REVIEW

β-Carboline-based molecular hybrids as anticancer agents: a brief sketch

Jay Prakash Soni, [iD]† Yogesh Yeole† and Nagula Shankaraiah [iD]*

Cancer is a huge burden on the healthcare system and is foremost cause of mortality across the globe. Among various therapeutic strategies, chemotherapy plays an enormous role in overcoming the challenges of treating cancer, especially in late stage detection. However, limitations such as extreme side/adverse effects and drug resistance associated with available drugs have impelled the development of novel chemotherapeutic agents. In this regard, we have reviewed the development of β-carboline-based chemotherapeutic agents reported in last five years. The review mainly emphasizes on the molecular hybrids of β-carbolines with various pharmacophores, their synthetic strategies, and *in vitro* anticancer evaluation. In addition, the mechanisms of action, *in silico* studies, structural influence on the potency and selectivity among diverse cancer cell lines have been critically presented. The review updates readers on the diverse molecular hybrids prepared and the governing structural features of high potential molecules that can help in the future development of novel cytotoxic agents.

1. Introduction

Cancer is now a leading cause of mortality and a huge burden to the human healthcare system world-wide. It may originate from genetic interferences or environmental causes or a combination of both. Among more than 100 different type of cancers, breast, lung, prostate, and colorectal cancer cases are dominant. Oral and laryngopharynx cancer are another notable cause of

Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad, 500037, India.
E-mail: shankar.niperhyd@gov.in, shankarnbs@gmail.com
† These authors contributed equally.

mortality, which might be attributed to the use of tobacco.[1] In this regard, natural products with great diversity play dominant roles in human life and daily activities including the treatment of health problems.[2a,b] Among such natural products, β-carboline alkaloids such as harmine, vasicine, and harmaline are present in the seeds of *Peganum harmala* (*Zygophyllaceae*), which are basically tricyclic pyrido[3,4-*b*]indole ring systems.[2c] The different class of β-carbolines belong to completely aromatized, dihydro, or tetrahydro pyridine moiety with diverse biological significance as anti-inflammatory,[2d] antidepressant,[2e] antimalarial,[2f] antileshmeniasis,[2g] antidiabetic,[2h] anticancer,[2f,i–k] antioxidant,[2l] and antianxiety[2m] (Fig. 1).[2]

Mr. Jay Prakash Soni was born in 1993, in Madhya Pradesh, India. He completed his graduation in Pharmacy from VNS Institution of Pharmacy, Bhopal in 2015 and received his M.S. (Pharm) degree from the National Institute of Pharmaceutical Education and Research (NIPER) S.A.S. Nagar, Punjab, India. Later, he joined Dr. N. Shankaraiah's research group in July 2017 as a PhD scholar at NIPER-Hyderabad. His

Jay Prakash Soni

research interest involves the design and synthesis of NCEs with potential cytotoxic profile and molecular modelling studies.

Mr. Yogesh Bapusaheb Yeole was born in Maharashtra, India. He graduated with a B. Pharma degree from Dr. D. Y. Patil Institute of Pharmaceutical Science and Research, Pune, India. Currently, he is pursuing his M.S. (Pharm.) degree at the National Institute of Pharmaceutical Education and Research, Hyderabad, India; and working in Dr. N. Shankaraiah's group.

Yogesh Yeole

The β-carboline scaffold constitutes the chief motif of several pharmacological agents and commercially available drugs with different therapeutic applications, such as yohimbine and tadalafil (used in erectile dysfunction), vinpocetine (used for cerebrovascular disorder), abecarnil (used as antianxietic), vincamine (used as peripheral vasodilator), rescinnamine (used as ACE inhibitor), ajmaline (used as antiarrhythmiatic), reserpine (used as antihypertensive), and deserpidine (used as antipsychotic and antihypertensive) (Fig. 2).[3] The anticancer/cytotoxic effect of β-carboline alkaloids is mainly attributed to their ability to target DNA,[4a] the modulation of p-53 signaling pathways,[2j] inhibition of topoisomerase I and II,[4b] histone deacetylase,[4c] telomerase,[4d] minor groove binding,[4a] kinesin spindle protein (KSP-ATPase),[4e] and other kinases.[4f] The photolytic cleavage of DNA has also been reported for various β-carboline derivatives.[4g,h] Apart from simple derivatives and metal complexes of natural β-carbolines, the molecular hybridization approach has been extensively explored with several pharmacophoric scaffolds to achieve more potent and promising molecules, especially with potential cytotoxic profile.[5a,b] Molecular hybridization not only helps to improve the potency/efficacy of molecules but also important to overcome drug resistance due to the involvement of more than one biological target and different mechanisms of action.[5c–e]

Some representative reviews published previously revolve around certain aspects of β-carbolines obtained either from natural source or synthetic molecules.[6] Zhang et al., in 2015,[5b] collated the anticancer activity of β-carbolines with their structure–activity relationship (SAR) and mechanism of actions, followed by a review on pharmacological and toxicological aspects of this scaffold by Khan et al., in 2017.[6a] Similarly, Kumar and co-workers updated the development of anticancer β-carboline derivatives and metal complexes

reported during 2011–2016.[6b] Wang and co-authors reviewed the monomeric and dimeric units of β-carboline alkaloids with an emphasis on their occurrences, structural diversity, and associated biological responses.[6c] Sahoo et al., in 2019, focused on natural norharmane alkaloids regarding their synthesis and SAR in connection to their cytotoxic abilities.[6d] Recently, Manasa et al., provided an assessment regarding β-carboline natural alkaloids and their derivatives with anticancer potential along with the SAR.[6e] An interesting review published by Piechowska et al., emphasized the presence of β-carbolines in food items, which reduces the risk of neurodegenerative diseases.[6f] In the present review, we aim to provide an update on the recent developments (2015–2020) of β-carboline-based hybrid molecules with anticancer potential. The review describes the synthetic strategies, in vitro anticancer potential, and mechanisms of action of such hybrids.

2. Synthesis of functionalized β-carboline framework

The synthesis of β-carboline core structures bearing different functional groups has been summarized in Scheme 1. The presence of multiple functionalities and their straight-forward conversion to other functional groups has enabled the demanding coupling of β-carboline core with additional pharmacophores.[7] L-Tryptophan (1, an amino acid) is the most often used starting material, which was readily esterified with an alcohol in acidic medium, followed by Pictet–Spengler cyclization, which afforded tetrahydro-β-carbolines (3). Alternatively, the cyclization of tryptamine (6) also delivered tetrahydro-β-carbolines (7), which on aromatization yielded β-carboline 4.[8a–c] The tetrahydro-β-carbolines 3 were either subjected to decarboxylative aromatization to form simple β-carboline core (4)[8d–f] or aromatized using different oxidative reagents such as KMnO$_4$ or S$_8$ to obtain aromatic β-carboline esters (5).[8g] The reduction of esters (5) using LiBH$_4$ or LiAlH$_4$ furnished alcohol (8), which upon subsequent oxidation with DMP/MnO$_2$ allowed the formation of β-carboline aldehyde (9).[8f] On the other hand, the azidation of alcohol (8) directly with DPPA or sulphonation with mesyl/tosyl to 10, followed by azidation with NaN$_3$, produced β-carboline azides (11), which upon further reduction afforded β-carboline amine (12, Scheme 1).[8h,i]

As briefed, the primary functional groups present in β-carboline are acid/ester, alcohol, aldehyde, and amine, which can be coupled with different pharmacophoric moieties to produce molecules with potential biological profile. Apart from these functionalities, β-carboline ester (5) was converted to its corresponding carbohydrazides (13) by treatment with hydrazine hydrate, followed by diazotization to carbonyl azides (14), which further transformed to β-carboline amine (15) by means of Curtius rearrangement (Scheme 1).[8j,k] The literature discussed in the successive part describes the complete synthetic strategies using any of the

Nagula Shankaraiah

Dr. Nagula Shankaraiah was born in 1976 in India. He obtained his B.Sc. and M.Sc. degree in Chemistry from Kakatiya University, Warangal. He completed his Ph.D. under the guidance of Dr. Ahmed Kamal at CSIR-IICT, Hyderabad. Later, he worked with Prof. Leonardo S. Santos at the University of Talca as a post-doctoral fellow and also worked in UINICAMP, Brazil. Currently, he is an Associate Professor at the Department of Medicinal Chemistry at NIPER-Hyderabad. His prime research interest focuses on the design and synthesis of cytotoxic NCEs for cancer chemotherapy and the development of sustainable methodologies involving click reactions, C–H activation, and one-pot domino reactions.
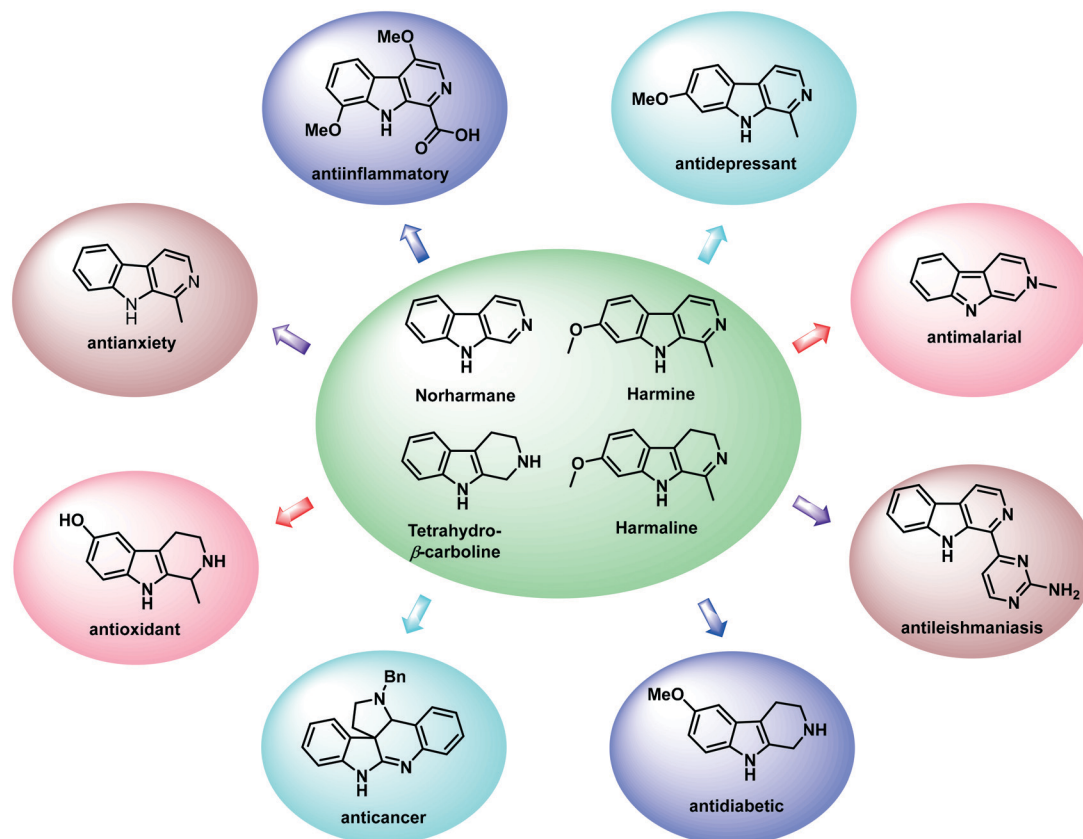
Fig. 1 Structural diversity of the β-carboline scaffold and various biologically-relevant molecules.
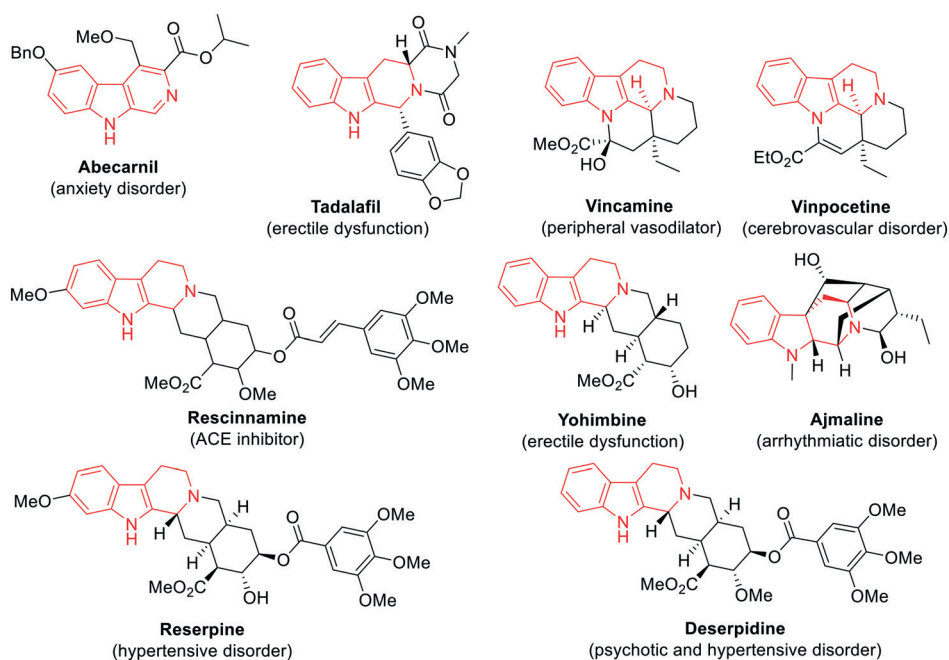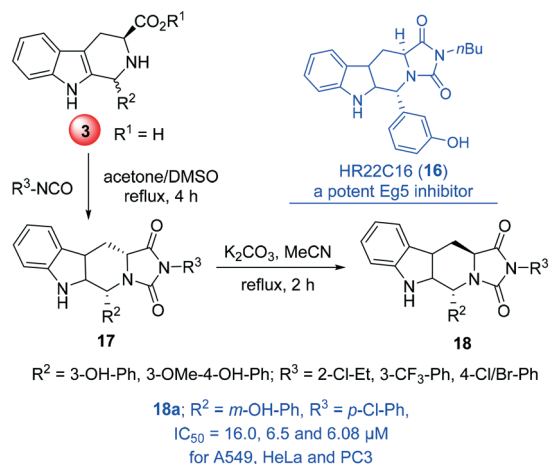

Fig. 2 Representative β-carboline containing molecules in clinical use.
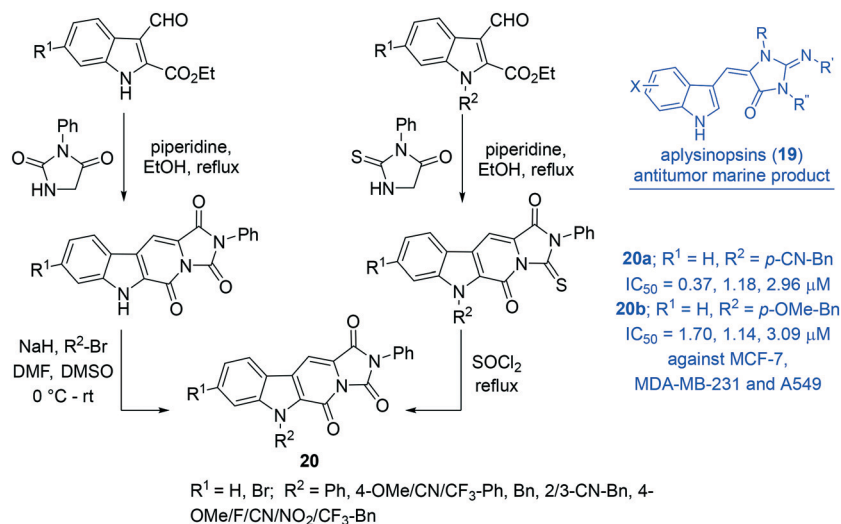
reagents/conditions presented in Scheme 1 and should be considered for citations. However, only some of the synthetic works/reviews that have not been involved in biological studies are cited in this section.

