem. Soc.*, 1991, **113**, 361–363; (*b*) A. J. Arduengo III, *Acc. Chem. Res.*, 1999, **32**, 913–921; (*c*) D. Bourissou, O. Guerret, F. P. Gabba and G. Bertrand, *Chem. Rev.*, 2000, **100**, 39–92; (*d*) L. Benhamou, E. Chardon, G. Lavigne, S. Bellemin-Laponnaz and V. Cesar, *Chem. Rev.*, 2011, **111**, 2705–2733; (*e*) D. Tapu, D. A. Dixon and C. Roe, *Chem. Rev.*, 2009, **109**, 3385–3407; (*f*) A. J. Arduengo III and L. I. Iconaru, *Dalton Trans.*, 2009, 6903–6914.

30 (*a*) R. M. de Figueiredo and M. Christmann, *Eur. J. Org Chem.*, 2007, 2575–2600; (*b*) E. Marques-Lopez, R. P. Herrera and M. Christmann, *Nat. Prod. Rep.*, 2010, **27**, 1138–1167; (*c*) C. Vaxelaire and P. W. M. Christmann, *Angew. Chem., Int. Ed.*, 2011, **50**, 3605–3607.

31 (*a*) N. Marion, S. Diez-Gonzalez and S. P. Nolan, *Angew. Chem., Int. Ed.*, 2007, **46**, 2988–3000; (*b*) A. J. Arduengo III and L. I. Iconaru, *Dalton Trans.*, 2009, 6903–6914; (*c*) A. T. Biju, N. Kuhl and F. Glorius, *Acc. Chem. Res.*, 2011, **44**, 1182–1195; (*d*) V. Nair, R. S. Menon, A. T. Biju, C. R. Sinu, R. R. Paul, A. Jose and V. Sreekumar, *Chem. Soc. Rev.*, 2011, **40**, 5336–5346; (*e*) A. Grossmann and D. Enders, *Angew. Chem., Int. Ed.*, 2012, **51**, 314–325; (*f*) X. Bugaut and F. Glorius, *Chem. Soc. Rev.*, 2012, **41**, 3511–3522.

32 C. Burstein and F. Glorius, *Angew. Chem., Int. Ed.*, 2004, **43**, 6205–6208.

33 (*a*) S. S. Sohn and J. W. Bode, *Angew. Chem., Int. Ed.*, 2006, **45**, 6021–6024; (*b*) M. He, B. J. Beahm and J. W. Bode, *Org. Lett.*, 2008, **10**, 3817–3820.

34 H. U. Vora and T. Rovis, *J. Am. Chem. Soc.*, 2010, **132**, 2860–2861.

35 J. M. Obrien and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2011, **133**, 7712–7715.

36 (*a*) T. L. Amyes, S. L. Diver, J. P. Richard, F. M. Rivas and K. J. Toth, *J. Am. Chem. Soc.*, 2004, **126**, 4366–4374; (*b*) O. Holloczki, P. Terleczky, D. Szieberth, G. Mourgas, D. Gudat and L. Nyulaszi, *J. Am. Chem. Soc.*, 2011, **133**, 780–789.

37 R. Breslow, *J. Am. Chem. Soc.*, 1958, **80**, 3719.

38 W. Wen, Y. Leong, X. Chen and Y. R. Chi, *Green Chem.*, 2013, **15**, 1505–1508.

39 (*a*) N. S. Thirukovela, R. Balaboina, V. Botla, R. Vadde, S. B. Jonnalagadda and C. S. Vasam, *Catal. Sci. Technol.*, 2019, **9**, 6471–6481; (*b*) N. S. Thirukovela, R. Balaboina, S. Kankala, R. Vadde and C. S. Vasam, *Tetrahedron*, 2019, **75**, 2637–2641; (*c*) N. S. Thirukovela, R. Balaboina, R. Vadde and C. S. Vasam, *Tetrahedron Lett.*, 2018, **59**, 3749–3752; (*d*) M. Muqeed, R. Manchal, V. Botla, Md. Azam, C. S. Vasam and N. S. Thirukovela, *Asian J. Org. Chem.*, 2023, **12**, e202300356; (*e*) R. R. Sagam, S. K. Nukala, R. Nagavath, N. Sirassu, M. Mohammod, R. Manchal and N. S. Thirukovela, *J. Mol. Struct.*, 2022, **1268**, 133692; (*f*) R. Nagavath, S. K. Nukala, N. Sirassu, R. R. Sagam, R. Manchal, S. Paidakula and N. S. Thirukovela, *J. Mol. Struct.*, 2022, **1250**, 131724; (*g*) P. K. Kannekanti, S. K. Nukala, M. Bangaru, N. Sirassu, R. Manchal and N. S. Thirukovela, *ChemistrySelect*, 2023, **8**, e202204010; (*h*) M. Bangaru, S. K. Nukala, P. K. Kannekanti, N. Sirassu, R. Manchal and N. S. Thirukovela, *ChemistrySelect*, 2023, **8**, e202204414; (*i*) N. S. Thirukovela, S. K. Nukala, N. Sirassu, R. Manchal, P. Gundepaka and S. Paidakula, *ChemistrySelect*, 2020, **5**, 12317–12319; (*j*) R. R. Sagam, S. K. Nukala, R. Nagavath, N. Sirassu, P. Gundepaka, R. Manchal and N. S. Thirukovela, *ChemistrySelect*, 2021, **6**, 7670–7673; (*k*) R. Nagavath, S. K. Nukala, R. R. Sagam, N. Sirassu, V. Guguloth, P. Kamarajugadda, S. Paidakula and N. S. Thirukovela, *ChemistrySelect*, 2022, **7**, e202202200.