Scheme 1 Synthesis of the β-carboline core with various functional groups (*reg = reagents).

Reagents and conditions: *reg 1*: SOCl$_2$, MeOH, rt, 12 h; *reg 2*: (a) 5% TFA in CH$_2$Cl$_2$, rt, 8 - 12 h; or (b) *p*TSA, toluene, reflux, 8 - 10 h; or (c) AcOH, reflux, 3 h; or (d) 0.1N H$_2$SO$_4$ reflux, 5 h; or (e) NaOH, H$_2$O, reflux, 3 h; or (f) H$_2$SO$_4$, H$_2$O, rt, 3 h; *reg 3*: (a) NCS, TEA, DMF, rt, 2 - 4 h; or (b) AcOH, K$_2$CrO$_7$, NaHSO$_3$, NaOH, 100 °C, 30 min; *reg 4*: (a) S$_8$, xylene, reflux, 12 - 15 h; or (b) KMnO$_4$, THF/DMF, rt, 8 - 12 h; or (c) TCC, DMF, TEA, -20 °C - rt, 2 h; or (d) TCICA, TEA, DMF, 0 °C - rt, 2 h; or (f) CuBr, DBU, DMSO, rt; *reg 5*: LiBH$_4$/LiAlH$_4$, THF, 0 °C - rt, 4 h; *reg 6*: (a) MnO$_2$, MeCN, reflux, 2 h; or (b DMP, CH$_2$Cl$_2$, rt, 3 h; *reg 7*: DPPA, DBU, dry THF, rt, 12 - 16 h; *reg 8*: (a) MsCl, TEA, DMF, 4 h; or (b) TsCl, DMAP, TEA, CH$_2$Cl$_2$, 0 °C - rt, 2 h; *reg 9*: NaN$_3$, DMF, rt, 5 h; *reg 10*: TPP, MeCN:H$_2$O (1:1), rt, 12 - 16 h; *reg 11*: DIBAL-H, THF/dioxane, -78 °C, 4 - 6 h ; *reg 12*: NH$_2$NH$_2$·H$_2$O, EtOH, reflux, 4 h; *reg 13*: NaNO$_2$, HCl, H$_2$O, 0 °C, 6 h; *reg 14*: AcOH, H$_2$O, reflux, 6 h.

3. β-Carboline hybrids as cytotoxic agents

3.1. β-Carboline-hydantoin hybrids

In continuation of our extensive efforts for finding new potent cytotoxic scaffolds based on different heterocycles,[9a–i] in 2014, we started working on derivatives/hybrids of β-carboline with the same integrity. These β-carboline hybrids may hold great promise as therapeutic agents for intervention in human cancers. HR22C16 (compound **16**, Scheme 2), a small-molecular probe for studying the dynamics of cell division, was identified in 2003.[10a] Based on this potent Eg5 inhibitor, new tetrahydro-β-carboline-hydantoin hybrids were prepared and their *in vitro* anticancer potential was evaluated. The synthesis was accomplished by the reaction of tetrahydro-β-carboline acid (**3**) with isocyanates (compounds **17** and **18**, Scheme 2). Among the synthesized library; compound **18a**

(with *meta*-hydroxyphenyl at C1 and *para*-chlorophenyl in hydantoin) was found to be significantly cytotoxic with IC$_{50}$ values of 16.0, 6.5, and 6.08 μM against the A549, HeLa, and PC3 cell lines, respectively, which were close to the IC$_{50}$ of etoposide (50.0, 4.71, and 14.4 μM). In addition, compound **18a** was found to be 5-fold more potent against the PC3 cell line as compared to normal prostate epithelial cells (RWPE), which indicated superior selectivity towards the cancer cells. The morphological changes of the tested cells such as membrane blebbing, cell shrinkage, apoptotic body formation, and chromatin condensation with the destructive fragmentation of the nucleus indicated the induction of cell apoptosis.[10b]

Inspired from natural marine product aplysinopsins (**19**), aromatic N^9-substituted β-carboline-hydantoin hybrids were designed and synthesized to restrict the conformational flexibility (compounds **20**, Scheme 3).[10c] The prepared

Scheme 2 Synthesis of β-carboline-hydantoin hybrids with anticancer activity.

Fig. 3 Structural features of β-carboline-hydantoin hybrids producing highly potent frameworks.

library was evaluated against various human cancer cell lines and their potency was compared with that of 5-fluorouracil (5-FU). The hybrids produced satisfactory results during the *in vitro* assay, wherein compound **20a** and **20b** showed potent IC_{50} values of 0.37, 1.18, 2.96 and 1.70, 1.14, 3.09 μM on MCF-7, MDA-MB-231, and A549, respectively. Interestingly, compound **20a** (N^9-*p*-CN-Bn) was found to be 83-fold more potent for MCF-7 compared to 5-FU and displayed high selectivity towards cancer cells. Electron-rich benzyl substitution with N^9-*p*-OMe-Bn also produced the highly potent molecule **20b**. The molecules with strong electron-withdrawing groups such as *p*-NO_2-Bn and *p*-CF_3-Bn were poorly active, while the slight electro-negative substitutions, such as *p*-CN/F, were showed a potency similar to that of **20b**. An attempt was also made to check the effect of substitution at various positions, where in contrast to the *para*-position, substitution at other positions decreased the potency (Fig. 3).[10d]

3.2. β-Carboline-chalcone/pyrazole hybrids

In the field of medicinal chemistry, chalcones have a unique impact on the broad spectrum of biological activity including anticancer potential.[11a] The hybrids of chalcones with β-carboline were designed and synthesized through the reaction of β-carboline aldehydes (**9**) with aryl/heteroaryl ketones in the presence of barium hydroxide (compounds **21**, Scheme 4). Further, the hybrids were evaluated for their *in vitro* cytotoxicity against various cancer cell lines and tested for their safety profile on normal cells. Among the series (with Ar ≠ heterocycles), some of the compounds showed an effective cytotoxic profile against A549 cells in less than 10 μM inhibitory concentrations. However, the compounds showed a significant elevation in the thermal denaturation (ΔT_m) of DNA in comparison to doxorubicin. Moreover, compound **21a** (Ar = 2-furanyl) showed a potent IC_{50} of 1.86 μM for MCF-7 (doxorubicin IC_{50} = 2.18 μM); also, significant potency was observed for other cancer cell lines. In addition, these hybrids showed no significant toxicity towards normal human embryonic kidney cells (HEK-293). The biological activity of these hybrids was attributed to the

Scheme 3 Synthesis of β-carboline-1-one-hydantoin hybrids with restricted conformation.

Ar = 4-F/OH/NO$_2$/NH$_2$/OMe-Ph, 3,4,5-OMe$_3$-Ph, 2-pyrrolyl/furanyl/thienyl
R^2 = 3-Cl-Ph, 4-F/CF$_3$-Ph, 3,4-OMe$_2$/F$_2$-Ph, 4-F-3-OMe-Ph, 3,4,5-OMe$_3$-Ph

21a; Ar = 2-furanyl, R^2 = *p*-CF$_3$-Ph; IC$_{50}$ = 1.86 μM (MCF-7)
22a; Ar = 2-furanyl, R^2 = *p*-CF$_3$-Ph; IC$_{50}$ = 2.39, 1.94, 2.63 μM for A549, MCF-7, HeLa
Act by • Induction of apoptosis • Photocleavage of DNA • Inhibition of Topo I

Scheme 4 Synthesis of β-carboline-chalcone/pyrazole hybrids.

ability to induce apoptosis, photo-cleavage of DNA, and inhibition of topoisomerase I.[11b] 4-Fluoro-3-methoxy-phenyl at the C1 of β-carboline with 4-methoxyphenylchalcone displayed the highest potency on A549. Similarly, the trimethoxyphenyl group at C1-β-carboline and chalcones produced significant activity with IC$_{50}$ of 5.30 μM and 3.67 μM for A549 and B-16, respectively. The heteroaryl group (2-furanyl) on chalcone with *p*-CF$_3$-Ph at C1-β-carboline resulted in a highly potent molecule. The *in silico* molecular modelling studies were in agreement with the experimental results and showed DNA intercalation as the binding mode. Barring the stacking of the β-carboline moiety within the DNA base pairs, alternative binding to the minor groove of DNA was also observed. Further, the best orientation from the DNA–ligand complex was subjected to molecular dynamics simulation. The study revealed the stability of the complex up to 5 ns without significant fluctuation from the initial orientation, which indicated strong DNA intercalation.[11b]

Further, these molecules were transformed into pyrazole hybrids as the bioisostere of chalcones through an easy reaction with hydrazine hydrate (compounds **22**, Scheme 4). Nevertheless, similar potency and selectivity was observed as chalcones, in which the most potent compound **22a** produced an IC$_{50}$ of 2.39, 3.63, 1.94, 2.63, and 2.75 μM for A549, DU-145, MCF-7, HeLa, and ACHN, respectively. The high IC$_{50}$ concentration of 95.37 μM for HEK-293 indicated the excellent selectivity of **22a** towards the cancer cells rather than the normal cells.[11c]

R. Cao and group explored *N*^{2}-alkylated salts of β-carbolines as cytotoxic agents.[12a–c] The results revealed the high potency of such *N*^{2}-quaternized salts (IC$_{50}$ ≤ 10 μM) compared to the respective neutral β-carbolines (IC$_{50}$ = 10–100 μM).[12c] Chauhan *et al.*, reported highly potent β-carboline grafted with chalcones at C1-β-carboline having an IC$_{50}$ of 2.25 μM towards the MCF-7 cell line (compound **23** and **24**, Scheme 5).[12d] Encouraged by these efforts, a new series of β-carboline-chalcone hybrids and their *N*^{2}-alkylated salts were designed, synthesized, and tested for their anti-proliferative activity against human cancer cell lines (compounds **25** and **26**, Scheme 5). The *in vitro* cytotoxic assay showed that the neutral hybrids (**25**) were less potent as compared to the *N*^{2}-alkylated salts of these hybrids (**26**) in the tested cell lines,

viz., BxPC-3, HeLa, C4–2, PC-3, HEK293T, MDA-MB-231, and NIH3T3. Interestingly, compound **26a** showed good anti-proliferative activity in all the mentioned cell lines with IC$_{50}$ values of 20.0, 22.1, 16.13, 22.02, 17.18, 15.95, and 55.23 μM, respectively. Compound **26a** showed induced apoptotic cell death in MDA-MB-231 cells. The β-carboline-C1-chalcone hybrids with *N*^{2}-quaternary ammonium salts showed better potency compared to the corresponding neutral hybrids; for example, the *N*^{2}-benzyl/propargyl groups produced significant response. The other aromatic enaminones, tested with the *N*^{2}-benzyl group, had marginal or no activity except for 3,4,5-trimethoxyphenyl chalcone. *N*^{2}-Butyl substitution with 3,4-dimethoxyphenylenaminone was completely inactive (Fig. 4).[12e]
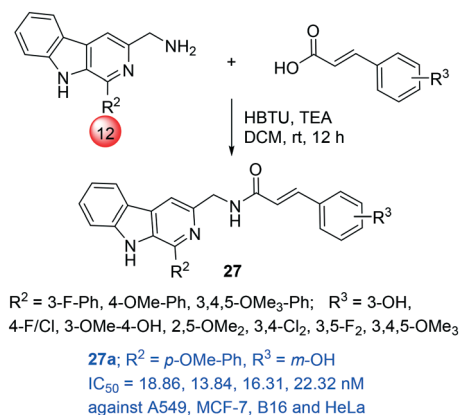
3.3. β-Carboline-cinnamide hybrids

Cinnamic acid and its derivatives have been well-known for their promising anticancer potential.[13a,b] Encouraged by the potential of this naturally occurring scaffold, hybrids of β-carboline-linked C3-*trans*-cinnamides were designed and

23; *N*^{2}-alkylated salts of β-carboline

24; Ar = 3,4-OMe$_2$-Ph IC$_{50}$ = 2.25 μM (MCF-7)

R^2 = -COCH$_3$

Ar = Ph, 4-Me/OMe/CF$_3$-Ph, 3,4-OMe$_2$-Ph, 4-NMe$_2$-Ph, 3,4,5-OMe$_3$-Ph
R^3 = *n*Bu, Bn, propargyl

(i) di-tert-butyldicarbonate, DMAP, THF, 24 h
(ii) ArCHO, Aq. NaOH, EtOH 0 °C - 25 °C, 16 h

R^3-Br, DMF 50 °C, 12 h

26a; R^3 = Bn, Ar = 3,4,5-OMe$_3$-Ph
IC$_{50}$ = 16.13, 17.18, 15.95 μM against C4-2, HEK293T, MDA-MB-231
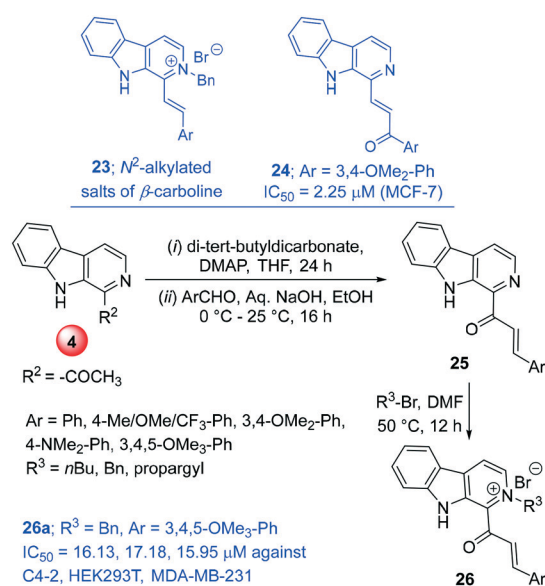
Scheme 5 Synthesis of β-carboline-chalcone hybrids and their quaternary salts.

Fig. 4 Structural features of quaternary salts of β-carboline-chalcone hybrids producing highly potent frameworks.

Scheme 6 Synthesis of β-carboline-cinnamide hybrids.

synthesized by the amide coupling of β-carboline amine (**12**) and cinnamic acids using hexafluorophosphate benzotriazole tetramethyl uronium (HBTU) (compounds **27**, Scheme 6). These hybrids were evaluated for their anticancer activity against different human cancer cell lines and were found to be highly potent with activity in the nanomolar range. In particular, compound **27a** showed the highest potency with IC_{50} values 18.86, 13.84, 16.31, and 22.32 nM for A549, MCF-7, B16, and HeLa cell lines, respectively. In addition, the selectivity towards the cancer cells was indicated by the high IC_{50} of 37.16 nM for normal cells (NIHT3T). Notably, the biological effect of these hybrids was attributed to apoptosis induction, cleavage of pBR-322 DNA, and inhibition of topoisomerase I. Together, the DNA binding experiments as well as molecular modelling studies revealed DNA intercalation at the topoisomerase I binding site. Moreover, the cinnamide part at the C3 position extended towards the
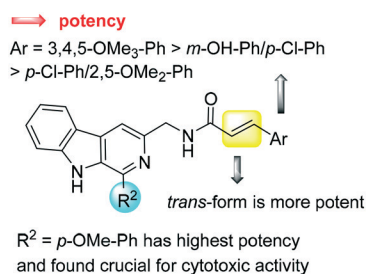
Fig. 5 Structural features of the most potent β-carboline-cinnamide hybrid.