40 N. Srinivas, K. Shravan and G. Brahmeshwari, *Nat. Prod. Res.*, 2019, **35**, 9–16.

41 (*a*) G. C. Senadi, M. R. Mutra, T. Y. Lu and J. J. Wang, *Green Chem.*, 2017, **19**, 4272–4277; (*b*) Y. H. T. Sai, C. M. B. Etichetti, S. Cicetti, J. E. Girardini, R. A. Spanevello, A. G. Suarez and A. M. Sarotti, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127247.

42 (*a*) V. Nair, A. N. Pillai, P. B. Beneesh and E. Suresh, *Org. Lett.*, 2005, **7**, 4625–4628; (*b*) V. Nair, A. N. Pillai, R. S. Menon and E. Suresh, *Org. Lett.*, 2005, **7**, 1189–1191; (*c*) V. Nair, S. Bindu, V. Sreekumar and N. P. Rath, *Org. Lett.*, 2003, **5**, 665–667; (*d*) Y. L. Wu, P. D. Jarowski, W. B. Schweizer and F. Diederich, *Chem.–Eur. J.*, 2010, **16**, 202–211; (*e*) V. Nair, R. S. Menon, P. B. Beneesh, V. Sreekumar and S. Bindu, *Org. Lett.*, 2004, **6**, 767–769; (*f*) X. F. Zhu, C. E. Henry and O. Kwon, *J. Am. Chem. Soc.*, 2007, **129**, 6722–6723; (*g*) M. R. Siebert, A. K. Yudin and D. J. Tantillo, *Org. Lett.*, 2008, **10**, 57–60.

43 S. J. Martin and D. R. Green, *Cell*, 1995, **82**, 349–352.

44 J. F. Kerr, A. H. Wyllie and A. R. Currie, *Br. J. Cancer*, 1972, **26**, 239–257.
45 S. Arandjelovic and K. S. Ravichandran, *Nat. Immunol.*, 2015, **16**, 907–917.
46 M. A. Jordan and L. Wilson, *Nat. Rev. Cancer*, 2004, **4**, 253–265.
47 (*a*) J. Ceramella, A. Caruso, M. A. Occhiuzzi, D. Iacopetta, A. Barbarossa, B. Rizzuti, P. Dallemagne, S. Rault, H. El. Kashef, C. Saturnino and M. S. Sinicropi, *Eur. J. Med. Chem.*, 2019, **1**, 111583; (*b*) K. H. Downing, *Annu. Rev. Cell Dev. Biol.*, 2000, **16**, 89–111; (*c*) H. S. Mohamed, N. H. Amin, M. T. El-Saadi and H. M. A. Rahman, *Bioorg. Chem.*, 2022, **16**, 105687.
48 H. Chen, Z. Lin, K. E. Arnst, D. D. Miller and W. Li, *Molecules*, 2017, **22**, 1281.
49 G. Wang, C. Li, L. He, K. Lei, F. Wang, Y. Pu, Z. Yang, D. Cao, L. Ma, J. Chen, Y. Sang, X. Liang, M. Xiang, A. Peng, Y. Wei and L. Chen, *Bioorg. Med. Chem.*, 2014, **22**, 2060–2079.
50 S. Messaoudi, B. Treguier, A. Hamze, O. Provot, J. F. Peyrat, J. R. De Losada, J. M. Liu, J. Bignon, J. W. Bakala, S. Thoret, J. Dubois, J. D. Brion and M. Alami, *J. Med. Chem.*, 2009, **52**, 4538–4542.
51 (*a*) I. Hyder, Y. Deepthi, K. Shasi, J. Khazir, N. Naresh, M. Sreekanth, M. Halmuthur and S. Kumar, *Bioorg. Med. Chem. Lett.*, 2015, **14**, 2860–2863; (*b*) Y. Liu, D. Wei, Y. Zhao, W. Cheng, Y. Lu, Y. Ma, L. Xin, C. Han, Y. Wei, H. Cao and Z. Chunyan, *Bioorg. Med. Chem. Lett.*, 2012, **20**, 6285–6295; (*c*) L. Zhang, X. Zeng, X. Ren, N. Tao, C. Yang, Y. Xu, Y. Chen and J. Wang, *Med. Chem. Res.*, 2019, **28**, 81–94; (*d*) Z. Zhen Wang, W. Xue Sun, X. Wang, Z. Y. Han, Q. H. Yue, Q. J. Liang, P. Y. Jun, L. G. Hua, X. W. Ming, Y. F. Gen and Y. Y. H. Yang, *Chem. Biol. Drug Des.*, 2017, **2**, 236–243; (*e*) M. V. P. S Vishnuvardhan, V. S. Reddy, K. Chandrasekhar, V. Lkshma Nayak, S. D. I. Bin, A. Abdullah and A. Kamal, *MedChemComm*, 2017, **8**, 1817–1823; (*f*) Y. Chengli, X. Qiongli, X. Zeng, T. Nengyin, Y. Xu, C. Yongzhen, W. Jing and L. Zhang, *Bioorg. Chem.*, 2019, **85**, 445–454.
52 (*a*) D. J. Fu, S. M. Liu, F. H. Li, J. J. Yang and J. Li, *J. Enzyme Inhib. Med. Chem.*, 2020, **35**, 1050–1059; (*b*) G. Wang, W. Liu, Y. Huang, Y. Li and Z. Peng, *Arabian J. Chem.*, 2020, **13**, 5765–5775; (*c*) K. D. Shin, Y. J. Yoon and Y. R. Kang, *Biochem. Pharmacol.*, 2008, **75**, 383–394.
53 R. Ravelli, B. Gigant, P. Curmi, I. Jourdain, S. Lachkar, A. Sobel and M. Knossow, *Nature*, 2004, **428**, 198–202.