DNA minor groove and formed π–π stacking. In the series, hybrids with *p*-OMe-Ph at C1-β-carboline or 3,4,5-tri-OMe-Ph on cinnamide were considerably more active with $IC_{50} < 24$ nM compared to the other conjugates. In particular, *p*-OMe-Ph at C1-β-carboline with 3-OH/4-Cl/3,4,5-tri-OMe-Ph on cinnamide exhibited the highest potency (Fig. 5). However, the replacement of cinnamide with acrylamide diminished the activity.[13c]

3.4. β-Carboline-hydroxamic acid hybrids

Vorinostat (**28**) (suberanilohydroxamic acid – SAHA) has been tested for the treatment of cutaneous T-cell lymphoma, where unsatisfactory clinical trials invigorated medicinal chemists to proselytize and develop molecules containing hydroxamic acid.[14a] In this regard, Zhang and co-workers designed, synthesized β-carboline-hydroxamic acid hybrids (Schemes 7–9), and revealed their cytotoxicity by targeting the HDAC and DNA *via* the regulation of the p53 signalling pathway. The hybrids have four major categories of linkers, namely, aliphatic amide/urea,[14b] aromatic amide,[14c] aromatic amine,[14d] and (iv) aliphatic cinnamic acid.[14e]

The first class of molecules with aliphatic amide/urea linker (compounds **29** and **30**, Scheme 7) were found to have significant *in vitro* anti-proliferative activity in human colorectal cancer cell lines and were found to have potent HDAC inhibition. Among the series of hybrids, compound **30a** exhibited the most potent *in vitro* anti-proliferative effect with an IC_{50} of 0.83, 0.94, 1.63, and 1.16 μM for HCT116, SW620, LOVO, and SW480 cell lines, respectively (7-fold higher potent than SAHA). Compound **30a** demonstrated an IC_{50} of 0.27 μM for HDAC inhibition and was found to be a strong cell apoptosis inducer. Moreover, the cleavage of PARP and caspase-3 along with increased histone H3/α-tubulin acetylation and the activation of the p53 signalling pathway against HCT116 cells was also credited for its biological responses.[14b]

The replacement of aliphatic amide/urea linker with aromatic amide linker led to a second series of hydroxamic acid hybrids (compounds **31**, Scheme 8), which were tested for their anti-proliferative activity. Among the evaluated compounds, **31a** exhibited highly potent anti-proliferative activity with IC_{50} of 1.79, 1.60, 2.46, and 4.25 μM for the cell lines HCT116, HepG2, SMMC-7721, and H1299, respectively. In addition, compound **31a** was found to be a highly effective inhibitor of HDAC with an IC_{50} of 0.092 μM, which is 5-folds more efficient than SAHA ($IC_{50} = 0.48$ μM). Moreover, no significant toxicity was observed for normal human LO2 cells.[14c]

The third class of hybrids was designed by replacing the amidic linker with aromatic amine linker (compounds **32**, Scheme 9). These hybrids were synthesized and evaluated against different human cancer cells for their anti-proliferative activity and HDAC inhibition. Among the synthesized library, compound **32a** showed the high potency with IC_{50} values of 0.78, 0.87, 0.53, 1.56, and 0.96 μM for the cell lines HCT116, SUMM-7721, HepG2, MCF-7, and Huh7,
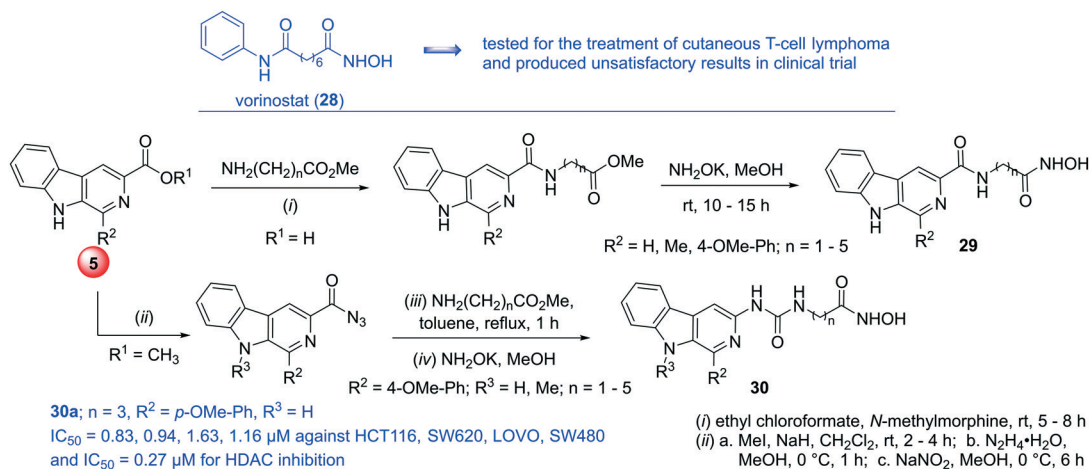
Scheme 7 Synthesis of β-carboline–hydroxamic acid hybrids with the aliphatic amide/urea linker.
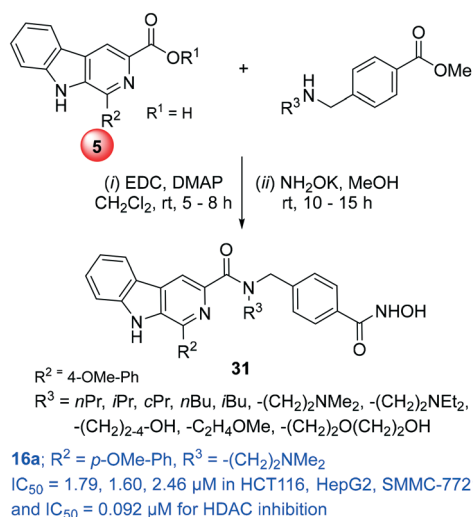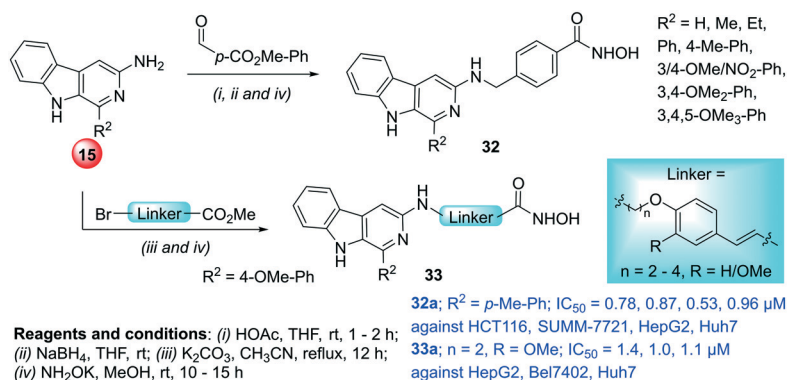

Scheme 8 Synthesis of β-carboline–hydroxamic acid hybrids with the aromatic amide linker.

respectively. It also demonstrated a 10-fold increase in the anti-proliferative potency compared to that of vorinostat **28**.[14d] It also showed potent HDAC inhibition with IC_{50} values

of 2.8, 0.75, and 1.1 nM for HDAC1/C3/C6. These molecules inhibited HDAC by inhibiting histone H3 and α-tubulin acetylation. In addition, hybrid **32** showed anti-metastatic activity by reducing the protein level of MMP2 and MMP9 as well as by inhibiting the MAPK signalling pathway.[14d]

Recently, in 2019, hydroxyl cinnamic acid linker was introduced in place of aliphatic/aromatic amide/amine linker, which led to the fourth series of hybrids (compounds **33**, Scheme 9). The hybrids were prepared and evaluated against human cancer cell lines, amongst which compound **33a** produced potent activity with IC_{50} values of 2.9, 1.4, 1.0, and 1.1 μM for SMMC-7721, HepG2, Bel7402, and Huh7 cell lines, respectively. Compound **33a** was also found to be very effective as an HDAC inhibitor with an IC_{50} value of 27 nM by the acetylation of histone H3 and α-tubulin. Perhaps these molecules induced apoptosis by regulating the expression of apoptotic proteins Bax, Bcl-2, and caspase-3. Interestingly, these compounds significantly inhibited the PI3K/Akt/mTOR signalling pathways, which are involved in cancers.[14e]

The replacement of the phenyl ring of vorinostat (**28**, Scheme 7) with β-carboline produced highly potent anti-proliferative scaffolds (**29–33**) *via* HDAC inhibition. The terminal hydroxamic acid group was found to be crucial for

Scheme 9 Synthesis of β-carboline–hydroxamic acid hybrids with the aromatic amine linker (**32**) and the aliphatic cinnamic acid linker (**33**).
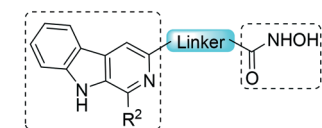
- terminal β-carboline and hydroxamic acid produced high potent congeners
  R^2 = p-OMe-Ph > H/Me
- Linkers effect on HDAC inhibition potency:
  aryl amines (32a; IC_{50} = 2.8 nM) >
  cinnamic acids (33a; IC_{50} = 28 nM) >
  aryl amides (31a; IC_{50} = 0.092 μM) >
  urea based amides (30a; IC_{50} = 0.27 μM) >
  aliphatic amide linkers

Fig. 6 Structural features of potent hydroxamic acid hybrids having different linker units.

the chelation of zinc ion at the active pocket of HDAC.[14f] In addition, the linker between these two pharmacophores was found to have distinctive influence on the potency as well as the selectivity of the congeners. Among the linkers screened, the following order of potency was observed: aryl amine (2.8 nM) > cinnamic acid (28 nM) > aryl amide (0.092 μM) > urea amide (0.27 μM) (Fig. 6).[14b–e]

3.5. β-Carboline-dithiocarbamate hybrids

Dithiocarbamates express important natural or synthetic class of chemopreventive agents such as brassinin[15a] (34) and sulforamate[15b] (35), which act via the inhibition of the proteasome enzyme(s).[15c,d] Moreover, the structure–activity relationship (SAR) indicated the significance of the dithiocarbamate motif towards the chemopreventive nature of brassinin.[15a] Enthused by potent activities of dithiocarbamates, Kamal et al., disclosed the synthesis and in vitro cytotoxicity evaluation of β-carboline-dithiocarbamate
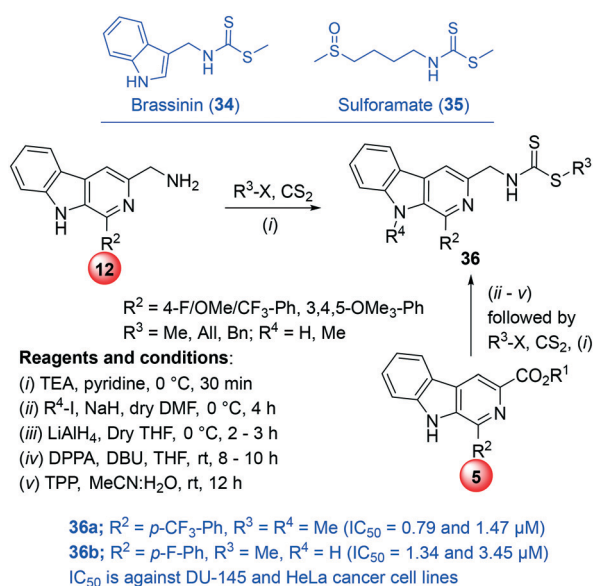
hybrids (compounds 36, Scheme 10). The synthesis was accomplished by treating the β-carboline amine (12) with carbon-disulphide and alkyl halide. The in vitro cell-based assays proved the potent cytotoxicity of the synthesized compounds on selected human cancer cell lines. However, the corresponding amine precursors (12, Scheme 10) were also evaluated but they exhibited poor potency, demonstrating the substantial role of dithiocarbamates towards the cytotoxic profile. Among the hybrids evaluated, compound 36a produced highest potency with IC_{50} of 0.79 and 1.47 μM for the DU-145 and HeLa cancer cells (found more potent than doxorubicin), respectively. Moreover, compound 36b also showed excellent potency with IC_{50} of 1.34 and 3.45 μM for the same cancer cell lines, respectively. Believably, these molecules had different binding modes with DNA from the usual β-carboline alkaloids. They displayed promising apoptosis induction and DNA topoisomerase II inhibition, unlike other β-carboline hybrids. The molecular docking analysis was also consistent with the experimental results. These molecules bound well to the ATP binding site of topoisomerase II and displayed good interactions at the binding pocket similar to the co-crystal.[15e]

The structural composition distinctly varied the pharmacological potency and the incorporation of dithiocarbamates increased the potency of congeners. The electronic nature of β-carboline-C1-aryl had a larger influence, where p-F/CF3-Ph produced better potency probably due to the lipophilic nature of fluorine. Methyl-dithiocarbamates were found to be the most appropriate with remarkable cytotoxicity. Moreover, the N^9-methyl substitution displayed increased activity (IC_{50} < 20 μM) compared to free-N^9-H (IC_{50} < 60 μM) (Fig. 7).[15e]

3.6. β-Carboline-imidazole/benzimidazole hybrids

Benzimidazoles were explored as potent cytotoxic agents by various mechanisms of actions such as the inhibition of receptor tyrosine kinase, topoisomerase II, and protein kinase CK2.[16] The bis-benzimidazole derivative 37 was found to inhibit DNA topoisomerase I and helicase, and has undergone phase I clinical evaluation. The exhilarating potential of imidazole/benzimidazole led to the development of several hybrids of such scaffolds with β-carboline having good anti-proliferative activity.[17,18d,e] A series of β-carboline-benzimidazole conjugates were designed, synthesized, and
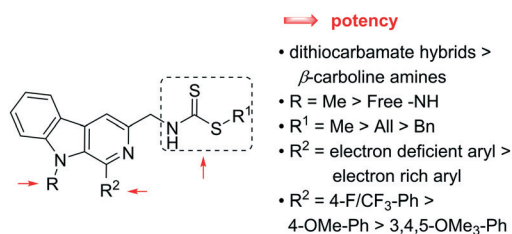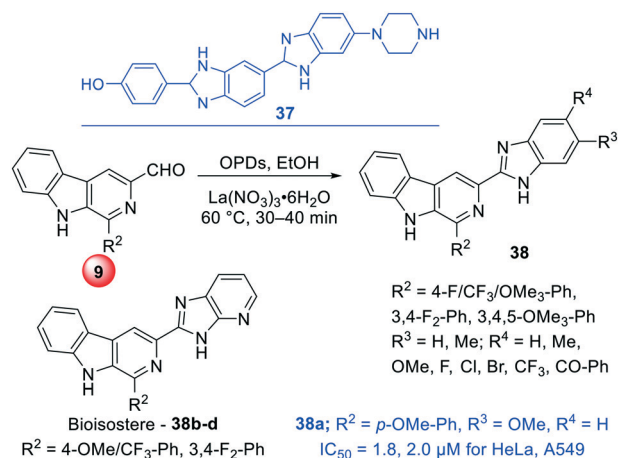

Brassinin (34)   Sulforamate (35)

R^2 = 4-F/OMe/CF3-Ph, 3,4,5-OMe3-Ph
R^3 = Me, All, Bn; R^4 = H, Me

Reagents and conditions:
(i) TEA, pyridine, 0 °C, 30 min
(ii) R^4-I, NaH, dry DMF, 0 °C, 4 h
(iii) LiAlH4, Dry THF, 0 °C, 2 - 3 h
(iv) DPPA, DBU, THF, rt, 8 - 10 h
(v) TPP, MeCN:H2O, rt, 12 h

(ii - v) followed by R^3-X, CS2, (i)

36a; R^2 = p-CF3-Ph, R^3 = R^4 = Me (IC_{50} = 0.79 and 1.47 μM)
36b; R^2 = p-F-Ph, R^3 = Me, R^4 = H (IC_{50} = 1.34 and 3.45 μM)
IC_{50} is against DU-145 and HeLa cancer cell lines

Scheme 10   Synthesis of β-carboline-dithiocarbamate hybrids.

→ potency

- dithiocarbamate hybrids > β-carboline amines
- R = Me > Free -NH
- R^1 = Me > All > Bn
- R^2 = electron deficient aryl > electron rich aryl
- R^2 = 4-F/CF3-Ph > 4-OMe-Ph > 3,4,5-OMe3-Ph

Fig. 7 Structural features of potent β-carboline-dithiocarbamate hybrids.

Scheme 11 Synthesis of β-carboline-benzimidazole hybrids.

Scheme 12 Synthesis of β-carboline-N-heterocyclic hybrids with the ability to generate carbene.

evaluated as anti-proliferative agents. The synthesis of these conjugates was accomplished by coupling the β-carboline aldehyde (**9**) with *ortho*-phenylenediamines (OPDs) using lanthanum nitrate as the catalyst. The bioisosteric replacement of benzimidazole with pyridoimidazole produced the bioisosteres **38b–38d** (compounds **38**, Scheme 11). The *in vitro* anti-proliferative evaluation of conjugates **38** against human cancer cell lines produced potent biological responses. In particular, compound **38a** showed potent cytotoxic activity with IC_{50} values of 1.8, 2.4, and 2.0 μM for HeLa, DU145, and A549 cell lines, respectively. The growth inhibition for hybrids **38** was also observed in the concentration range from 0.3 to 63 μM. Indeed, compound **38a** exhibited remarkable anticancer activity against leukemia cancer cells with GI_{50} of 0.3 and 0.8 μM for RPMI-8226 and CCRF-CEM, respectively. The hybrids dominantly cleaved the pBR322 plasmid DNA by the irradiation of UV light and also showed the inhibition of topoisomerase I. The congeners also showed G-quadruplex DNA stabilization, inhibition of telomerase, and induced apoptosis towards the anticancer activity.[4d] The binding mode and interactions with DNA/topoisomerase I were examined by biophysical assays and molecular docking analysis. The results were consistent with DNA intercalation and minor groove binding, where the molecules were properly fitted in the active site of topoisomerase I.[17] The best docked orientation displayed electrostatic interactions with Arg364, Lys374 (H-bonding), C112, A113, C10, G11 (planar π–π stacking) along with hydrophobic interactions involving Asp533, Ile535, Asn722, Arg364, Glu365, and Lys425 amino acids present close to the binding site.

Among the hybrids **38**, the substitution of *p*-OMe-Ph at C1 along with 6-F/OMe-benzimidazole at C3 of β-carboline produced the highest potency as the cytotoxic agent. In addition, other benzimidazole activating groups such as methyl substitution produced moderate cytotoxicity, while electron-withdrawing group (6-CF₃) on C3-benzimidazole demolished the activity. Perhaps, the replacement of benzimidazoles with pyridoimidazole (compound **38b–38d**,
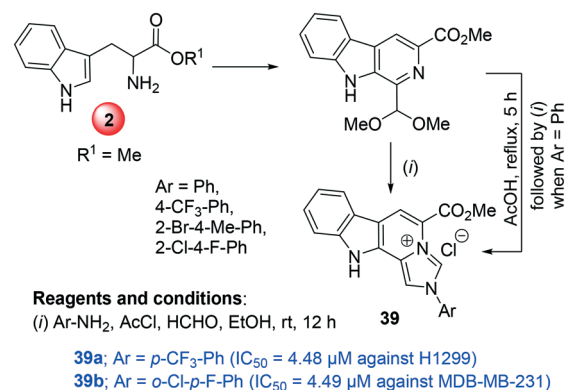
Scheme 11) drastically hindered the biological potency of the congeners.[17]

N-Heterocyclic carbenes, their precursors, and metal complexes have played crucial role towards the development of anticancer agents.[18a–c] In this strategy, β-carboline-based N-heterocyclic hybrids having the ability to generate carbene under biological conditions were synthesized and evaluated for their anti-proliferative/metastatic activities against breast and lung cancer cells (compounds **39**, Scheme 12). Among the hybrids, some of the molecules produced high potency with IC_{50} of <10 μM; for example, **39a** showed an IC_{50} of 4.48 μM against H1299 and **39b** has an IC_{50} of 4.49 μM for MDA-MB-231. These hybrids exhibited anti-invasive effects against highly metastatic human breast cancer cells MDA-MB-231 *via* the anomaly of the MAP-kinase signalling pathway and the inhibition of matrix metalloproteinases.[18d]

The imidazolium salts have an remarkable array of biological activities including the anticancer potential. Likely, an
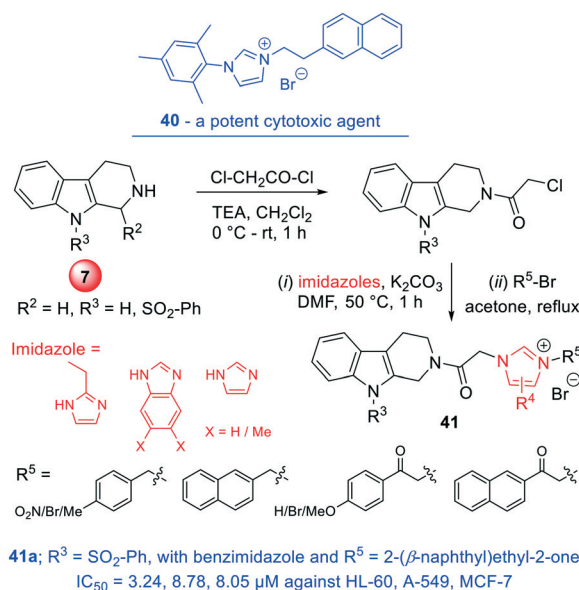
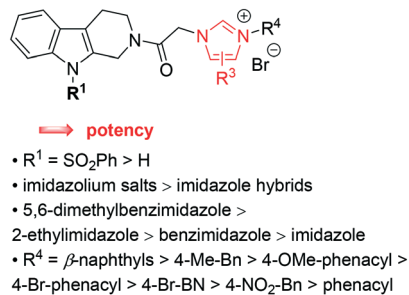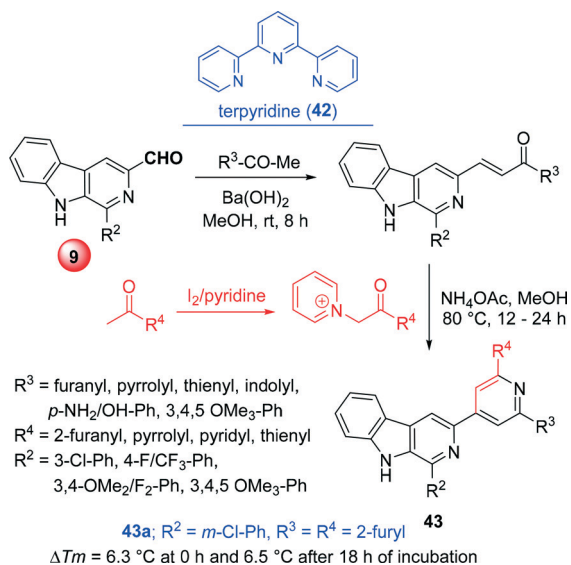Scheme 13 Synthesis of hybrids of β-carboline-imidazolium salts.

Fig. 8 Structure–activity relationship (SAR) of the hybrids of the β-carboline-imidazolium salt.

Scheme 14 Synthesis of β-carboline-pyridine hybrids.

imidazolium salt (compound **40**, Scheme 13) has produced the potent cytotoxicity.[18e,f] In this context, the hybrids of β-carboline-imidazolium salts were designed, synthesized, and evaluated against different cancer cell lines (compounds **41**, Scheme 13). The *in vitro* cytotoxicity evaluation revealed the good potency of these congeners as anticancer agents. In particular, compound **41a** was proved to be a highly cytotoxic molecule with IC_{50} of 3.24, 15.03, 8.78, 8.05, and 11.01 μM for HL-60, SMMC-7721, A549, MCF-7, and SW480 cancer cell lines, respectively. The biological responses of these hybrids were credited to apoptosis induction *via* the arrest of the G1 phase of the cell cycle. However, the neutral imidazole hybrids were poorly active compared to their corresponding imidazolium salts (Fig. 8).[18g]

3.7. β-Carboline-pyridine hybrids

Pyridine, being an excellent bioisostere of phenyl as well as pyrimidine, has a considerable influence on its biological activities. A terpyridine (**42**, Scheme 14) and its metal complexes exhibited astonishing cytotoxic property *via* DNA intercalation.[19a,b] In the same fashion, β-carboline-pyridine hybrids were designed, synthesized, and evaluated for their biological activity. The synthesis involved the reaction of

β-carboline aldehydes (**9**) with aryl/heteroaryl ketones in presence of barium hydroxide, which furnished the corresponding chalcone derivatives. These chalcones were subjected to Khronke reaction in the presence of ammonium acetate to obtain the designed β-carboline-pyridine hybrids (compounds **43**, Scheme 14). The hybrids were evaluated for their DNA binding ability by assessing the thermal denaturation (ΔT_m) of DNA from calf-thymus. Interestingly, among the hybrids, compound **43a** showed strong DNA binding ability with highest melting stabilization, as indicated by ΔT_m (6.3 °C at 0 h and 6.5 °C after 18 h of incubation). However, the reference standard doxorubicin was found to have 3-folds depressed ΔT_m (2.4 °C at 0 h and 2.6 °C after 18 h of incubation), which indicated weaker DNA binding. However, most of the hybrids produced good DNA binding affinity, in particular, *m*-Cl-Ph at C1-β-carboline and 2,6-(*bis*-furan-2-yl)pyridyl at C3 exhibited the highest affinity towards DNA interactions. Molecular docking analysis revealed DNA intercalation as the binding mode of these ligands stabilized by planar π–π stacking and H-bonding of heteroatoms.[19c]

3.8. β-Carboline-salicylic acid hybrids

As salicylic acid and its precursors are associated with the prevention and treatment of cancers,[20a,b] its hybrids with β-carboline were prepared and evaluated for their anticancer potential and apoptosis induction (compounds **44**, Scheme 15). The *in vitro* cytotoxicity study of these hybrids along with 5-FU and harmine was performed against various human cancer cell lines. Interestingly, most of the designed molecules were found to be more potent than harmine as well as 5-FU. Among all, compound **44a** was the most potent molecule with IC_{50} of 6.97, 7.12, 8.25, 7.89, and 13.1 μM for SMMC-7721, HepG2, HCT116, Ej, and H460, respectively. It showed excellent selectivity towards liver cancer cells (SMMC-7721) along with decreased mitochondrial membrane potential, which intervened with apoptotic proteins such as Bcl-2 and Bax in a dose-dependent manner. The influence of the structural variations of these hybrids was clearly reflected by the potency, where C1-methyl (**44**, R^2 = Me) produced the
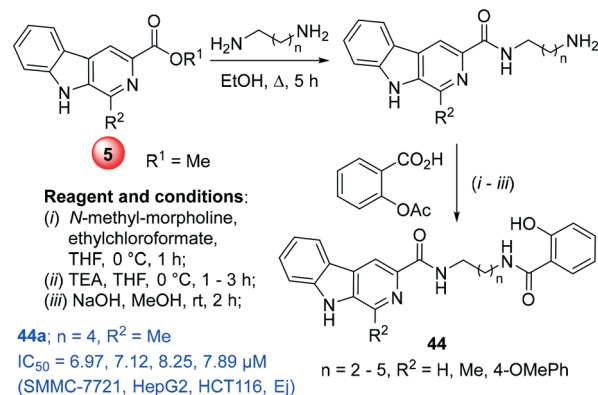
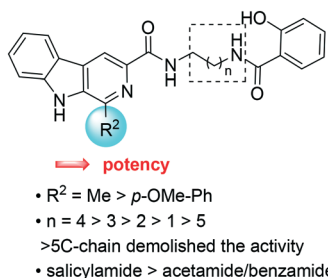**Scheme 15** Synthesis of β-carboline-salicylic acid hybrids.

Fig. 9 Structural features of β-carboline-salicylic acid hybrids influencing the biological potency.

most potent molecule, while the bulky substitution (4-OMe-Ph) decreased the activity. The length of the alkyl chain between the two pharmacophores was crucial; the activity increased until 5-carbon chain elongation, while further lengthening the linker decreased the potency. The replacement of salicylamide either with an acetamide or benzamide decreased the antitumor activity and directed the significance of the salicylic acid pharmacophore (Fig. 9).[20c]

3.9. β-Carboline-thiazolidinedione hybrids

Thiazolidinedione and its derivatives have great impact on the development of anticancer agents, such as compounds 45a (IC$_{50}$ = 1.3 and 3.1 μM for HL-60 and L1210, respectively) and 45b (IC$_{50}$ = 0.13 and 0.05 μM for A549 and MDB-MB-231, respectively) have shown potent activity.[21a–c] In continuation, β-carboline-thiazolidinedione hybrids were designed, synthesized, and evaluated for their antitumor activity. The synthesis was accomplished using β-carboline-carbohydrazide (13) with isothiocyanates or alternatively treated with
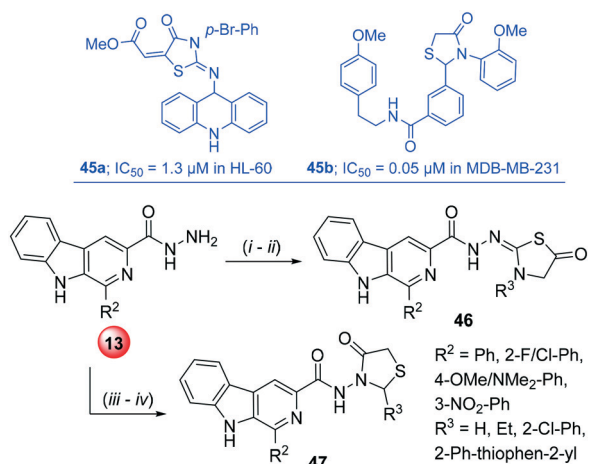
Reagents and conditions: (i) KSCN/EtNCS/PhNCS, EtOH, reflux; (ii) BrCH$_2$CO$_2$C$_2$H$_5$, AcONa, KOH, EtOH, reflux; (iii) R^{3}CHO, DMF, MW; (iv) HSCH$_2$CO$_2$H, p-TsOH, toluene, reflux

46a: R^2 = m-NO$_2$-Ph, R^3 = Ph;
IC$_{50}$ = 1.59, 2.36, 0.19, 1.53, 0.83 and 2.28 μM against U251, MCF-7, NCI/ADR-RES, 786-0, OVCAR-3 and HT-29

Scheme 16 Synthesis of β-carboline-thiazolidinedione hybrids.
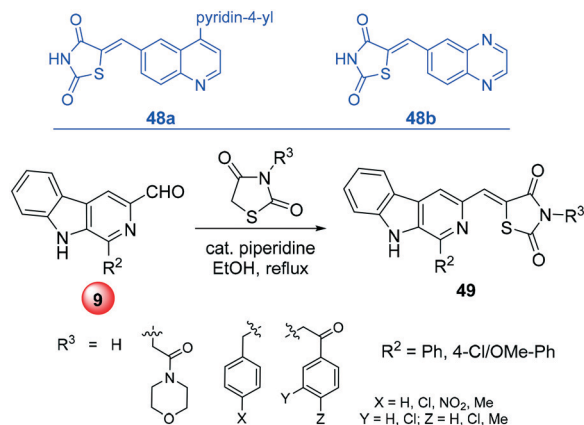
49a; R^2 = p-Cl-Ph, R^3 = 2-(morpholin-4-yl)ethyl-2-one
IC$_{50}$ = 1.34, 1.40, 1.80, 0.97 μM (PC-3, MG-63, HCT-15, MDA-MB-231)

Scheme 17 Synthesis of β-carboline-thiazolidine-2,4-dione hybrids.

aldehydes, followed by reaction with 2-marcapto-acetic acid (compounds 46 and 47, Scheme 16). The hybrids were examined for in vitro cytotoxicity, among which compound 46a especially showed potent cytotoxic activity, having an IC$_{50}$ of 1.59, 2.36, 0.19, 1.53, 3.91, 0.83, and 2.28 μM against U251, MCF-7, NCI/ADR-RES, 786-0, NCI-H460, OVCAR-3, and HT-29, respectively. The hybrids were credited for arresting the sub-G1 phase and the loss of cell membrane integrity.[21d]

The thiazolidinedione hybrid with quinoline produced potent anti-tumor agents and among them, two are currently under clinical investigation (compound 48a–b, Scheme 17).[22a] The highly potent derivatives 48a–b encouraged the design of β-carboline-thiazolidinedione hybrids in identical fashion, barring the replacement of quinoline/quinoxaline.[22b] The synthesis was achieved by the simple coupling of β-carboline aldehydes (9) with active methylene of thiazolidinedione in basic ethanol medium (compounds 49, Scheme 17). The in vitro cytotoxicity of hybrids 49 was examined in different human cancer cell lines and exhibited considerable activity. In particular, compound 49a displayed the best cytotoxicity with an IC$_{50}$ of 1.34, 6.66, 1.40, 1.80, 0.97, 4.51, 5.08, and 3.33 μM against PC-3, A549, MG-63, HCT-15, MDA-MB-231, A431, PANC-1, and L132, respectively. The high potency of 49a may also be credited to the presence of morpholine, which has its own remarkable potential as a cytotoxic agent.[22c] The hybrids were found to have the ability to induce apoptosis by membrane blebbing, chromatin condensation, and apoptotic body formation. The induction of apoptosis was evident from cell–cell adhesion, cell wall deformation, shrinkage of cells, and reduction in the number of viable cells. The different staining techniques such as AO/EB and DAPI along with the depolarization of mitochondrial membrane potential were also in support of the induction of apoptosis. The generation of reactive oxygen species (ROS), cell cycle arrest at the G1-phase, and the intercalation of CT-DNA were also been observed. The biophysical DNA binding affinity studies (relative viscosity, circular dichroism, and UV spectral study) along with molecular modelling analysis

revealed the DNA intercalative property of these ligands. The best docked orientation of the ligand indicated the intercalation of planar β-carboline supported by strong π–π interactions. Morpholine substitution over thiazolidinedione oriented into the active site and the ligand was stabilized by different electrostatic and hydrophobic interactions. Further, MD simulation for 5 ns indicated the stability of the ligand–DNA complex without significant deviation from the initial orientations. The structural variations were found to have a distinct role in governing the potency and selectivity of these hybrids. The electron-withdrawing group on C1-aryl, such as 4-Cl-Ph, showed higher potency compared to electron-rich aromatic substitution such as 4-OMe-Ph and, which followed the order p-Cl-Ph > Ph > p-OMe-Ph. Among the thiazolidine N-substitutions, the potency followed the orders of morpholine > benzyl > phenacyl > free-NH.[22b]

3.10. β-Carboline-podophyllotoxin hybrids

Podophyllotoxins are a well-known class of scaffolds exerting their anticancer activity via different mechanisms of action, such as inhibition of microtubule, topoisomerase, and DNA intercalation.[23a,b] The β-carboline-podophyllotoxin hybrids were synthesized by amide coupling between β-carboline acids (5) and the corresponding amine (compounds 50, Scheme 18). The synthesized hybrids were evaluated for their in vitro cytotoxic potential against a panel of human cancer cell lines. Among the hybrids examined, compound 50a produced potent cytotoxic effects with IC_{50} of 1.87, 1.07, 2.64, 2.68, and 2.92 μM for A549, DU-145, MDA MB-231, HT-29, and HeLa, respectively. Notably, this compound has an IC_{50} of 103.28 μM for HEK-293 cells (human embryonic kidney cells), which directly imitates the high selectivity towards cancer cells rather than the normal cells. Hybrid 50a showed high efficacy towards DU-145 cancer cells, followed by potency towards A549. These hybrids led to cancer cell apoptosis by the inhibition of the cell cycle at the S and G2/M phases and act as catalytic inhibitors of topoisomerase IIα. Moreover, the biophysical DNA binding studies such as UV and fluorescence spectroscopy along with circular dichroism demonstrated the non-intercalative
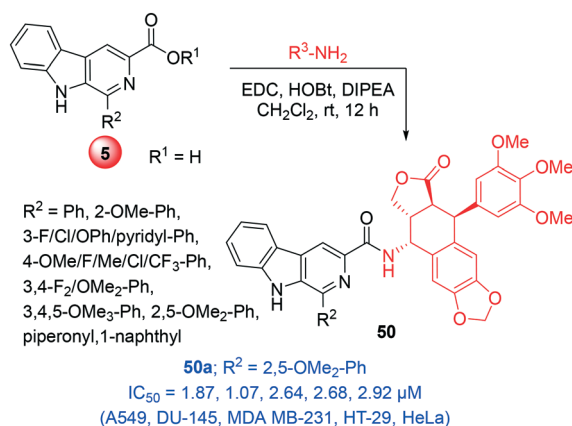
interactions of ligands such as groove or surface binding, causing the unwinding of CT-DNA. The in silico molecular modelling results were in correlation with DNA topoisomerase IIα inhibition assay, where the compounds occupied the ATP-binding site and formed strong interactions with the surrounding residues. The entry of ATP into the binding site was hindered due to the interaction of ligands with the residues below the ATP binding site and magnesium ions. Molecular dynamics revealed the ability of the amide functionality to orient the molecules perfectly in a curved shape in the minor groove and reflected the significance of the amide bond between two pharmacophores.[23c]

3.11. β-Carboline-combretastatin hybrids

Combretastatin derivatives are well-known cytotoxic agents acting via varied mechanisms such as apoptosis induction, DNA intercalation, and topoisomerase II inhibition. The potent anti-proliferative activity of combretastatin A-4 (CA-4) was marked as the lead candidate in the discovery of anticancer agents. The flexible double bond configuration has limited its clinical use since only the cis-conformation is active.[24a,b] In effort to develop the rigid cis-isomer, the hybrids of β-carboline-combretastatin were designed, synthesized and further tested for the in vitro cytotoxic activity against human cancer cell lines. The synthesis of these hybrids involved amide coupling between the β-carboline amine (12) and CA-4 acids using EDC-HOBt as the coupling agent (compounds 51, Scheme 19). The hybrids were examined for their in vitro cytotoxicity, which exhibited considerable cytotoxicity with <2 μM IC_{50} for many of the analogues. For instance, compound 51a showed the highest potency with an IC_{50} of 1.01 and 1.51 μM against the A549 and DU-145 cell lines, respectively.[24c] However, another compound 51b from the series also produced potent activity


Scheme 19 Synthesis of β-carboline–combretastatin hybrids.
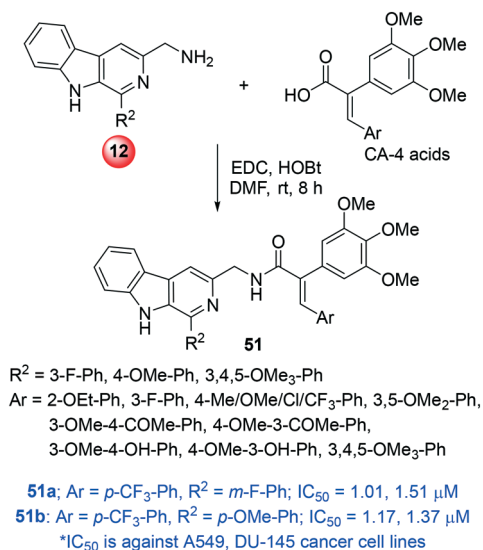
Scheme 18 Synthesis of β-carboline-podophyllotoxin hybrids.

with an IC_{50} of 1.17 and 1.37 μM against the A549 and DU-145 cell lines, respectively.

The cytotoxic effect of these hybrids was attributed to cell cycle arrest at the G2/M phase, apoptosis induction, DNA intercalation, and topoisomerase II inhibition. Molecular modelling analysis unfolded the understating of interactions of these hybrids and the results were consistent with DNA topoisomerase II inhibition assay. The compounds were occupied in the ATP binding pocket of topoisomerase II and showed strong electrostatic interactions. These ligands also displayed DNA intercalation due to the β-carboline ring, whereas the combretastatin moiety was extended out to the DNA minor groove. The prevailing role of structural variations of hybrids **51** has been well established and observed for their biological response. The 3,4,5-tri-OMe-Ph motif was found to be crucial for its efficacy. The other aromatic ring of combretastatin with Ar = 4-CF$_3$/Me-Ph substitutions showed the highest potency. Moreover, the substitutions such as R^2 = 4-OMe-Ph or 3,4,5-tri-OMe-Ph at C1-β-carboline also produced excellent cytotoxicity.[24c]

3.12. β-Carboline-sulfonyl piperazine hybrids

A new hybrid class of β-carboline-linked sulfonyl piperazine derivatives were prepared by amide coupling between β-carboline acids (**5**) and 4-(arylsulfonyl)piperazine using EDC-HOBt (compounds **52**, Scheme 20). The anticancer activity of these congeners was examined with the help of *in vitro* cytotoxic studies. Among the series, most of the compounds showed good potency; in particular, compounds **52a** and **52b** exhibited highly potent activity with the respective IC_{50} values of 0.59 and 2.80 μM towards the MG-63 cancer cells. Fortunately, these hybrids showed least toxicity towards normal monkey kidney cells (Vero), having an IC_{50} of 8.41 μM and 9.95 μM for **52a** and **52b**, respectively. These compounds manifested the ability to inhibit topoisomerase II along with the binding to DNA, thus inhibiting the cell cycle. The experimental results of topoisomerase II inhibition and DNA binding were also supported by the *in silico* molecular modelling studies. Similar to other β-carboline-based topoisomerase II inhibitors, these molecules suitably docked in the ATP binding site of the enzyme topoisomerase IIα and produced consistent electrostatic interactions. Different H-bond interactions were observed for N^9-H with Asp99 and –CO with Arg98 at the
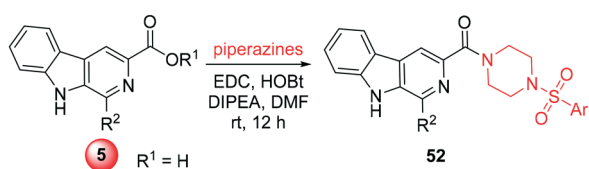
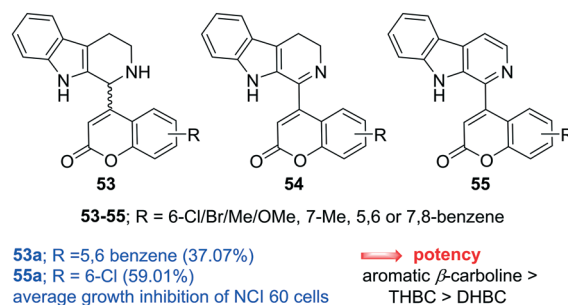distance of 2.79 Å and 2.99 Å, respectively. Also, the π–cation interaction of sulfonyl phenyl was observed with Arg98 and Lys157 along with multiple hydrophobic interactions. Similarly, the DNA ligand docking simulation displayed strong N^9-H bond interaction with DT8 (DNA base pair) along with β-carboline π–π stacking interactions. The C1-aryl substitutions with phenyl or *para*-chlorophenyl on sulfonyl produced the most potent congeners. The C1-phenyl on β-carboline produced better activity rather than the substituted phenyls.[25]
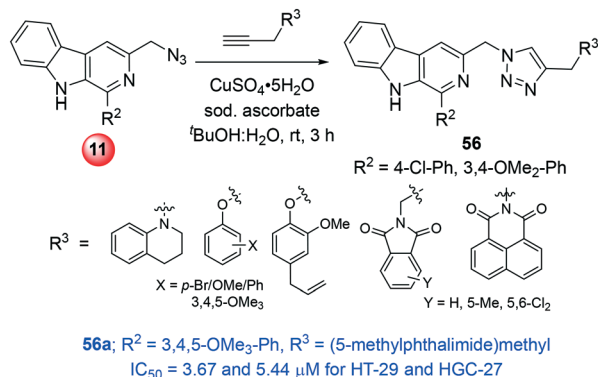
3.13. β-Carboline-coumarin hybrids

Coumarin is another well-distinguished pharmacophore with a wide range of biological activities along with cytotoxic/anti-cancer potential.[26a,b] In the pursuit to obtain highly efficacious cytotoxic agents, the hybrids of β-carboline-coumarins were designed. The tetrahydro-β-carboline hybrids were synthesized using Pictet–Spengler cyclization, followed by controlled oxidation to dihydro-β-carboline to aromatic β-carboline hybrids (compounds **53–55**, Scheme 21). Among the hybrids evaluated for their cytotoxic potential towards 60 different cancer cell lines, most of the molecules produced considerable cell growth inhibition. In particular, compound **53a** and **55a** showed 37.07% and 59.01% average growth inhibition of the NCI 60 cancer cell lines, respectively, at a single concentration of 10 μM. Moreover, these compounds exhibited significant cytotoxicity in the HeLa cancer cells with the GI_{50} of 33.33 μM and 23.4 μM. In the series of aromatic β-carboline, 6-Cl or 6-OMe-coumarin produced maximum potency. Similarly, tetrahydro-β-carboline was found to be more active than that of the corresponding dihydro-β-carboline towards the tested cancer cell lines. The molecular docking analysis with tubulin (1SA0) and KSP (1Q0B) displayed good electrostatic interaction (H-bond) with Ala250, Asp251, Leu252 of tubulin, or Asn29 of KSP.[26c]

3.14. β-Carboline-triazole hybrids

Triazole(s) are well-known pharmacophore with a broad range of biological activities. The triazole derivatives have been considerably explored as cytotoxic agents and have met with encouraging results.[27a,b] In the effort to achieve better potency and selectivity, β-carboline-triazole hybrids were
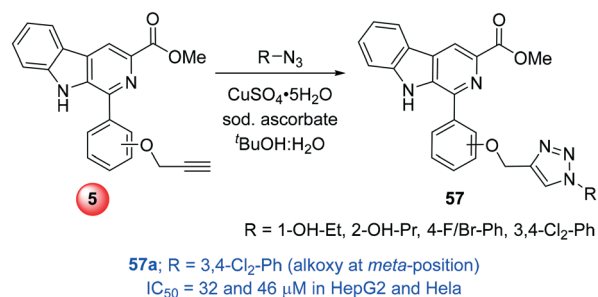

R^2 = H, Ph, 4-F/CF$_3$-Ph, 3,3,5-OMe$_3$-Ph; Ar = Ph, 4-Me/OMe/Cl/^{t}Bu-Ph

52a; R^2 = Ar = Ph, IC_{50} = 0.59 μM (MG-63)
52b; R^2 = Ph, Ar = *p*-ClPh, IC_{50} = 2.8 μM (MG-63)

Scheme 20 Synthesis of β-carboline-sulfonyl piperazine hybrids.

53-55; R = 6-Cl/Br/Me/OMe, 7-Me, 5,6 or 7,8-benzene

53a; R =5,6 benzene (37.07%)
55a; R = 6-Cl (59.01%)
average growth inhibition of NCI 60 cells

➡ **potency**
aromatic β-carboline >
THBC > DHBC

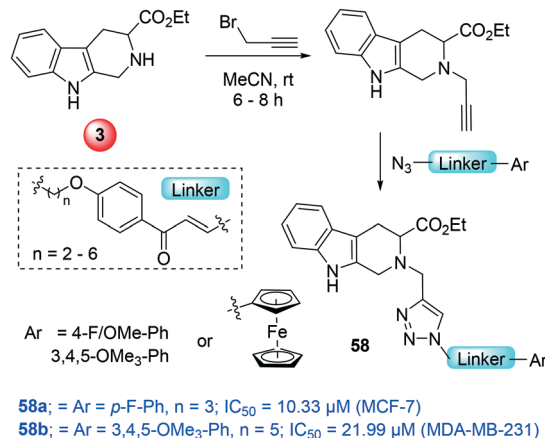**Scheme 21** β-Carboline-coumarin hybrids and their anticancer activity.

Scheme 22 Synthesis of C3-thetered β-carboline-triazole hybrids.

Scheme 24 Synthesis of tetrahydro-β-carboline-triazole hybrids grafted with chalcone.

prepared and evaluated for their anti-proliferative activity.[9c] For instance, C3-tethered β-carboline-triazole congeners were synthesized *via* the click coupling of β-carboline-azides (**11**) with different alkynes in the presence of copper catalyst (compounds **56**, Scheme 22). These hybrids were tested for their *in vitro* cytotoxicity against different human cancer cell lines, where most of the hybrids exhibited good cytotoxic profile. The representative compound **56a** produced the best potency with an IC$_{50}$ of 3.67 and 5.44 μM, respectively, for HT-29 and HGC-27 cancer cells compared to harmine (IC$_{50}$ = 4.28 and 10.56 μM). The structure–activity relationship revealed that the 3,4,5-tri-OMe-Ph at C1-β-carboline and (5-methylphthalimide)methyl on C4-triazole produced the best potency. A similar activity range was observed for 3,4-di-OMe-Ph at C1-β-carboline with naphthalimide on C4-triazole. The biophysical DNA binding assays (UV, CD, fluorescence spectral analysis, and DNA viscosity measurement) along with molecular modelling studies substantiated these hybrids as DNA minor groove binding agents.[27c]

Another series of β-carboline-triazole hybrids have been prepared by connecting the C1-β-carboline and C4-triazoles with an aryl-alkyl-ether linker (compounds **57**, Scheme 23). The *in vitro* cytotoxicity results revealed considerable potency of compound **57a** with an IC$_{50}$ of 32.0 and 46.0 μM for HepG2 and HeLa cancer cell lines, respectively. The *in vitro* testing results were found to be the least potent for unaromatized β-carboline, while 3,4-di-Cl-Ph in triazole displayed the best activity among the hybrids. However, these

molecules have also displayed significant antibacterial property against *E. coli*, *S. aureus*, *B. cereus*, and vancomycin-resistant strains *E. faecium* and *E. faecalis*.[27d]

The hybrids of tetrahydro-β-carboline-triazole grafted with chalcones were designed, synthesized, and evaluated for their anticancer potential against breast cancer cell lines (compounds **58**, Scheme 24). All the compounds from the series were examined against the MCF-7 and MDA-MB-231 cancer cell lines and resulted in interesting activity. For instance, compound **58a** was 5-fold more potent than tamoxifen with an IC$_{50}$ of 10.33 μM on the MCF-7 cell line. Compound **58b** showed 3-fold higher potency than tamoxifen against the MDA-MB-231 cell line with an IC$_{50}$ of 21.99 μM. The analysis of the structural relationship with the activity demonstrated that the nature of aryl chalcone and the length of alkyl-aryl-ether played a critical role towards the cytotoxic potency of the hybrids. Thus, trimethoxyphenyl-chalcone and long 5C-alkyl chain afforded high selectivity and potency towards the MDA-MB-231 cells, while *para*-fluorophenyl-chalcone and shorter 3C-alkyl chain were dominant towards the MCF-7 cells. The hybrids with ferrocenyl-chalcone were found to be less potent rather than other aryl–chalcone hybrids.[27e]

The polycyclic hybrids of tetrahydro-β-carboline-triazoles were synthesized and evaluated for their *in vitro* anticancer activity on MCF-7 and B16F10 (compounds **59** and **60**, Scheme 25). Compound **59** showed potent cytotoxic activity

Scheme 23 Synthesis of β-carboline-triazole hybrids with aryl-alkyl-ether linker.
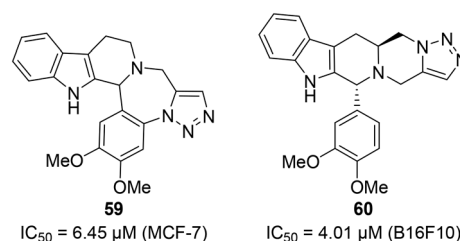
Scheme 25 Polycyclic tetrahydro-β-carboline-triazole hybrids.

with an IC$_{50}$ of 6.45 μM against the MCF-7 cells and compound **60** also exhibited significant cytotoxicity with an IC$_{50}$ of 4.01 μM against the B16F10 cells. The molecules demonstrated the ability to bind the DNA minor groove, induce apoptosis, arrest cell cycle, inhibit cell migration, and colony formation. Molecular docking with DNA indicated non-covalent binding of the ligands into the DNA minor groove, which lengthen the minor groove and distorted the helical axis by about 4 Å. The β-carboline N^9-H, methoxy, and triazole participated in electrostatic H-bonding. In addition, π–π stacking was also observed with triazole moiety. In case of molecule **60**, the *trans*-diastereomer was more efficient in adopting the crescent form to bind along the minor groove curvature. Further, molecular dynamics revealed the stability of the best-docked orientation for 4 ns without inducing large-scale conformational changes in the DNA double helix.[27f]

3.15. β-Carboline-indole hybrids

Indole is a privileged pharmacophore as it has been remarkably explored with pronounced biological potential.[28a–c] The indole-containing marketed drug, sunitinib, was used for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumor by the inhibition of multi-target receptor tyrosine kinase (RTK).[28d,e] In the pursuit of highly potent cytotoxic agents, the hybrids of β-carboline-indole were prepared by the Knoevenagel condensation of β-carboline aldehyde **9** with 2-oxindole and their *in vitro* cytotoxicity was examined (compounds **61**, Scheme 26). The *E*-configuration of the newly formed double bond was characterized with the help of NOE experiments (^{1}H gDQFCOSY NMR). The primary MTT assay of the hybrids displayed promising cytotoxic activity against a panel of human cancer cell lines, such as HCT-15, HCT-116, A549, NCI-H460, and MCF-7. Among the congeners evaluated, hybrid **61a** exhibited distinguished potency towards the entire tested cancer cell lines with IC$_{50}$ of 1.43, 4.18, 2.50, 6.71, and 3.36 μM, respectively. Notably, it displayed high selectivity towards the cancer cells and least toxicity towards the normal cells (IC$_{50}$ = 49.79 μM for HFL-1). Moreover, a
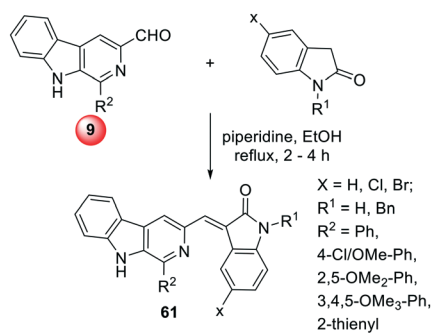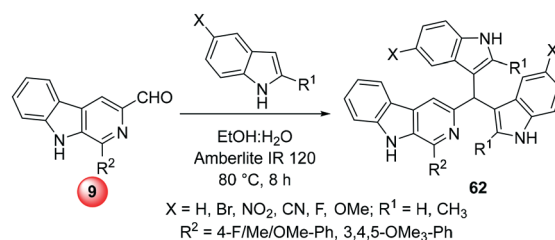
critical relationship was observed with the structural modifications; the C1 substitutions with phenyls produced potent activity, while the heteroaryl rings decreased the activity. The presence of electron-donating groups over C1 phenyl enhanced the activity. In addition, free-NH or benzyl substitution of oxindole had a decisive impact on the activity and C5-halo-substitution also exhibited excellent cytotoxic activity. On treatment, the morphological changes in the tested cells, AO/EB staining, DAPI nucleic acid staining, and the reduction of mitochondrial membrane potential suggested the induction of apoptosis. The cell cycle analysis showed the elevation of the G0/G1 phase cell population from 68.7% to 80.61% in the 1–2.5 μM concentration range. Further, the molecular docking analysis with DNA demonstrated the intercalation of planar β-carboline towards the GC base pairs, assisted by the right alignment of the C1-substituents. In addition, the oxindole moiety was also oriented perfectly between the DNA base pairs to have strong π-π interactions. Molecular dynamics revealed the stability of the best-docked orientation up to 20 ns without significant deviation from the initial orientation. The biophysical assays, molecular docking, and simulation studies were consistent with the DNA intercalation of these ligands.[28f]

Further, a class of β-carboline-bis-indole hybrids was prepared and evaluated for their *in vitro* anti-proliferative activity against various cancer cell lines (compounds **62**, Scheme 27). Among the hybrids examined, most of the compounds exhibited considerable anti-proliferative activity. For example, compounds **62a** and **62b** were proved to be highly active with the respective IC$_{50}$ of 1.80 and 1.86 μM for the DU-145 cell line. Moreover, these compounds were recognized as apoptosis inducers, inhibitors of the cell cycle (G2/M phase), and topoisomerase I, and cleaved the pBR322 plasmid under UV light. However, different substituents have a distinguishable impact on the biological activity. In particular, the presence of electron-deficient C1-aryl has higher potency compared to electron-rich C1-aromatic groups. C2-methylated indole has reduced activity than C2-unsubstituted molecules, while substitution at C5 followed the activity pattern of H > Br > NO$_2$ > CN > OMe.[28g]

Recently, a series of β-carboline-indole hybrids adjoined with thiadiazole-carboxamide was prepared by amide coupling between β-carboline acid (**5**) and 2-amino-5-(indol-2-yl)thiadiazole with the help of EDC-HOBt (compounds **63**,

61a; X = Br, R^1 = Bn, R^2 = 2,5-OMe$_2$-Ph
IC$_{50}$ = 1.43, 2.50 μM (HCT-15, A549)

Scheme 26 Synthesis of β-carboline–indole hybrids.

62a: X = R^1 = H, R^2 = *p*-F-Ph; IC$_{50}$ = 1.80 μM (DU-145)
62b: X = Br, R^1 = H, R^2 = *p*-Me-Ph; IC$_{50}$ = 1.86 μM (DU-145)
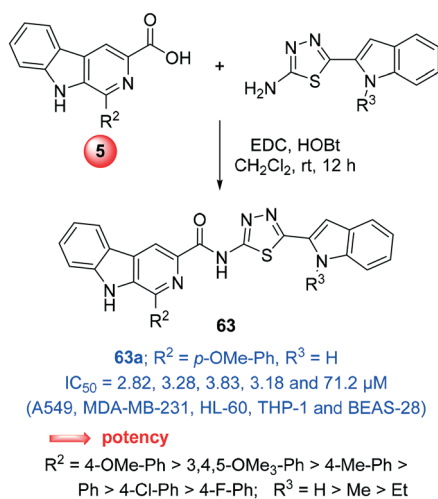
Scheme 27 Synthesis of β-carboline–bis-indole hybrids.

Scheme 28). Preceding this, 2-amino-5-(indol-2-yl)thiadiazole was prepared from indole-2-carboxylic acid in a sequence of four-steps, *viz.*, esterification, *N*-alkylation, ester hydrolysis, and reaction with thiosemicarbazide in phosphorous-oxychloride. Further, these hybrids exhibited DNA intercalative topoisomerase IIα inhibition and thus anti-proliferative activity. The *in vitro* cytotoxicity and apoptosis assays revealed promising results with nuclear and morphological alterations, and mitochondrial membrane depolarization against a panel of human cancer cell lines. Particularly, compound **63a** exhibited most potent IC_{50} of 2.82, 3.28, 4.09, 4.78, 3.83, 3.18 μM for A549, MDA-MB-231, BT-474, HCT-116, HL-60 and THP-1, respectively.[28h]
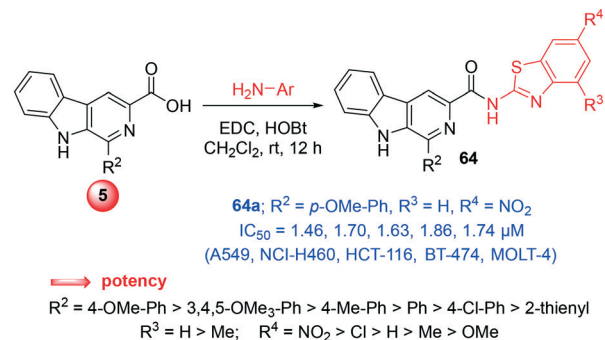
Notably, the safety/selectivity index of **63a** against normal human lung epithelial cells (BEAS-28) was found in the range of 14.9–25.26 with an IC_{50} of 71.2 μM. The enzyme inhibition assay demonstrated the induction of nicks and linearized the kDNA, indicating the catalytic inhibition of topoisomerase IIα. Further, the relative viscosity experiment and absorbance spectroscopy revealed the DNA intercalation of these ligands. The induction of cell apoptosis was inferred from different nuclear/non-nuclear staining such as AO or DAPI along with the changes in mitochondrial membrane potential and the arrest of cell cycle in the G0/G1 phase. In addition, molecular modelling studies affirmed the intercalative binding of these molecules in the active pocket of topoisomerase IIα. The critical analysis of structural features and biological response revealed that the electron-rich β-carboline-C1-aryl with unsubstituted indole-NH produced the best potency.[28h]

3.16. β-Carboline-benzothiazole hybrids

Benzothiazole (BT) is an important pharmacophore, which shows a broad range of therapeutic profile including anti-proliferative action.[29a–c] In the quest to obtain promising anticancer agents, β-carboline-benzothiazole hybrids were synthesized *via* the amide coupling of β-carboline acid (**5**)

63a; R^2 = *p*-OMe-Ph, R^3 = H
IC_{50} = 2.82, 3.28, 3.83, 3.18 and 71.2 μM
(A549, MDA-MB-231, HL-60, THP-1 and BEAS-28)
➡ potency
R^2 = 4-OMe-Ph > 3,4,5-OMe₃-Ph > 4-Me-Ph >
Ph > 4-Cl-Ph > 4-F-Ph; R^3 = H > Me > Et

Scheme 28 Synthesis of β-carboline-indole hybrids adjoined with thiadiazole.

64a; R^2 = *p*-OMe-Ph, R^3 = H, R^4 = NO₂
IC_{50} = 1.46, 1.70, 1.63, 1.86, 1.74 μM
(A549, NCI-H460, HCT-116, BT-474, MOLT-4)
➡ potency
R^2 = 4-OMe-Ph > 3,4,5-OMe₃-Ph > 4-Me-Ph > Ph > 4-Cl-Ph > 2-thienyl
R^3 = H > Me; R^4 = NO₂ > Cl > H > Me > OMe

Scheme 29 Synthesis of β-carboline-benzothiazole hybrids.

and various substituted 2-aminobenzothiazoles (compounds **64**, Scheme 29). The *in vitro* cytotoxic ability of the hybrids was established on adherent (A549, NCI-H460, HCT-116, BT-474) and suspension (MOLT-4, THP-1, HL-60) human cancer cell lines. Among the hybrids, compound **64a** displayed potent cytotoxicity with an IC_{50} of 1.46, 1.70, 1.63, 1.86, 1.74, 2.07, and 9.98 μM against the cancer cell lines. In addition, hybrid **64b** also exhibited similar potency with IC_{50} of 1.81, 2.01, 1.97, 2.06, 1.93, 4.51, and 11.05 μM for the same cell lines. Notably, these congeners were found to be 13-folds more potent than harmine and were found to have comparable IC_{50} to doxorubicin as well. In addition, biophysical assays and molecular modelling studies unveiled these hybrids as intercalative topoisomerase II inhibitors and DNA binding agents with the ability to induce apoptosis.[29d] The structural influence on the biological response was distinguished as C1-β-carboline aryls, which were more active when compared to the heteroaryl groups. Moreover, unsubstituted C4-BT (R^3 = H) or C6-BT substitutions (R^4) with electron-withdrawing groups were also more potent.[29d]

4. Conclusions

In summary, the current review demonstrates the design of molecular hybrids, their synthesis, and evaluation of *in vitro* anti-cancer potential in the last 5 years (2015–2020). The cytotoxic potential of the β-carboline motif grafted with various pharmacophores such as indole, imidazole, benzimidazole, benzothiazole, coumarin, pyridine, and hydantoin has been unfolded. Interestingly, hybrids with lead molecules such as combretastatin and podophyllotoxin that appeared in the literature have also been discussed. Moreover, the well-known chemical motifs such as dithiocarbamate, hydroxamic acid, salicylic acid, chalcone, cinnamide, pyrazole, thiazolidinedione, piperazine, and triazole were also very well explored as anticancer agents by employing β-carboline as the core. Interestingly, these hybrids not only produced excellent cytotoxic effects against most of the human cancer cell lines but were also adequately selective towards the cancer cells and exhibited excellent *in vitro* response in sub-micromolar/nanomolar concentrations. For instance, the β-carboline-chalcone hybrid **12a** has shown

highly potent cytotoxicity with an IC_{50} of 13.84 nM against the breast cancer cell line MCF-7. However, these agents can be further examined *in vivo*, which may assist in the identification of suitable lead molecules for the future development of novel anticancer agents with high efficacy and safety.

Note: Cell lines present in this review

Cell lines name	Description
A549/H1299/NCI-H460	Lung adenocarcinoma cells
HeLa	Cervical carcinoma cells
PC-3/DU-145	Prostate cancer cells
MCF-7/MDA-MB-231/BT-474	Breast cancer cells
ACHN/786-0	Renal cancer cells
BxPC-3/PANC-1	Pancreatic cancer cells
C4–2	Castration-resistant prostate cancer cells
B-16	Mouse melanoma cancer cells
HCT116/HCT15/HT-29	Colon cancer cells
SW-620/SW-480/LOVO	Colon cancer cells
HepG2/Huh7/Bel7402	Hepatocellular carcinoma cells
SMMC-7721/SUMM-7721	Hepatocellular carcinoma cells
RPMI-8226/CCRF-CEM/HL-60	Leukemia cancer cells
Ej	Bladder carcinoma cells
U251	Glioma cancer cells
NCI-ADR-RES/OVCAR-3	Ovarian cancer cells
MG-63	Osteosarcoma cells
A-431/B16F10	Skin cancer cells
L-132	Human pulmonary epithelial cells
HGC-27	Human gastric carcinoma cells
THP-1	Acute monocytic leukemia cells
MOLT-4	Acute lymphoblastic leukemia cells
RWPE	Normal prostate epithelial cells
HEK-293/HEK293T	Normal human embryonic kidney cells
NIH3T3	Normal murine fibroblast cells
HFL-1	Normal lung fibroblasts cells
BEAS-28	Normal human lung epithelial cells
Vero	Normal monkey kidney cells

Conflicts of interest

There is no conflict of interest to declare.

Acknowledgements

References

1 (*a*) R. L. Siegel, K. D. Miller and A. Jemal, *Ca-Cancer J. Clin.*, 2019, **69**, 7; (*b*) R. L. Siegel, K. D. Miller and A. Jemal, *Ca-Cancer J. Clin.*, 2020, **70**, 7; (*c*) P. Mathur, K. Sathish Kumar, M. Chaturvedi, P. Das, K. L. Sudarshan, S. Santhappan, V. Nallasamy, A. John, S. Narasimhan and F. S. Roselind, *JCO Glob. Oncol.*, 2020, **6**, 1063.

2 (*a*) S. Bernardini, A. Tiezzi, V. Laghezza Masci and E. Ovidi, *Nat. Prod. Res.*, 2018, **32**, 1926; (*b*) D. Singh, N. Devi, V. Kumar, C. C. Malakar, S. Mehra, S. Rattan, R. K. Rawal and V. Singh, *Org. Biomol. Chem.*, 2016, **14**, 8154; (*c*) R. A. Abramovitch and I. D. Spenser, *Adv. Heterocycl. Chem.*, 1964, **3**, 79; (*d*) W. H. Jiao, H. Gao, F. Zhao, H. W. Lin, Y. M. Pan, G. X. Zhou and X. S. Yao, *Chem. Pharm. Bull.*, 2011, **59**, 359; (*e*) C. A. A. Ferraz, R. G. de Oliveira Júnior, L. Picot, J. R. G. da Silva Almeida and X. P. Nunes, *Fitoterapia*, 2019, **137**, 104196; (*f*) G. Van Baelen, S. Hostyn, L. Dhooghe, P. Tapolcsanyi, P. Matyus, G. Lemiere, R. Dommisse, M. Kaiser, R. Brun, P. Cos, L. Maes, G. Hajos, Z. Riedl, I. Nagy, B. U. W. Maes and L. Pieters, *Bioorg. Med. Chem.*, 2009, **17**, 7209; (*g*) E. V. Costa, M. L. B. Pinheiro, C. M. Xavier, J. R. A. Silva, A. C. F. Amaral, A. D. L. Souza, A. Barison, F. R. Campos, A. G. Ferreira, G. M. C. Machado and L. L. P. Leon, *J. Nat. Prod.*, 2006, **69**, 292; (*h*) E. J. Cooper, A. L. Hudson, C. A. Parker and N. G. Morgan, *Eur. J. Pharmacol.*, 2003, **482**, 189; (*i*) R. Frederick, C. Bruyere, C. Vancraeynest, J. Reniers, C. Meinguet, L. Pochet, A. Backlund, B. Masereel, R. Kiss and J. Wouters, *J. Med. Chem.*, 2012, **55**, 6489; (*j*) Y. Chen, M. Y. Qin, L. Wang, H. Chao, L. N. Ji and A. L. Xu, *Biochimie*, 2013, **95**, 2050; (*k*) T. Akabli, F. Lamchouri, S. Senhaji and H. Toufik, *Struct. Chem.*, 2019, **30**, 1495; (*l*) T. Herraiz and J. Galisteo, *Free Radical Res.*, 2002, **36**, 923; (*m*) F. Aricioglu and H. Altunbas, *Ann. N. Y. Acad. Sci.*, 2003, **1009**, 196; (*n*) H. Khan, S. Patel and M. A. Kamal, *Curr. Drug Metab.*, 2017, **18**, 853.

3 (*a*) K. K. Banoth, Faheem, K. V. G. Chandrasekhar, N. Adinarayana and S. Murugesan, *Heliyon*, 2020, **6**, e04916; (*b*) A. Samala, S. M. Murthy and K. M. Gottumukkala, *Res. J. Pharm. Technol.*, 2018, **11**, 3547.

4 (*a*) S. Nekkanti, R. Tokala and N. Shankaraiah, *Curr. Med. Chem.*, 2017, **24**, 2887; (*b*) A. M. Deveau, M. A. Labroli, C. M. Dieckhaus, M. T. Barthen, K. S. Smith and T. L. MacDonald, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 1251; (*c*) F. Yang, N. Zhao, D. Ge and Y. Chen, *RSC Adv.*, 2019, **9**, 19571; (*d*) K. Yadav, P. N. R. Meka, S. Sadhu, S. D. Guggilapu, J. Kovvuri, A. Kamal, R. Srinivas, P. Devayani, B. N. Babu and N. Nagesh, *Biochemistry*, 2017, **56**, 4392; (*e*) T. Takeuchi, S. Oishi, T. Watanabe, H. Ohno, J. Sawada, K. Matsuno, A. Asai, N. Asada, K. Kitaura and N. Fujii, *J. Med. Chem.*, 2011, **54**, 4839; (*f*) P. A. Barsanti, W. Wang, Z. J. Ni, D. Duhl, N. Brammeier, E. Martin, D. Bussiere and A. O. Walter, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 157; (*g*) K. Toshima, Y. Okuno, Y. Nakajima and S. Matsumura, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 671; (*h*) H. Guan, X. Liu, W. Peng, R. Cao, Y. Ma, H. Chen and A. Xu, *Biochem. Biophys. Res. Commun.*, 2006, **342**, 894.

5 (*a*) S. Gopu, V. Ravi Kumar, K. Laxma Reddy, P. Venkat Reddy and S. Sirasani, *Nucleosides, Nucleotides Nucleic Acids*, 2019, **38**, 349; (*b*) M. Zhang and D. Sun, *Anti-Cancer Agents Med. Chem.*, 2015, **15**, 537; (*c*) C. Viegas Jr, E. J. Barreiro and C. A. M. Fraga, *Curr. Med. Chem.*, 2007, **14**, 1829; (*d*) C. A. M. Fraga, *Expert Opin. Drug Discovery*, 2009, **4**, 605; (*e*) V. Ivasiv, C. Albertini, A. E. Goncalves, M. Rossi and M. L. Bolognesi, *Curr. Top. Med. Chem.*, 2019, **19**, 1694.

6 (*a*) H. Khan, S. Patel and M. A. Kamal, *Curr. Drug Metab.*, 2017, **18**, 853; (*b*) S. Kumar, A. Singh, K. Kumar and V. Kumar, *Eur. J. Med. Chem.*, 2017, **142**, 48; (*c*) J. Dai, W. Dan, U. Schneider and J. Wang, *Eur. J. Med. Chem.*, 2018, **157**, 622; (*d*) C. R. Sahoo, S. K. Paidesetty and R. N. Padhy, *Eur. J. Med. Chem.*, 2019, **162**, 752; (*e*) K. L. Manasa, S. S. Yadav, D. Srikanth, N. Nagesh and M. Alvala, *IOSR J. Pharm. Biol. Sci.*, 2020, **15**, 1; (*f*) P. Piechowska, R. Zawirska-Wojtasiak and S. Mildner-Szkudlarz, *Nutrients*, 2019, **11**, 814.

7 (*a*) M. Milen and P. Abranyi-Balogh, *Chem. Heterocycl. Compd.*, 2016, **52**, 996; (*b*) C. E. P. Galvis and V. V. Kouznetsov, *Synthesis*, 2017, **49**, 4535; (*c*) N. Devi, S. Kumar, S. K. Pandey and V. Singh, *Asian J. Org. Chem.*, 2018, **7**, 6; (*d*) P. Maity, D. Adhikari and A. K. Jana, *Tetrahedron*, 2019, **75**, 965; (*e*) B. E. Love, *Org. Prep. Proced. Int.*, 1996, **28**, 1.

8 (*a*) K. Pulka, *Curr. Opin. Drug Discovery Dev.*, 2010, **13**, 669; (*b*) B. Saha, S. Sharma, D. Sawant and B. Kundu, *Tetrahedron Lett.*, 2007, **48**, 1379; (*c*) L. Gupta, K. Srivastava, S. Singh, S. K. Puri and P. M. S. Chauhan, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3306; (*d*) A. Kamal, M. Sathish, A. V. G. Prasanthi, J. Chetna, Y. Tangella, V. Srinivasulu, N. Shankaraiah and A. Alarifi, *RSC Adv.*, 2015, **5**, 90121; (*e*) B. Shi, R. Cao, W. Fan, L. Guo, Q. Ma, X. Chen, G. Zhang, L. Qiu and H. Song, *Eur. J. Med. Chem.*, 2013, **60**, 10; (*f*) Q. Wu, R. Cao, M. Feng, X. Guan, C. Ma, J. Liu, H. Song and W. Peng, *Eur. J. Med. Chem.*, 2009, **44**, 533; (*g*) R. Cao, W. Peng, H. Chen, X. Hou, H. Guan, Q. Chen, Y. Ma and A. Xu, *Eur. J. Med. Chem.*, 2005, **40**, 249; (*h*) S. Gaikwad, D. Kamble and P. Lokhande, *Tetrahedron Lett.*, 2018, **59**, 2387; (*i*) B. Zheng, T. H. Trieu, T. Z. Meng, X. Lu, J. Dong, Q. Zhang and X. X. Shi, *RSC Adv.*, 2018, **8**, 6834; (*j*) M. Zhao, L. Bi, W. Wang, C. Wang, M. Baudy-Floc'h, J. Ju and S. Peng, *Bioorg. Med. Chem.*, 2006, **14**, 6998; (*k*) R. Cao, H. Chen, W. Peng, Y. Ma, X. Hou, H. Guan, X. Liu and A. Xu, *Eur. J. Med. Chem.*, 2005, **40**, 991.

9 (*a*) A. Kamal, V. Srinivasulu, M. Sathish, Y. Tangella, V. L. Nayak, M. P. N. Rao, N. Shankaraiah and N. Nagesh, *Asian J. Org. Chem.*, 2014, **3**, 68; (*b*) A. Kamal, T. S. Reddy, M. V. P. S. Vishnuvardhan, V. D. Nimbarte, A. V. S. Rao, V. Srinivasulu and N. Shankaraiah, *Bioorg. Med. Chem.*, 2015, **23**, 4608; (*c*) S. Nekkanti, K. Veeramani, S. S. Kumari, R. Tokala and N. Shankaraiah, *RSC Adv.*, 2016, **6**, 103556; (*d*) P. Sharma, T. S. Reddy, N. Praveen, K. Ram, S. K. Bhargava and N. Shankaraiah, *Eur. J. Med. Chem.*, 2017, **138**, 234; (*e*) U. Yadav, A. P. Sakla, R. Tokala, S. T. Nyalam, A. Khurana, C. S. Digwal, V. Talla, C. Godugu, N. Shankaraiah and A. Kamal, *ChemistrySelect*, 2020, **5**, 4356; (*f*) S. Sana, R. Tokala, D. M. Bajaj, N. Nagesh, K. K. Bokara, G. Kiranmai, U. J. Lakshmi,

S. Vadlamani, V. Talla and N. Shankaraiah, *Bioorg. Chem.*, 2019, **93**, 103317; (*g*) G. S. Mani, P. Anchi, S. Sunkari, K. Donthiboina, C. Godugu, N. Shankaraiah and A. Kamal, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127432; (*h*) R. Tokala, D. Bora, S. Sana, F. M. Nachtigall, L. S. Santosh and N. Shankaraiah, *J. Org. Chem.*, 2019, **84**, 5504; (*i*) D. Bora, R. Tokala, S. E. John, B. Prasanth and N. Shankaraiah, *Org. Biomol. Chem.*, 2020, **18**, 2307.

10 (*a*) S. Hotha, J. C. Yarrow, J. G. Yang, S. Garrett, K. V. Renduchintala, T. U. Mayer and T. M. Kapoor, *Angew. Chem., Int. Ed.*, 2003, **42**, 2379; (*b*) N. Shankaraiah, S. Nekkanti, K. J. Chudasama, K. Ram, P. Sharma, M. Kumar, V. G. M. Naidu, V. Srinivasulu, G. Srinivasulu and A. Kamal, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 5413; (*c*) D. Bialonska and J. K. Zjawiony, *Mar. Drugs*, 2009, **7**, 166; (*d*) C. H. Yao, T. C. Hsieh, J. S. Song and J. C. Lee, *Future Med. Chem.*, 2020, **12**, 3.

11 (*a*) D. K. Mahapatra, V. Asati and S. K. Bharti, *Expert Opin. Ther. Pat.*, 2019, **29**, 385; (*b*) N. Shankaraiah, K. P. Siraj, S. Nekkanti, V. Srinivasulu, P. Sharma, K. Ram, M. Sathish, M. V. P. S. Vishnuvardhan, S. Ramakrishna, C. Jadala, N. Nagesh and A. Kamal, *Bioorg. Chem.*, 2015, **59**, 130; (*c*) A. Kamal, V. Srinivasulu, V. L. Nayak, M. Sathish, N. Shankaraiah, C. Bagul, N. V. S. Reddy, N. Rangaraj and N. Nagesh, *ChemMedChem*, 2014, **9**, 2084.

12 (*a*) R. Cao, W. Yi, Q. Wu, X. Guan, M. Feng, C. Ma, Z. Chen, H. Song and W. Peng, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 6558; (*b*) G. Zhang, R. Cao, L. Guo, Q. Ma, W. Fan, X. Chen, J. Li, G. Shao, L. Qiu and Z. Ren, *Eur. J. Med. Chem.*, 2013, **65**, 21; (*c*) R. Cao, W. Fan, L. Guo, Q. Ma, G. Zhang, J. Li, X. Chen, Z. Ren and L. Qiu, *Eur. J. Med. Chem.*, 2013, **60**, 135; (*d*) S. S. Chauhan, A. K. Singh, S. Meena, M. Lohani, A. Singh, R. K. Arya, S. H. Cheruvu, J. Sarkar, J. R. Gayen, D. Datta and P. M. S. Chauhan, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2820; (*e*) P. O. V. Reddy, M. Hridhay, K. Nikhil, S. Khan, P. N. Jha, K. Shah and D. Kumar, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 1278.

13 (*a*) P. De, M. Baltas and F. Bedos-belval, *Curr. Med. Chem.*, 2011, **18**, 1672; (*b*) P. Su, Y. Shi, J. Wang, X. Shen and J. Jhang, *Anti-Cancer Agents Med. Chem.*, 2015, **15**, 980; (*c*) M. Sathish, S. C. Dushantrao, S. Nekkanti, R. Tokala, S. Thatikonda, Y. Tangella, G. Srinivas, S. Cherukommu, N. H. Krishna, N. Shankaraiah, N. Nagesh and A. Kamal, *Bioorg. Med. Chem.*, 2018, **26**, 4916.

14 (*a*) A. K. Bubna, *Indian J. Dermatol.*, 2015, **60**, 419; (*b*) Y. Ling, C. Xu, L. Luo, J. Cao, J. Feng, Y. Xue, Q. Zhu, C. Ju, F. Li, Y. Zhang, Y. Zhang and X. Ling, *J. Med. Chem.*, 2015, **58**, 9214; (*c*) Y. Ling, J. Feng, L. Luo, J. Guo, Y. Peng, T. Wang, X. Ge, Q. Xu, X. Wang, H. Dai and Y. Zhang, *ChemMedChem*, 2017, **12**, 646; (*d*) Y. Ling, J. Guo, Q. Yang, P. Zhu, J. Miao, W. Gao, Y. Peng, J. Yang, K. Xu, B. Xiong, G. Liu, J. Tao, L. Luo, Q. Zhu and Y. Zhang, *Eur. J. Med. Chem.*, 2018, **144**, 398; (*e*) Y. Ling, Y. Li, R. Zhu, J. Qian, J. Liu, W. Gao, C. Meng, J. Miao, B. Xiong, X. Qiu, C. Ling, H. Dai and Y. Zhang, *J. Nat. Prod.*, 2019, **82**, 1442; (*f*) L. Zhang, J. Zhang, Q. Jiang, L. Zhang and W. Song, *J. Enzyme Inhib. Med. Chem.*, 2018, **33**, 714.

15 (*a*) P. Gaspari, T. Banerjee, W. P. Malachowski, A. J. Muller, G. C. Prendergast, J. Duhadaway, S. Bennett and A. M. Donovan, *J. Med. Chem.*, 2006, **49**, 684; (*b*) M. You, M. Moriarty, R. C. Moon and J. M. Pezzuto, *Cancer Res.*, 1997, **57**, 272; (*c*) D. Buac, S. Schmitt, G. Ventro, F. Rani Kona and Q. Ping Dou, *Mini-Rev. Med. Chem.*, 2012, **12**, 1193; (*d*) S. D. Shinde, A. P. Sakla and N. Shankaraiah, *Bioorg. Chem.*, 2020, **105**, 104346; (*e*) A. Kamal, M. Sathish, V. L. Nayak, V. Srinivasulu, B. Kavitha, Y. Tangella, D. Thummuri, C. Bagul, N. Shankaraiah and N. Nagesh, *Bioorg. Med. Chem.*, 2015, **23**, 5511.

16 (*a*) A. A. El Rashedy and H. Y. Aboul-Enein, *Mini-Rev. Med. Chem.*, 2013, **13**, 399; (*b*) H. M. Refaat, *Eur. J. Med. Chem.*, 2010, **45**, 2949; (*c*) P. Singla, V. Luxami and K. Paul, *Bioorg. Med. Chem.*, 2015, **23**, 1691; (*d*) S. Samundeeswari, B. Chougala, M. Holiyachi, L. Shastri, M. Kulkarni, S. Dodamani, S. Jalalpur, S. Joshi, S. Dixit, V. Sunagar and R. Hunnur, *Eur. J. Med. Chem.*, 2017, **128**, 123.

17 A. Kamal, M. P. N. Rao, P. Swapna, V. Srinivasulu, C. Bagul, A. B. Shaik, K. Mullagiri, J. Kovvuri, V. S. Reddy, K. Vidyasagar and N. Nagesh, *Org. Biomol. Chem.*, 2014, **12**, 2370.

18 (*a*) S. Babaji, P. Narayan, K. Thenmozhi and P. Rambhau, *Eur. J. Med. Chem.*, 2014, **81**, 408; (*b*) M. Porchia, M. Pellei, M. Marinelli, F. Tisato, F. Del and C. Santini, *Eur. J. Med. Chem.*, 2018, **146**, 709; (*c*) N. Y. S. Lam, D. Truong, H. Burmeister, M. V. Babak, H. U. Holtkamp, S. Movassaghi, D. M. Ayine-tora, A. Zafar, M. Kubanik, L. Oehninger, T. Sohnel, J. Reynisson, S. M. F. Jamieson, C. Gaiddon, I. Ott and C. G. Hartinger, *Inorg. Chem.*, 2018, **57**, 14427; (*d*) S. U. Dighe, S. Khan, I. Soni, P. Jain, S. Shukla, R. Yadav, P. Sen, S. M. Meeran and S. Batra, *J. Med. Chem.*, 2015, **58**, 3485; (*e*) J. Liu, M. Wang, Y. Zhou, J. Yan, L. Yang, Y. Li, H. Zhang and X. Yang, *RSC Adv.*, 2015, **5**, 63936; (*f*) L. X. Liu, X. Q. Wang, B. Zhou, L. J. Yang, Y. Li, H. B. Zhang and X. D. Yang, *Sci. Rep.*, 2015, **5**, 13101; (*g*) B. Zhou, Z. Liu, G. Deng, W. Chen, M. Li, L. Yang, Y. Li, X. Yang and H. Zhang, *Org. Biomol. Chem.*, 2016, **14**, 9423.

19 (*a*) A. A. Altaf, A. Shahzad, Z. Gul, N. Rasool, A. Badshah, B. Lal and E. Khan, *J. Drug Des. Med. Chem.*, 2015, **1**, 1; (*b*) S. Zheng, Q. Zhong, M. Mottamal, Q. Zhang, C. Zhang, E. Lemelle, H. McFerrin and G. Wang, *J. Med. Chem.*, 2014, **57**, 3369; (*c*) N. Shankaraiah, P. Sharma, S. Pedapati, S. Nekkanti, V. Srinivasulu, N. P. Kumar and A. Kamal, *Lett. Drug Des. Discovery*, 2016, **13**, 335.

20 (*a*) H. G. Yu, J. A. Huang, Y. N. Yang, H. Huang, H. S. Luo, J. P. Yu, J. J. Meier, H. Schrader, A. Bastian, W. E. Schmidt and F. Schmitz, *Eur. J. Clin. Invest.*, 2002, **32**, 838; (*b*) Y. Zhu, J. Fu, K. L. Shurlknight, D. N. Soroka, Y. Hu, X. Chen and S. Sang, *J. Med. Chem.*, 2015, **58**, 6494; (*c*) Q. B. Xu, X. F. Chen, J. Feng, J. F. Miao, J. Liu, F. T. Liu, B. X. Niu, J. Y. Cai, C. Huang, Y. Zhang and Y. Ling, *Sci. Rep.*, 2016, **6**, 36238.

21 (*a*) A. Kumar, A. Vaidya, V. Ravichandran, S. Kumar and R. Kishore, *Bioorg. Med. Chem.*, 2012, **20**, 3378; (*b*) J. Wu, L. Yu, F. Yang, J. Li, P. Wang, W. Zhou, L. Qin, Y. Li, J. Luo, Z. Yi, M. Liu and Y. Chen, *Eur. J. Med. Chem.*, 2014, **80**, 340; (*c*) H. Paulikova, Z. Vantova, L. Hunakova, L. Cizekova, M. Carna, M. Kozurkova, D. Sabolova, P. Kristian, S. Hamuľakova and

J. Imrich, *Bioorg. Med. Chem.*, 2012, **20**, 7139; (*d*) V. A. Barbosa, P. Barea, R. S. Mazia, T. Ueda-Nakamura, W. F. da Costa, M. A. Foglio, A. L. T. Goes Ruiz, J. E. de Carvalho, D. B. Vendramini-Costa, C. V. Nakamura and M. H. Sarragiotto, *Eur. J. Med. Chem.*, 2016, **124**, 1093.

22 (*a*) S. D. Knight, N. D. Adams, J. L. Burgess, A. M. Chaudhari, M. G. Darcy, C. A. Donatelli, J. I. Luengo, K. A. Newlander, C. A. Parrish, L. H. Ridgers, M. A. Sarpong, S. J. Schmidt, G. S. Van Aller, J. D. Carson, M. A. Diamond, P. A. Elkins, C. M. Gardiner, E. Garver, S. A. Gilbert, R. R. Gontarek, J. R. Jackson, K. L. Kershner, L. Luo, K. Raha, C. S. Sherk, C. M. Sung, D. Sutton, P. J. Tummino, R. J. Wegrzyn, K. R. Auger and D. Dhanak, *ACS Med. Chem. Lett.*, 2010, **1**, 39; (*b*) R. Tokala, S. Thatikonda, S. Sana, P. Regur, C. Godugu and N. Shankaraiah, *New J. Chem.*, 2018, **42**, 16226; (*c*) A. P. Kourounakis, D. Xanthopoulos and A. Tzara, *Med. Res. Rev.*, 2020, **40**, 709.

23 (*a*) Y. You, *Curr. Pharm. Des.*, 2005, **11**, 1695; (*b*) F. Cortese, B. Bhattacharyya and J. Wolff, *J. Biol. Chem.*, 1977, **252**, 1134; (*c*) M. Sathish, B. Kavitha, V. L. Nayak, Y. Tangella, A. Ajitha, S. Nekkanti, A. Alarifi, N. Shankaraiah, N. Nagesh and A. Kamal, *Eur. J. Med. Chem.*, 2018, **144**, 557.

24 (*a*) A. Chaudhary, S. N. Pandeya, P. Kumar, P. P. Sharma, S. Gupta, N. Soni, K. K. Verma and G. Bhardwaj, *Mini-Rev. Med. Chem.*, 2007, **7**, 1186; (*b*) N. Nam, *Curr. Med. Chem.*, 2003, **10**, 1697; (*c*) C. Jadala, M. Sathish, T. S. Reddy, V. G. Reddy, R. Tokala, S. K. Bhargava, N. Shankaraiah, N. Nagesh and A. Kamal, *Bioorg. Med. Chem.*, 2019, **27**, 3285.

25 K. Lakshmi Manasa, S. Thatikonda, D. K. Sigalapalli, A. Sagar, G. Kiranmai, A. M. Kalle, M. Alvala, C. Godugu, N. Nagesh and B. Nagendra Babu, *Bioorg. Chem.*, 2020, **101**, 103983.

26 (*a*) A. Thakur, R. Singla and V. Jaitak, *Eur. J. Med. Chem.*, 2015, **101**, 476; (*b*) L. Zhang and Z. Xu, *Eur. J. Med. Chem.*, 2019, **181**, 111587; (*c*) S. Samundeeswari, M. V. Kulkarni, S. D. Joshi, S. R. Dixit, S. Jayakumar and R. M. Ezhilarasi, *ChemistrySelect*, 2016, **1**, 5019.

27 (*a*) A. Kamal, S. Prabhakar, M. Janaki Ramaiah, P. Venkat Reddy, C. Ratna Reddy, A. Mallareddy, N. Shankaraiah, T. L. N. Reddy, S. N. C. V. L. Pushpavalli and M. Pal-Bhadra, *Eur. J. Med. Chem.*, 2011, **46**, 3820; (*b*) N. Shankaraiah, A. P. Sakla, K. Laxmikeshav and R. Tokala, *Chem. Rec.*, 2020, **20**, 253; (*c*) N. Shankaraiah, C. Jadala, S. Nekkanti, K. R. Senwar, N. Nagesh, S. Shrivastava, V. G. M. Naidu, M. Sathish and A. Kamal, *Bioorg. Chem.*, 2016, **64**, 42; (*d*) P. Salehi, K. Babanezhad-Harikandei, M. Bararjanian, A. Al-Harrasi, M. A. Esmaeili and A. Aliahmadi, *Med. Chem. Res.*, 2016, **25**, 1895; (*e*) B. Sharma, L. Gu, R. P. Pillay, N. Cele, P. Awolade, P. Singh, M. Kaur and V. Kumar, *New J. Chem.*, 2020, **44**, 11137; (*f*) S. Nekkanti, V. Pooladanda, M. Veldandi, R. Tokala, C. Godugu and N. Shankaraiah, *ChemistrySelect*, 2017, **2**, 7210.

28 (*a*) Y. Wan, Y. Li, C. Yan, M. Yan and Z. Tang, *Eur. J. Med. Chem.*, 2019, **183**, 111691; (*b*) S. Dadashpour and S. Emami, *Eur. J. Med. Chem.*, 2018, **150**, 9; (*c*) A. K. Singh, V. Raj and S. Saha, *Eur. J. Med. Chem.*, 2017, **142**, 244; (*d*) G. D. Demetri, A. T. van Oosterom, C. R. Garrett, M. E. Blackstein, M. H. Shah, J. Verweij, G. McArthur, I. R. Judson, M. C. Heinrich,

J. A. Morgan, J. Desai, C. D. Fletcher, S. George, C. L. Bello, X. Huang, C. M. Baum and P. G. Casali, *Lancet*, 2006, **368**, 1329; (*e*) R. J. Motzer, T. E. Hutson, P. Tomczak, M. D. Michaelson, R. M. Bukowski, S. Oudard, S. Negrier, C. Szczylik, R. Pili, G. A. Bjarnason, X. Garcia-del-Muro, J. A. Sosman, E. Solska, G. Wilding, J. A. Thompson, S. T. Kim, I. Chen, X. Huang and R. A. Figlin, *J. Clin. Oncol.*, 2009, **27**, 3584; (*f*) R. Tokala, S. Thatikonda, U. S. Vanteddu, S. Sana, C. Godugu and N. Shankaraiah, *ChemMedChem*, 2018, **13**, 1909; (*g*) J. Kovvuri, B. Nagaraju, V. L. Nayak, R. Akunuri, M. P. N. Rao, A. Ajitha, N. Nagesh and A. Kamal, *Eur. J. Med.*

Chem., 2018, **143**, 1563; (*h*) R. Tokala, S. Sana, U. J. Lakshmi, P. Sankarana, D. K. Sigalapalli, N. Gadewal, J. Kode and N. Shankaraiah, *Bioorg. Chem.*, 2020, **105**, 104357.

29 (*a*) M. Singh and S. K. Singh, *Anti-Cancer Agents Med. Chem.*, 2014, **14**, 127; (*b*) A. Kamal, M. A. H. Syed and S. M. Mohammed, *Expert Opin. Ther. Pat.*, 2015, **25**, 335; (*c*) P. C. Sharma, D. Sharma, A. Sharma, K. K. Bansal, H. Rajak, S. Sharma and V. K. Thakur, *Appl. Mater. Today*, 2020, **20**, 100783; (*d*) R. Tokala, S. Mahajan, G. Kiranmai, D. K. Sigalapalli, S. Sana, S. E. John, N. Nagesh and N. Shankaraiah, *Bioorg. Chem.*, 2021, **106**, 10448.